

EPHAR2024

The Federation of European Pharmacological Societies

9th European Congress of Pharmacology



23-26
June
2024



ATHENS
GREECE

Megaron Athens
International
Conference Centre

ephar2024.org

**ABSTRACT
BOOK**

Oral Communications

OC01. E2F Transcription Factor 2 (E2F2) Facilitates Atherosclerosis Through CCAAT Enhancer Binding Protein β Mediated Endothelial Cell Inflammation

Wu Y¹, Huang X¹, Huang Y¹, Wang L¹

¹*Department of Biomedical Sciences, City University of Hong Kong, Kowloon, China*

Introduction: Atherosclerosis, the leading cause of most cardiovascular diseases, begins with damage to the vascular endothelium. E2F transcription factor 2 (E2F2) is a crucial regulator of cell proliferation and is involved in multiple pathways related to atherosclerosis, including apoptosis, differentiation, metabolism, and inflammation. To clarify the mechanism behind endothelial damage and resulting atherosclerosis, RNA-seq was conducted on the endothelium derived from atherosclerotic mice, and the result suggests a correlation between E2F2 and atherosclerosis. The study hypothesizes that E2F2 worsens atherosclerosis by causing damage to endothelial cells.

Methods: RNA-seq analysis was used to identify the mechanisms causing endothelial cell damage during atherosclerosis in ApoE-/- mice. To confirm the involvement of E2F2 in endothelial cell damage and subsequent atherosclerosis, we utilized carotid artery surgery-induced atherosclerotic mice and ApoE-/- mice with endothelial cell-specific E2F2 knockdown. HUVECs were treated with either the E2F2 inhibitor HLM006474 or the E2F2 overexpression adenovirus to identify and confirm downstream pathways and molecules involved in E2F2-induced endothelial cell damage.

Results: This study found a potential association between E2F2 and atherosclerosis, as evidenced by the activation of the E2F target pathway in the endothelium of atherosclerotic mice and the significant increase of E2F2 in the aorta of atherosclerotic mice and renal arteries of patients with plaque. Additionally, HLM006474 and endothelial cell-specific E2F2 knockdown decreased the plaque area in atherosclerotic mice, highlighting the pivotal role of E2F2 in atherosclerosis. (n=3-10)

In HUVECs, HLM006474 inhibits pathways related to endothelial cell inflammation and reduces levels of pro-inflammatory cytokines. On the other hand, overexpression of E2F2 enhances pathways related to endothelial cell inflammation and increases levels of pro-inflammatory cytokines. These results suggest that E2F2 can trigger inflammation in endothelial cells. (n=3) CCAAT Enhancer Binding Protein β (cebp/ β) is a confirmed transcription factor that mediates the release of multiple pro-inflammatory cytokines in monocytes induced by oxidized LDL. Our results indicate that overexpression of E2F2 escalates the expression of CCAAT Enhancer Binding Protein β (cebp/ β). Oppositely, interfering with cebp/ β expression via siRNA can reverse the increase in pro-inflammatory cytokines due to E2F2 overexpression. This suggests that E2F2-induced endothelial cell inflammation is mediated by cebp/ β . (n=3-6)

Conclusion: This study confirms that elevated E2F2 in atherosclerotic endothelium promotes atherosclerosis through cebp/ β -mediated endothelial cell inflammation. The E2F2 inhibitor HLM006474 also shows potential as a therapeutic agent for atherosclerosis.

OC02. Cyst(e)ine import and catabolism preserve endothelial chromatin accessibility and promote vessel growth

Mettner J^{1,2}, Drekolia M^{1,2}, Wittig J^{1,2}, Siragusa M², Weigert A³, Fleming I², Günther S⁴, Looso M⁴, Hecker D⁵, Schulz M⁵, Hu J^{1,6}, Bibli S^{1,2}

¹*Department for Vascular Dysfunction, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany*, ²*Institute for Vascular Signalling, Goethe University, Frankfurt, Germany*,

³*Institute for Biochemistry I, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany*,

⁴*Cardio-Pulmonary Institute (CPI) Bioinformatics and Deep Sequencing Platform, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany*, ⁵*Institute for Cardiovascular Regeneration, Computational Epigenomics & System Cardiology, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany*, ⁶*Department of Histology and Embryology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*

Aim: Metabolic rewiring of the quiescent vasculature is responsible for endothelial growth [1]. This study set out to investigate the importance of the Solute Carrier (SLC) Transporter-family SLC7A11-CSE axis in metabolically active endothelial cells and how cyst(e)ine catabolism controls endothelial cell identity and proliferation.

Methods: RiboTag sequencing was employed to identify alterations of key SLC transporter and metabolic proteins in proliferating and quiescent human endothelial cells. Expression of target SLCs was further validated with immunohistochemistry *in vitro*, as well as by flow cytometry of postnatal day 6 (P6) retina of Fluorescent Ubiquitin Cell Cycle Indicator (FUCCI) mice *in vivo*. ¹³C metabolic flux analysis of ¹³C-Cystine was also performed, coupled with ¹³C histone proteomics, ATAC-seq, Cut&Run, and RNA-sequencing analysis. Functional angiogenesis assays were performed *in vitro* in cells silenced for CSE or SLC7A11. *In vivo* angiogenesis was characterized in the postnatal retina of the endothelial specific inducible CSE knock out mice (CSEfl/flxPdgfb Cre).

Results: RiboTag sequencing revealed that among the 1000 metabolic transcripts, more than 280 belonged to the Solute Carrier Transporter family. Interestingly, 5 SLC transporter were significantly enriched in the proliferating endothelium, including the cystine transporter SLC7A11. FUCCI analysis of endothelial cells confirmed that surface expression of SLC7A11 was higher in G2/M than in G1/G0 arrested cells. Transported cystine from the SLC7A11 is rapidly reduced to cysteine and can flux to acetyl-CoA through the enzyme cystathionine gamma lyase (CSE). Indeed, in the SLC7A11high proliferative endothelium, utilized cystine carbons support more than 30% of the endothelial acetyl-CoA pool. Subsequent ¹³C histone proteomics linked the cystine-derived acetyl-moiety to the acetylation of histone 3 (H3). ATACseq of CSE deleted endothelial cells of inducible CSE knock out mice showed that cysteine carbons are responsible for maintaining the chromatin accessible to key transcriptional factors that support angiogenesis. RNAseq analysis confirmed that CSE preserves transcriptional marks that support endothelial cell fate commitment, proliferation and identity. Functionally, *in vitro* and *ex vivo* models of angiogenesis showed that cystine catabolism is essential for endothelial proliferation. Simultaneously, *in vivo*, depletion of CSE and induction of endothelial cysteinostasis reduced the proliferative capacity of the vascular front.

Conclusions: Cystine import through the SLC7A11 transporter and subsequent metabolic rewiring to acetyl-CoA through the CSE enzyme, regulate endothelial cell transcription and proliferative capacity by preserving the endothelial chromatin architecture.

[1] Falkenberg, K. D., Rohlenova, K., Luo, Y. & Carmeliet, P. The metabolic engine of endothelial cells. *Nature metabolism* 1, 937–946; (2019).

OC03. Investigating the cardioprotective potential of novel Sirtuin agonists. Sirtuin 5 emerges as a new target in mitigating myocardial ischemia-reperfusion injury

Castiello C¹, **Efentakis P¹**, Symeonidi L¹, Chania C¹, Tasouli A², Kostomitsopoulos N³, Mikros E⁴, Zwergel C⁵, Valente S⁵, Mai A⁵, Andreadou I¹

¹Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece, ²Onassis Cardiac Surgery Center, Athens, Greece, ³Biomedical Research Foundation of the Academy of Athens, Athens, Greece, ⁴Department of Pharmaceutical Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece, ⁵Department of Drug Chemistry and Technologies; University of Rome; Sapienza, Rome, Italy

Introduction: Myocardial infarction is the leading cause of mortality. Sirtuins (SIRTs) exhibit numerous physiological actions, including redox and metabolic regulation of the myocardium. Seven SIRTs have been described, however their role in myocardial ischemia/reperfusion injury (IRI) is elusive and is based on studies exploiting non-specific SIRT agonists. Herein, we investigated i. the isoform-dependent cardioprotective potential of SIRTs using newly-synthesized agonists in vitro, in cardiomyoblasts (H9c2 cells) and human endothelial cells (EA.hy-926 cells) ii. the cardioprotective potential of SIRT agonists in an in vivo model of IRI iii. the underlying cardioprotective mechanism.

Methods: We synthesized three novel SIRT agonists, namely MC2606, MC2789 and MC3215, exhibiting SIRT1, SIRT3 and SIRT5 agonism respectively. H9c2 and EA.hy-926 cells were incubated with the compounds at 1-20µM and were subjected to 24h hypoxia and 2 hours reoxygenation. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The compounds' selectivity on SIRT isoforms was assessed by immunoblotting analysis of acetyl- and malonyl-lysine. In vivo, male C57BL/6J mice underwent 30min ischemia (I) and 2h reperfusion (R). Mice received MC2606 and MC3215 (30mg/kg) at the 20th min of I and Infarct size (IS) was quantified by Triphenyl-Tetrazolium Chloride (TTC)-EVAN's Blue staining (n=5-7/group). Additionally, we challenged the cardioprotective potential of the compounds in an in vivo model of 24h R at 20mg/kg (n=7/group). Experiments were repeated up to the 10th min of R, solely for MC3215, for myocardial tissue sampling and immunoblotting analysis. Seahorse-Mitostress® analysis was conducted in the EA.hy-926 cells for the investigation of MC3215's metabolic profile.

Results: Sirt1 (MC2606) and Sirt5 (MC3215) agonists mitigated hypoxia/reoxygenation injury in both cell lines. Compounds' SIRT-agonism specificity was favorable in the EA.hy-926 cells. In vivo, both compounds reduced IS at 2h R compared to Controls (27.57 ± 2.16 and 24.72 ± 2.37 vs 34.85 ± 1.79 respectively), whilst only the Sirt5 agonist spared IS at 24h R compared to Controls (25.18 ± 2.72 vs 38.80 ± 4.71 respectively). Immunoblotting analysis deduced that MC3215 activates Reperfusion Injury Salvage Kinase (RISK) pathway, by increasing endothelial NO synthase (eNOS), protein kinase-B (Akt) and Glycogen Synthase Kinase-3β (GSK3β) phosphorylation. Additionally, MC3215 increased AMP-activated kinase-α (AMPKα) and Acetyl-CoA carboxylase (ACC) phosphorylation indicating an induction of fatty acids β-oxidation. The metabolic effect of MC3215, leading to an enhanced energetic phenotype characterized by increased aerobic and glycolytic metabolism was also confirmed by Seahorse-Mitostress® analysis.

Conclusions: SIRT5 agonism arises as a novel cardioprotective target, leading to activation of RISK pathway and mitochondria-related metabolic effects, towards salvaging ischemic myocardium from IRI.

OC04. Effect of SGLT2i on heart tissue in diabetic rats: SIRT1 connection

Kizrak U¹, **Özşarlak-Sözer G¹**, Shamamedov K¹, Alan-Albayrak E¹, Cilaker-Micili S², Cavdar Z³, Ural C³, Cetinkalp S⁴, Bilici-Guler G², Kerry Z¹

¹Ege University Faculty of Pharmacy Department of Pharmacology, Izmir, Turkey, ²Dokuz Eylul University, Faculty of Medicine, Department of Histology and Embryology, Izmir, Turkey, ³Dokuz Eylul University, Faculty of Medicine, Department of Molecular Medicine, Izmir, Turkey, ⁴Ege University, Faculty of Medicine, Department of Endocrinology and Metabolic Diseases, Izmir, Turkey

Introduction: Sodium glucose cotransporter 2 inhibitors (SGLT2i) are a novel group of antihyperglycemic drugs that are used for the treatment of T2DM by inhibiting glucose reabsorption from the kidneys. Recently, SGLT2i's are more prominent because of their cardioprotective effect, especially by significantly reducing the risk of hospitalization and death due to heart failure (HF). In our study, we aimed to demonstrate an explanatory mechanism for empagliflozin (EMPA) to prevent diabetic cardiomyopathy (DCM).

Methods: Rats (n=30) were divided into 3 groups: control (n=10), diabetic (n=10) and diabetes+EMPA treatment (n=10). The type 2 diabetes model was established by feeding a high-fat diet (HFD, 35% kcal) for 4 weeks followed by low-dose streptozotocin (STZ) (35 mg/kg, i.p.). Empagliflozin (10 mg/kg/day) was administered to the treatment group via drinking water for 12 weeks. Blood glucose levels of the rats were measured weekly during the experiment (18 weeks). In isolated heart tissues, SIRT1 and SIRT6 expression levels, total reactive oxygen species (ROS) levels, GRP78 and Troponin-I expression levels, MMP-2/-9 activity levels and protein levels of tissue inhibitors (TIMP-1/2) were measured. Additionally, Hematoxylin-Eosin and Masson Trichrome stainings were performed on heart tissue.

Results: Blood glucose level ($E_{max}=252.1\pm6.97$, $p<0.001$) increased in diabetic rats and EMPA ($E_{max}=116\pm3.74$, $p<0.001$) reduced this increase. EMPA caused a decrease in ROS levels in heart tissue ($p<0.001$). GRP78 ($E_{max}=322.9\pm0.91$, $p<0.001$) and Troponin-I expression levels ($E_{max}=321.6\pm0.40$, $p<0.001$), which increase in diabetes, decreased with EMPA treatment ($E_{max}=250.0\pm1.09$; $E_{max}=241.7\pm0.57$ $p<0.001$). It was observed that MMP-2 activity level increased in the diabetic group ($E_{max} = 73.43\pm17.54$, $p = 0.0036$) and this increase was reversed with EMPA ($E_{max} = 16.07\pm1.63$, $p = 0.0037$). Histological scoring due to myocyte degeneration, vacuolization and collagen accumulation increased in the diabetic group (2.5 ± 0.16) and decreased in the EMPA group (1.3 ± 0.16 , $p<0.001$). Collagen accumulation observed in the diabetic group was reduced by EMPA. The decrease in the expression levels of SIRT1 and SIRT6 in the diabetes group compared to the control group was found to be statistically significant ($p = 0.0009$; $p = 0.0007$); On the contrary, EMPA treatment reversed this decrease in SIRT1 expression level ($p = 0.0006$).

Conclusion: EMPA may have a protective effect against DCM by reducing MMP-2 activity, ROS level, GRP78 and Troponin-I expression levels, and collagen accumulation in heart tissue, which are increased in diabetes. These beneficial effects of EMPA on heart tissue can be explained by the increase in SIRT1 expression.

OC05. The functional interaction of Apolipoprotein A2 with the metabolic system of apolipoprotein E -rich- lipoproteins in the regulation of the lipid profile

Kakafoni G¹, Mparnia V¹, Giannatou K¹, Zvintzou E^{1,2}, Kypreos K¹, Gafencu A², Ioana Madalina F²

¹Pharmacology Laboratory, Department of Medicine, School of Health Sciences, University of Patras, Patras, Greece, ²Institute of Cellular Biology and Pathology "Nicolae Simionescu" of the Romanian Academy, Bucharest, Romania

Recently we showed that hepatic production of human apolipoprotein A2 (APOA2) in wild-type mice causes an increase in both triglycerides and total cholesterol [1]. Conversely, our preliminary data also indicate that APOA2, in the absence of functional apolipoprotein E (APOE), leads to reduced triglyceride levels, due to a reduced rate of their hepatic secretion. Thus, a possibility of a functional interaction of APOA2 with the APOE-lipoprotein metabolic system has arisen, the mechanism of which remains unclear.

Here we studied the effect of APOA2 on the metabolism of triglyceride-rich lipoproteins (TRLs) and metabolic activation of adipose tissue. We used APOE (apoe-/-) or LDLR (ldlr-/-) deficient mice fed a high-lipid diet for 2 weeks, which were subsequently infected with a recombinant adenovirus expressing either APOA2 or green fluorescent protein alone.

In apoe-/- mice, APOA2 expression causes a reduction in both cholesterol and VLDL-triglycerides, which was also observed in ldlr-/- mice, indicating that this reduction in apoe-/- mice is independent of clearance through low density lipoprotein receptor (LDLR). In apoe-/- mice APOA2 causes significantly reduced hepatic triglyceride secretion, which did not appear to be the case in ldlr-/- mice. Gene expression analysis of liver enzymes showed that APOA2 affects de novo triglyceride biosynthesis and TRL formation. This was accompanied by a significant reduction of hepatic triglycerides in the apoe-/- mice and a significant increase in ldlr-/- mice. Finally, the study of the mitochondrial metabolic activation of the brown adipose tissue showed that the expression of APOA2 leads to a decrease in the rate of oxidative phosphorylation in apoe-/- mice, and to an increase in ldlr-/- mice. A similar study in white adipose tissue showed that APOA2 causes a reduction in metabolic mitochondrial activity in both groups of experimental animals.

Hypertriglyceridemia is an important and independent factor in the development of atherosclerotic disease [2]. The mechanism by which APOA2 interacts with the APOE-LDLR system and regulates the lipid profile is under investigation. Taken together, our data suggests that apolipoprotein A2 could be a possible therapeutic target for hypertriglyceridemia and other metabolic disorders related to the development of atherosclerosis.

[1] Zvintzou E, Xepapadaki E, Kalogeropoulou C, Filou S, Kypreos KE. Pleiotropic effects of apolipoprotein A-II on high-density lipoprotein functionality, adipose tissue metabolic activity and plasma glucose homeostasis. *The Journal of Biomedical Research.* 2020;34(1):14. Doi:10.7555/JBR.33.20190048

[2] Handelman Y, Shapiro MD. Triglycerides, atherosclerosis, and cardiovascular outcome studies: Focus on omega-3 fatty acids. *Endocrine Practice.* 2017;23(1):100-112. Doi:10.4158/EP161445.RA

OC06. Potential repurposing of the SYK inhibitor Fostamatinib in a diet-induced model of insulin resistance: an in vivo study

Aimaretti E¹, Porchietto E², Ferreira Alves G², Einaudi G², Collotta D³, Rubeo C¹, Marzani E³, Mastrocola R¹, Cifani C², Aragno M¹, Collino M³

¹*Department of Clinical and Biological Sciences, University of Turin, Turin, Italy*, ²*School of Pharmacy, University of Camerino, Camerino, Italy*, ³*Department of Neuroscience "Rita Levi Montalcini", University of Turin, Turin, Italy*

Introduction: A low-grade chronic inflammation ("metaflammation") is a mechanism leading to insulin resistance, diabetes, and obesity. Recent evidence suggests a key role for the spleen tyrosine kinase (SYK)[1].

The potential of pharmacological modulation of SYK within the context of diet-induced metabolic derangements has not yet been investigated. Here we test the effects of chronic administration of Fostamatinib disodium hexahydrate (R788)[2], a drug recently approved for treating chronic immune thrombocytopenia, in a high-fat-high-sugar diet murine model.

Methods: 4-weeks old male C57BL/6OlaHsd mice were fed either a standard diet (10% fat, SD, n=15) or a high-fat-high sugar diet (58% fat, 26% carbohydrate, HFHS, n=15) for 12 weeks. A subgroup of HFHS-fed mice was treated with Fostamatinib (30 mg/kg die, p.o., n=15) for the last 8 weeks (HFHS+FOST). Blood and gastrocnemius muscle were collected for biochemical analyses. The Shapiro-Wilk test was used to assess data distribution. The statistical analysis was performed by one-way ANOVA, followed by Bonferroni's post-hoc test. A p-value <0.05 was considered significant. Data are expressed as mean±S.E.M.

Results: HFHS feeding resulted in obesity (SD=25,15±0,90g, HFHS=31,59±1,47g; p<0,05vsSD; n=15/group) and insulin-resistance with an increased HOMA-IR (HOMeostasis Model Assessment - Insulin Resistance). Moreover, HFHS diet increased serum concentrations of interleukin-1beta (SD=0,25±0,05pg/mL, HFHS=1,13±0,14pg/mL; p<0,05vsSD; n=10/group). These metabolic and inflammatory abnormalities were dramatically reduced by Fostamatinib administration (body weight: HFHS+FOST=30,18±1,47g; interleukin-1beta: HFHS+FOST=0,31±0,07pg/mL; p<0,05vsHFHS; n=10-15/group). These effects were associated with a significant activation of the SYK pathway in the gastrocnemius muscle, leading to the formation of the inflammatory complex NLRP3 inflammasome and a higher local protein expression of interleukin-1beta (p<0,05 vs SD). When Fostamatinib was administered, these beneficial results were paralleled by a massive reduction in the SYK activity, which contributed to inhibiting NLRP3 inflammasome complex formation and activation. Interestingly, Fostamatinib restored effectively (p<0,05 vs HFHS) the molecular insulin pathway and GLUT4 translocation in gastrocnemius muscle of HFHS-fed mice, thus suggesting an effect of Fostamatinib on insulin sensitivity.

Conclusions: The downregulation of SYK-ASC-NLRP3 pathway may represent a novel pharmacological approach for the treatment of diet-related insulin resistance and obesity, with the potential for EMA- and FDA-approved Fostamatinib to be repurposed for the treatment of type 2 diabetes and/or its complications.

[1] Lin YC, Huang DY, Wang JS, et al. Syk is involved in NLRP3 inflammasome-mediated caspase-1 activation through adaptor ASC phosphorylation and enhanced oligomerization. J Leukoc Biol. 2015;97(5):825-835. doi:10.1189/jlb.3HI0814-371RR

[2] Markham A. Fostamatinib: First Global Approval. Drugs. 2018;78(9):959-963. doi:10.1007/s40265-018-0927-1

OC07. Pharmacological inhibition of cyclin-dependent kinase 9 (CDK9) ameliorates metabolic abnormalities evoked by chronic consumption of hypercaloric diet in mice

Porchetto E¹, Collotta D², Aimaretti E³, Ferreira Alves G¹, Einaudi G¹, Marzani E², Rubeo C³, Mastrocola R³, Aragno M³, Cifani C¹, Collino M²

¹*Pharmacology Unit, School of Pharmacy, University of Camerino, Camerino, Italy*, ²*Department of Neurosciences “Rita Levi Montalcini”, University of Turin, Turin, Italy*, ³*Department of Clinical and Biological Sciences, University of Turin, Turin, Italy*

Introduction: Cyclin-dependent-kinase 9 (CDK9) is a protein kinase whose abnormal upregulation has been reported to be involved in several pathologies, such as atherosclerosis and osteoarthritis. CDK9 was recently implicated in inflammatory conditions, where an increased interaction between CDK9 and NF κ B and MAPKs and subsequent activation of downstream signals were observed [1,2]. Here we explored the role of CDK9 in metaflammation and the potential beneficial effects of its pharmacological inhibition with a potent and selective inhibitor, LDC000067, in an in vivo model of diet-induced metabolic abnormalities.

Methods: 4 weeks old male C57BL/NHsd mice were fed with a standard diet (SD,n=12) or a high-fat-high-sugar diet (HFHS,n=24) for 16 weeks. A subgroup of HFHS were administered LDC000067 (10mg/kg/day/p.o., dissolved in 1% CMCNa, HFHS+LDC067,n=12) for the last 12 weeks. Body composition was measured with LF50 minispec analyser. At the end of the protocol, oral glucose tolerance test and insulin tolerance test were performed; organs and plasma were collected for the ex-vivo analyses. Shapiro-Wilk test was used to verify data distribution. One way and two ANOVA followed by the analysis of variance and Bonferroni's test were assessed for establishing a standard of significance level ($p<0.05$). Data are expressed as mean \pm SEM.

Results: HFHS consumption evoked body weight gain, specifically fat mass accumulation and lean mass depletion ($p<0.05$ vs SD); LDC000067 reduced body weight gain, decreasing fat accumulation, and preventing lean mass loss ($p<0.05$ vs HFHS). Hypercaloric diet impaired glucose tolerance and insulin sensitivity ($p<0.05$ vs SD), while LDC000067 administration restored glucose homeostasis and insulin sensitivity. Furthermore, inhibition of CDK9 affects hormonal and lipid profile, resulting in an improvement of their plasmatic levels ($p<0.05$ vs HFHS). HFHS group exhibited increased plasmatic levels of cholesterol, transaminases and pro-inflammatory cytokines such as IL-6(SD=13.77 \pm 0.84, HFHS=17.46 \pm 1.11 μ g/ml,n=10/group, $p<0.05$), along with decreased levels of anti-inflammatory cytokines i.e. IL-10(SD=70.72 \pm 2.1, HFHS=56.52 \pm 3.6 μ g/ml,n=10/group, $p<0.05$); noteworthy, CDK9 inhibition reduced these values back to the standard group IL-6(HFHS=17.46 \pm 1.11, HFHS+LDC067=13.57 \pm 0.94 μ g/ml,n=10/group, $p<0.05$), IL-10(HFHS=56.52 \pm 3.6, HFHS+LDC067=67.59 \pm 3.1 μ g/ml,n=10/group, $p<0.05$).

Conclusions: Pharmacological inhibition of CDK9 with LDC000067 reduced diet-induced harmful effects, thus suggesting a key role for this kinase as a new tool for counteracting metabolic abnormalities.

[1] Yang X, Luo W, Li L, et al. CDK9 inhibition improves diabetic nephropathy by reducing inflammation in the kidneys. *Toxicol Appl Pharmacol.* 2021;416:115465. doi:10.1016/j.taap.2021.115465

[2] Huang S, Luo W, Wu G, et al. Correction for: Inhibition of CDK9 attenuates atherosclerosis by inhibiting inflammation and phenotypic switching of vascular smooth muscle cells. *Aging (Albany NY)*. 2022;14(7):3329-3330. doi:10.18632/aging.204017

OC08. FKBP5 rs4713916 could serve as a genetic predictor of interindividual different response to inhaled corticosteroids in patients with chronic obstructive pulmonary disease

Ntenti C¹, Papakonstantinou E³, Grize L², Stoltz D², Goulas A¹

¹1st Laboratory of Pharmacology, Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, ²Clinic of Respiratory Medicine and Pulmonary Cell Research, University Hospital Basel, Basel, Switzerland, ³Clinic of Respiratory Medicine, Faculty of Medicine, University of Freiburg, Freiburg, Germany

Background: Inhaled glucocorticoids (ICS) are highly effective for asthma management but show considerably less efficacy in Chronic Obstructive Pulmonary Disease (COPD) treatment. This difference in response to ICS treatment could be due to genetic variations. Notably, multiple single-nucleotide polymorphisms (SNPs) have been identified in the FKBP5 gene. This gene is involved as a co-chaperone in the Hsp90 complex, interacting with the glucocorticoid receptor (GR) steroid, suggesting a genetic basis for the variability in ICS treatment outcomes.

Aim: Our research aimed to investigate any possible associations between common variants of this gene and aspects related to COPD, including disease traits, lung capacity, and the response to ICS treatment.

Methodology: A total of 165 patients with COPD, GOLD stage B-D, who were enrolled in the HISTORIC study (ISRCTN11017699)-a randomized, placebo-controlled, double-blind, investigator-initiated trial- were included in our study. Patients were genotyped for the rs3800373, rs4713916 and rs1360780 polymorphisms of the FKBP5 gene. The statistical analysis was carried out with the SPSS program and R Studio with a multivariate analysis with a 95% confidence interval.

Results: At the end of the study, patients carrying the heterozygous (AG) genotype for rs4713916 exhibited significant improvement, marked by a consistent and significant rise in FEV1 values ($p=0.020$, with an increase of 200 ml over 12 months). These individuals also reported a clinically relevant decrease in the self-reported Saint-George questionnaire score (-5.2 units) suggesting that the response to ICS in relation to the rs4713916 genotype exemplifies a heterozygote advantage. The same genetic subgroup of patients also displayed elevated parameters related to DLCO (Diffusing Capacity of the Lungs for Carbon Monoxide) at the beginning of the study ($p<0.020$). The findings from the other two polymorphisms present more complex results that justify additional research.

Conclusion: Our results are robust, derived from a well-defined cohort in a randomized clinical trial, and indicate that the polymorphism rs4713916 in the FKBP5 gene could be a reliable predictor of the effectiveness of ICS treatment and clinical outcomes in COPD patients.

OC09. The Role of DHEA(s) in Dementia: Insights from Cellular and Animal Models and Human Patients

Vuic B¹, Milos T¹, Tudor L¹, Konjevod M¹, Farkas V¹, Nikolac Perkovic M¹, Nedic Erjavec G¹, Knezovic A², Osmanovic Barilar J², Szabo A³, Farkas S³, Zelena D³, Uzun S⁴, Kozumplik O⁴, Mimica N⁴, **Svob Strac D¹**

¹Laboratory of Molecular Neuropsychiatry, Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia, ²Department of Pharmacology, School of Medicine, University of Zagreb, Zagreb, Croatia, ³Centre for Neuroscience, Szentagothai Research Centre, Institute of Physiology, Medical School, University of Pécs, Pecs, Hungary, ⁴Department for Biological Psychiatry and Psychogeriatrics, University Psychiatric Hospital Vrapce, Zagreb, Croatia

Introduction: Dementia is a syndrome characterized by progressive loss of cognitive functions, predominantly affecting older individuals, with Alzheimer's disease (AD) and vascular dementia (VaD) as the most common forms. Neurosteroids dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are involved in various brain functions such as neural survival, plasticity, cognition and behavior and therefore have been studied for their neuroprotective potential in dementia. In order to better understand the role of these neurosteroids in dementia, we have investigated potential neuroprotective effects of DHEA(S) in cellular and animal models, as well as human patients.

Methods: To investigate neuroprotective effects of DHEA(S) on cellular survival and viability in an in vitro model of AD and VaD, we have exposed primary mouse neurons and human neuroblastoma cells to toxic A β oligomers or to oxygen-glucose deprivation (OGD), respectively. In a genetic AD model, triple-transgenic AD (3xTg-AD) mice were chronically treated with DHEAS using subcutaneously implanted osmotic pumps. Pharmacologically induced AD model was established by intracerebroventricularly injecting the C57BL/6 mice with A β oligomers and chronically administered with DHEA via intraperitoneal injection. Cognitive and behavioral testing was conducted on both animal models. Changes in the expression of proteins involved in PI3K signaling following DHEA(S) treatment have been investigated in cellular and animal models of dementia using Western blot analysis. Cognitive symptoms of individuals with dementia and mild cognitive impairment (MCI) (comparative group) were evaluated by MMSE (Mini mental state examination). DHEA(S) plasma concentrations were determined by ELISA, whereas genotyping for rs2637125 polymorphism in SULT2A1 gene, coding for enzyme catalyzing the formation of DHEAS from DHEA, was conducted using qPCR.

Results: Findings obtained using in vitro models of dementia indicated potential neuroprotective effects of DHEA(S) probably via their antiapoptotic actions on cell survival. Behavioral analysis in mouse models of AD demonstrated protective effects of DHEA(S) on fear-conditioned memory, and trend towards beneficial effects on visual short-term and spatial memory, as well as anxiety-like behavior. Results also suggested that DHEA(S) might modulate the expression of key apoptosis regulators via PI3K-AKT signaling pathway and BDNF regulation. In human patients, there were no changes in DHEAS plasma concentration and no association of SULT2A1 rs2637125 polymorphism with dementia and cognitive function; however, patients with severe cognitive impairment were not included in the study.

Conclusions: Obtained results suggested the role of DHEA(S) in dementia and their potential beneficial effects in dementia prevention and treatment. However, these findings need to be confirmed in larger studies.

OC10. Dissecting the role of p75 pan-neurotrophin receptor in the hyperglycemia – induced neuropathology using a human iPSC-based model.

Chanoumidou K^{1,2}, Zota I^{1,2}, Papadopoulou M^{1,2}, Gravanis A^{1,2}, Charalampopoulos I^{1,2}

¹*Pharmacology Dept., Medical School, University of Crete, Heraklion, Greece*, ²*Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas (IMBB-FORTH), Heraklion, Greece*

Introduction: Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by high glucose levels. Accumulating evidence indicate T2DM as a high-risk factor for Alzheimer's Disease (AD), however the underlying interlink is still unclear. Increasing evidence support that neuroinflammation represents a central adversity in both pathologies. Here, we aim to study the neurological manifestations of hyperglycemia and investigate their relationship with AD pathogenesis with emphasis on the neurotrophin receptor p75 (p75NTR). p75NTR belongs to the TNF-receptor superfamily and signals apoptosis in a cell context dependent manner.

Methods: We use mono- and co-cultures of human iPSC-derived neurons and astrocytes in 2D and 3D (porous collagen scaffolds) conditions to investigate the involvement of p75NTR signaling in the direct and astrocyte-mediated effects of hyperglycemia on neurodegeneration. Human iPSC are initially differentiated into neural progenitor cells using a dual smad inhibition protocol and finally to neurons by using a growth factor based protocol (Ehrlich et al., 2017). Neural progenitor cells are differentiated into astrocytes as described by Perriot et al., 2021. Upon differentiation cells are exposed to high glucose (50mM and 100mM) considering as control condition the 20mM which is the glucose concentration of the culture medium.

Results: Our results show that p75NTR is a mediator of glucose neurotoxicity in neurons. High glucose triggers an increase in p75 NTR protein levels accompanied by neuronal cell death which is rescued when the activity of p75 NTR is impaired. In accordance, high glucose enhances neuronal susceptibility to cytotoxic stimuli such as Amyloid- β , suggesting a connection with AD. Furthermore, we show that high glucose leads to decreased expression of the synaptic proteins Synaptophysin and Synapsin indicating a deregulation of synaptic plasticity. On the contrary, high glucose has no effect on astrocyte survival and activation.

Conclusions: Our study provides insights into the brain deficits caused by hyperglycemia and highlights the involvement of p75NTR in glucose neurotoxicity.

- [1] Ehrlich M., et al. Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. Proc Natl Acad Sci U S A. 2017 Mar 14;114(11):E2243-E2252
- [2] Perriot S. et al., Differentiation of functional astrocytes from human-induced pluripotent stem cells in chemically defined media. Star Protocols, 2021, Oct 20;2(4):100902

OC11. The selective positive allosteric modulator (PAM) of the glutamate transporter EAAT2 efficiently relieves pain and pruritus of different origins and potentiates opioid-induced analgesia

Mogilski S¹, Kubacka M¹, Sapa J¹, Rapacz A¹, Jakubiec M², Abram M², Kamiński K²

¹*Department of Pharmacodynamics, Faculty of Pharmacy, Medical College, Jagiellonian University, Kraków, Poland*, ²*Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

Introduction: Pain and itch utilize similar mechanisms to transduce and transmit their signals, and there is an overlap in their proinflammatory mediators, neurotransmitters, and neuropeptides. Glutamate is crucial for the transmission of both sensations and acts as a neurotransmitter for pruriceptive and nociceptive sensory neurons [1].

Our group discovered a novel, first-in-class drug candidate compound, (R)-AS-1, which selectively enhances glutamate uptake by positive allosteric modulation (PAM) of (EAAT2). To the best of our knowledge, (R)-AS-1 is the first molecule with drug-like, safety, and pharmacokinetic profiles as well as potent *in vivo* activity in animal models used for antiseizure drug screening [2].

Considering the established mechanism of action for (R)-AS-1, we hypothesized that it may be a potent analgesic and antipruritic agent.

Methods: We tested R-AS-1 using a battery of procedures and animal models of pain and pruritus, including pain induced by thermal stimuli (hot plate and cold plate), chemical stimuli (formalin, capsaicin), and mechanical stimuli (von Frey method). Moreover, we evaluated this compound in models of neuropathic pain induced by oxaliplatin (OXPT) or streptozotocin STZ). We used pharmacological tools, such as WAY213613 (an EAAT2 inhibitor), to precisely confirm the role of the glutamate transporter in R-AS-1-dependent analgesia. Additionally, we used histamine-dependent and histamine-independent models of pruritus. We also tested the influence of R-AS-1 and WAY213613 on motor coordination and spontaneous locomotor activity.

Results: R-AS-1 significantly inhibited the nociceptive response in acute neurogenic pain and inflammatory pain. The compound showed potent analgesic effects on STZ- and OXPT-induced pain, influencing both mechanical and thermal stimuli. R-AS-1 significantly potentiated the analgesic effects of morphine. The analgesic effects of the compound were inhibited or completely reversed by WAY213613. In addition, R-AS-1 attenuated the scratching activity induced by chloroquine or histamine.

Conclusions: We proved that EAAT2 positive allosteric modulation by R-AS-1 resulted in analgesic effects on pain of different origins. Moreover, R-AS-1 augmented the analgesic effects of morphine and showed an antipruritic activity. These effects were attenuated by treatment with an EAAT2 inhibitor, which proved that increasing the activity of this transporter is beneficial in dealing with pain and pruritus.

[1] Anzelc, M.; Burkhardt, C. G. Pain and Pruritus: a study of their similarities and differences. *Int J Dermatol.* 2020, 59, 159–164.

[2] Abram, M., et al. Discovery of (R)-N-Benzyl-2-(2,5-dioxopyrrolidin-1-yl)propanamide [(R)-AS-1], a Novel Orally Bioavailable EAAT2 Modulator with Drug-like Properties and Potent Antiseizure Activity In Vivo. *J. Med. Chem.* 2022, 65, 11703–11725.

OC12. Design and biological characterization of new biologic (Ab-IPL-IL-17) for IL-17s-mediated diseases

Saviano A¹, Mansour A², Raucci F¹, Merlini F³, Marigliano N¹, Schettino A¹, Vellecco V³, Cirino G³, Bucci M³, McGettrick H⁴, Grieco P³, Iqbal A², Maione F¹

¹*ImmunoPharmaLab, Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*, ²*Institute of Cardiovascular Sciences (ICVS), College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*, ³*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*, ⁴*Institute of Inflammation and Ageing (IIA), College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*

Introduction: Interleukin (IL)-17A and IL-17F are key drivers of inflammation that are functionally dysregulated in several human immune-mediated inflammatory diseases (IMIDs), such as rheumatoid arthritis (RA), psoriasis and inflammatory bowel disease (IBD) [1]. Targeting these cytokines has some therapeutic benefits, but issues associated with low therapeutic efficacy and immunogenicity for subgroups of patients or IMIDs reduce their clinical use. Therefore, there is an urgent need to improve the coverage and efficacy of antibodies targeting IL-17A and/or IL-17F and IL-17A/F heterodimer [2].

Methods: Here, by docking and computational analysis, we initially identified a bioactive 20 amino acid IL-17A/F-derived peptide (nIL-17™) that mimics, *in vitro* and *in vivo*, the pro-inflammatory actions of the full-length protein. Subsequently, we generated a novel anti-IL-17 neutralising monoclonal antibody (Ab-IPL-IL17™) capable of effectively reversing the pro-inflammatory and pro-migratory actions of nIL-17™ and parental protein [3].

Results: We demonstrated that nIL-17™ displays more pro-inflammatory effects than parental protein IL-17A, in both NIH-3T3 mouse embryonic fibroblast cell line ($P \leq 0.05$; $N=3$) and human CD14+ derived macrophages ($P \leq 0.05$; $N=3$). Furthermore, nIL-17™ promoted leucocyte recruitment to preinflamed tissues *in vivo* (air pouch model, $P \leq 0.0001$; $N=7$) and *in vitro* (to inflamed endothelium $P \leq 0.001$; $N=3$). In addition, Ab-IPL-IL17™ not only was able to reduce significantly the biological effects evoked by both IL-17A ($P \leq 0.001$; $N=5$) and IL-17F ($P \leq 0.01$; $N=5$) but, more importantly, it displayed less off-target effects than the current gold-standard biologic, secukinumab. Finally, we compared the therapeutic efficacy of Ab-IPL-IL-17™ with reference anti-IL-17 antibodies in preclinical murine models and samples from RA and IBD patients. We found that Ab-IPL-IL17™ could effectively reduce clinical signs of arthritis ($P \leq 0.01$; $N=3$) and neutralise elevated IL-17 levels in IBD patient serum ($P \leq 0.05$; $N=6$).

Conclusions: Collectively, our preclinical and clinical evidence indicates high efficacy and therapeutic potency of Ab-IPL-IL17™, supporting the rationale for large-scale clinical evaluation of Ab-IPL-IL17™ in patients with IMIDs.

- [1] P. Miossec, J. K. Kolls. Targeting IL-17 and TH17 cells in chronic inflammation. *Nat Rev Drug Discov*, 2012;11:763-76.
- [2] F. Maione. Commentary: IL-17 in Chronic Inflammation: From Discovery to Targeting. *Front Pharmacol*, 2016;7:250.
- [3] A. Saviano, A. A. Mansour, F. Raucci, F. Merlini, N. Marigliano, A. Schettino et al. New biologic (Ab-IPL-IL-17) for IL-17-mediated diseases: identification of the bioactive sequence (nIL-17) for IL-17A/F function. *Ann Rheum Dis*, 2023;82:1415-28.

OC13. The anti-inflammatory cytokine IL-37 plus IL-15 enhances human NK cells anti-tumor functions by interacting with IL-1R8

Landolina N¹, Mariotti F¹, Pelosi A¹, D'Oria V², Ingegnere T¹, Alicata C¹, Vacca P³, Moretta L¹, Maggi E¹

¹Tumor Immunology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, ²Research Laboratories, Confocal Microscopy Core Facility, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, ³Immunology Research Area, Innate Lymphoid Cells Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

Introduction: IL-37 is a cytokine belonging to the IL-1 family, modulating immune responses in a large number of diseases including cancer [1]. Extracellular IL-37 interacts with the inhibitory receptor IL-1R8 that we have recently found to regulate multiple NK cellular pathways including the anti-tumor function [2]. Here, we studied the effects of exogenous IL-37 on human NK cells functions via IL-1R8.

Methods: Freshly isolated, mock- or IL-1R8-silenced human NK cells were stimulated for 18h with rhIL-37 in the presence of the activating cytokine IL-15. NK cell cytotoxicity was analyzed following 4h incubation with colon cancer cell lines as previously described [2]. Interferon γ (IFN- γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels were measured in cell supernatants using ELISA and Multiplex assays. Expression of IL-1R8 and phosphorylation of ERK, NF-kB and GSK3 β were assessed by western blot analyses. IL-37 gene expression in cancer tissues were generated by interrogating the integrated database of gene chip data on TNMplot.com web tool [3]. Kaplan-Meier curves representing the overall survival test on cancer patients, were built by the R2: Genomics Analysis and Visualization Platform. Statistical analyses were performed using the GraphPad Prism V.6.0 software and Student's t test.

Results: NK cells cytolytic and IFN γ /GM-CSF secretory functions were significantly enhanced following incubation with IL-37 plus IL-15. Phosphorylation of both ERK1/2 and NF-kB was increased in the samples treated with IL-15/IL-37 but not in controls. Treatment with IL-15/IL-37 reduced IL-1R8 protein levels and increased phosphorylation of GSK3 β , whose inhibition restored IL-1R8 expression. Upon treatment with IL-15/IL-37, IL-1R8 silenced NK cells did not exhibit any further improvement of NK cell cytotoxicity compared to their IL-15-treated controls. IL-37 gene expression was lower in both colon and skin cancer patients compared to healthy tissues. Colon adenocarcinoma and neuroblastoma patients with high IL-37 gene expression exhibited higher overall survival than those with low IL-37 gene expression.

Conclusions: These data indicate that IL-37 (in combination with IL-15) enhances NK cells anti-tumor activity by promoting the activation of both MAPK and NF-kB signaling pathways and controlling IL-1R8 degradation, through GSK3 β activity downregulation. IL-1R8-silenced NK cells stimulated with IL-37 did not exhibit any further increase of cytotoxicity if compared to IL-1R8-silenced NK cells stimulated with IL-15 alone, indicating that IL-37 cannot further downregulate IL-1R8. IL-37 reduction in cancer tissues is in line with the positive role played by IL-37 in promoting NK cell-dependent anti-tumor response.

OC14. Crizotinib inhibits mTORC1 activation, protein synthesis and endothelial cell activation by pleiotrophin or PTPRZ1 deletion

Mourkogianni E¹, Ntenekou D¹, Enake M¹, Xanthopoulos A¹, Kastana P¹, Papadimitriou E¹

¹*University of Patras, Patras, Greece*

Introduction: Protein tyrosine phosphatase receptor zeta 1 (PTPRZ1) is a transmembrane tyrosine phosphatase (TP) expressed in endothelial cells and required for stimulation of cell migration by pleiotrophin (PTN), suggesting that it may affect angiogenesis. Indeed, increased angiogenesis is observed in the retina, as well as the lungs and the heart of Ptprz1-/- compared to Ptprz1+/+ mice. The signaling pathway downstream of PTPRZ1 has not been extensively studied and is the aim of the present study.

Methods: Human umbilical vein endothelial cells (HUVEC) and lung microvascular endothelial cells (LMVEC) isolated from Ptprz1+/+ and Ptprz1-/- mice were used. Proximity ligation assay was used for c-Met and p70S6K phosphorylation levels quantification, and Western blot analysis for p70S6K or 4EBP1 phosphorylation levels quantification. Protein synthesis was quantified by a puromycylation assay. Migration was studied by using the transwell assay.

Results: Ptprz1-/- LMVEC have increased angiogenic features compared to Ptprz1+/+ LMVEC. PTN stimulates Ptprz1+/+ LMVEC migration but does not cause further increase in Ptprz1-/- LMVEC, suggesting that it acts through inhibition of PTPRZ1 TP activity. c-Met tyrosine kinase (TK) activity is enhanced in Ptprz1-/- LMVEC and c-Met is activated by PTN, in a PTPRZ1-dependent manner. Ptprz1-/- LMVEC have increased mTORC1 activity and enhanced protein synthesis compared to Ptprz1+/+ LMVEC. In the same line, downstream of PTPRZ1 in HUVEC, PTN activates mTORC1 and protein synthesis. The mTORC1 inhibitor rapamycin decreases protein synthesis levels of PTN-stimulated HUVEC to those of untreated HUVEC. The c-Met TK inhibitor, crizotinib, abolished the enhanced angiogenic phenotype, as well as the PTN-induced migration, mTORC1 activation and protein synthesis in HUVEC. Since the enhanced c-Met activity in Ptprz1-/- LMVEC depends on the decreased $\beta 3$ integrin levels, we tested the effect of the selective anti- $\alpha v\beta 3$ LM609 antibody or a PTN peptide that inhibits the PTN- $\alpha v\beta 3$ interaction; both abolish the effect of PTN in endothelial cell migration. Surprisingly, LM609 and the PTN peptide induced c-Met and mTORC1 activation.

Conclusions: PTPRZ1 deletion or TP inhibition by PTN activates c-Met, mTORC1, protein synthesis and migration in endothelial cells, all inhibited by crizotinib. Although inhibitors of $\alpha v\beta 3$ inhibit migration, they activate c-Met, mTORC1 and protein synthesis, providing a potential explanation for integrin inhibitors' failure in clinical trials.

OC15. Observational studies of repurposing old drugs for new indications: The example of metformin to treat breast cancer

Suissa S^{1,2}

¹McGill University, Montreal, Canada, ²Jewish General Hospital, Montreal, Canada

Background: Non-randomized observational studies using large healthcare databases provide real-world evidence (RWE) on the risks and benefits of drugs. The Food and Drug Administration (FDA) has called for observational studies to investigate the potential repurposing of old inexpensive drugs for new indications. An example is metformin, an effective drug to treat type 2 diabetes, for which several observational studies reported significant reductions in cancer outcomes, including breast cancer. However, these studies were affected by time-related biases.

Objective: To assess whether metformin is associated with lower breast cancer-related and all-cause mortality in women diagnosed with breast cancer using an observational new-user study design that avoids time-related biases.

Methods: The subjects were identified from the Clinical Practice Research Datalink (CPRD), a primary care database from the United Kingdom (UK) that contains medical records for over 50 million people. The base cohort included all women with a new diagnosis of breast cancer, 30 years of age or more between 2000 and 2020. The prevalent new-user cohort design, that emulates a randomized trial, was used to match each patient initiating metformin after the breast cancer diagnosis with a non-user on time and propensity scores, among those with a diagnosis of diabetes. Subjects were followed until all-cause and breast cancer-related death. Hazard ratios (HR) and 95% confidence intervals (CI) of these outcomes, comparing metformin use with non-use, were estimated.

Results: The study cohort included 4,925 new users of metformin who were matched to 4,925 non-users among women with breast cancer and type 2 diabetes. The women were 68 years of age, 44% were obese and 58% were ever smokers, followed for an average 5.7 years. The rates of breast cancer and all-cause death were 2.1 and 5.2 per 100 per year, respectively. The HR of breast cancer death with metformin use was 0.97 (95% CI 0.85-1.10), compared with non-use. In contrast, using the biased approach of previous observational studies, the HR of breast cancer death with metformin use was 0.55 (95% CI 0.51-0.60). The HR of all-cause mortality with metformin use was 0.89 (95% CI 0.82-0.97).

Conclusions: The observational studies reporting significant reductions in cancer mortality with metformin were affected by time-related biases. This study, using an observational new-user study design that avoids these biases, found no reduction in breast cancer mortality with metformin use. The small reduction in all-cause death with metformin among women with breast cancer and type 2 diabetes deserves further investigation.

OC16. Emerging roles of 3-MST in colon cancer

Ascencao K¹, Szabo C¹

¹*University of Fribourg, Fribourg, Suisse*

Colorectal cancer (CRC) results from the accumulation of consecutive ‘driver’ mutations in several key pathways, which transform normal cells into mesenchymal cells (EMT). The opposite transition, mesenchymal-to-epithelial transition, is named MET. Despite many advances achieved in therapeutic approaches, a significant number of patients develop resistance to therapies and/or cancer relapse occurs. Cancer’s ability to resist is mainly linked to a small sub-population of cells, cancer stem cells (CSCs), with stem cell characteristics such as self-renewal ability; pluripotent capacity to drive tumor initiation, growth and heterogeneity. Hydrogen sulfide (H₂S) is an important endogenous gasotransmitter, produced mainly by three enzymes cystathione-β-synthase (CBS), cystathione-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST). Several studies have shown that various cancer cells, included colon cancer cells, overexpressed one or more H₂S-producing enzymes. Cell-derived H₂S supports cancer cell metabolism, proliferation, movement, cytoprotection and stemness.

In our studies, we used an extensive list of methodologies, ranging from standard techniques (such as western blotting, PCR, FACS, proliferation and migrations assays, transduction) to more specialized techniques (such as cellular bioenergetics and 3D cell culture).

The results of our work show that inhibition of H₂S generation in HCT116 induces MET, partly via, the downregulation of ACLY and consequent modulation of the Wnt-β-catenin pathway; decreases CyR61 mRNA expression through the modulation of S1PR via ATF1 and CREB. Furthermore, 3-MST inhibition suppresses the secretion/release of CyR61 via Caveolin-1 and Snail1 downregulation; and promotes apoptosis of colon cancer cell which can be counteract by endogenously CyR61 production, at least in part via RhoA activation. In addition, consecutive ‘driver’ mutations in human colonic organoids produce gradual upregulation in CBS-in particular in its truncated (45 kDa) form-as well as in CSE and 3-MST expression. In metastatic organoids, the upregulation of H₂S-producing enzymes leads to an increase in H₂S generation. This effect is reflected in an increase in the proliferation rate, an upregulation of cellular bioenergetics (mitochondrial respiration and glycolysis) and an upregulation of the Wnt/β-catenin pathway. Furthermore, CSCs isolated from HCT116 cell line have increased H₂S-producing enzymes expression and their clonogenicity and viability is maintained by 3-MST-derived H₂S.

In conclusion, increased H₂S generation, by promoting EMT, cell bioenergetics, survival, signaling, dedifferentiation and stemness, plays a significant role in colon cancer pathogenesis. Therefore, it supports the idea that pharmacological inhibition of these processes, particularly via 3-MST inhibition, could be useful in patients with advanced colorectal cancer who relapse or fail to respond to conventional cancer therapies.

Poster Presentations

PP001. The role of the pharmacist in the management of cancer pain in Greece

Aliferis E¹, Garani-Papadatos S¹

¹*University of West Attica, Athens, Greece*

Introduction: Epidemiological data in the treatment of oncological patients requires close collaboration between the various specialties, for the best care and treatment of pain. The purpose of this study is to identify and describe how in Greece Pharmacists can contribute to more effective cancer pain management.

Methods: The survey was conducted with an electronic questionnaire with the help of the 3 major Greek Pharmaceutical Associations, addressing a) the role of the pharmacist in raising awareness of pain issues b) the contribution of the pharmacist to patient empowerment regarding management of pain c) the contribution of the pharmacist to the chain of clinical treatment.

Results. The results concern n=107 people who responded to the survey in the first 6 months. Most pharmacists are knowledgeable about palliative care. However, a very small percentage consults with local doctors about the available drugs (36%) or has discussed with the doctor possible interruption or modification of a drug (32%). A smaller percentage has participated in a cancer patient case meeting (8%). Regarding the obstacles in the management of cancer pain, the main ones mentioned are the non-participation in an interdisciplinary team, the lack of training of health professionals and the lack of information for patients and relatives.

Conclusions: There is a good level of information among pharmacists but in a context that does not favor the undertaking of further actions. There is a lack of policy towards the inclusion of pharmacists in the care of cancer patients. The enhancement of collaboration of pharmacists with other specialties will help to further explore the multiple dimensions of the issue. More investigation is needed with more pharmacists and focus on more and better-quality features.

- [1] Oliveira CS, Silva MP, Miranda Íngara KSPB, Calumby RT, De Araújo-Calumby RF. Impact of clinical pharmacy in oncology and hematology centers: A systematic review. *Journal of Oncology Pharmacy Practice*. 2020;27(3):679-692. Doi:10.1177/1078155220976801
- [2] Shrestha S, KC B, Blebil AQ, Teoh SL. Pharmacist Involvement in Cancer Pain Management: A Systematic Review and Meta-Analysis. *The Journal of Pain*. 2022;23(7):1123-1142. Doi:10.1016/j.jpain.2022.02.002
- [3] Veetttil SK, Darouiche G, Sawangjit R, Cox N, Lai NM, Chaiyakunapruk N. Effects of pharmacist interventions on pain intensity: Systematic review and meta-analysis of randomized controlled trials. *Journal of the American Pharmacists Association*. 2022;62(4):1313-1320.e6. doi:10.1016/j.japh.2022.02.015
- [4] Tait P, Chakraborty A, Jones K, Tieman J. What Is the Community Pharmacists' Role in Supporting Older Australians with Palliative Care Needs? *Healthcare*. 2021;9(5):489. Doi:10.3390/healthcare9050489

PP002. Effects of dapagliflozin on vascular reactivity of internal mammary artery and saphenous vein bypass grafts isolated from diabetic and nondiabetic patients

Vidin Sen A¹, Uydes Dogan B¹, Kisa U², Kocogullari C², Teskin O³, **Alp Yildirim I¹**

¹*Istanbul University Faculty of Pharmacy Department of Pharmacology, Istanbul, Turkey*, ²*Dr. Siyami Ersek Thoracic and Cardiovascular Education Research Hospital Department of Cardiovascular Surgery, Istanbul, Turkey*, ³*Biruni University Faculty of Medicine Department of Pharmacology, Istanbul, Turkey*

Introduction: Sodium-glucose co-transporter-2 (SGLT-2) inhibitors are a novel class of antidiabetic drugs reduce plasma glucose levels by inhibiting renal glucose reabsorption. It has been reported that SGLT-2 inhibitors reduce the incidence of major adverse cardiovascular events independently from glycemic control [1]. This study aimed to evaluate the effects of diabetes and hyperglycemic conditions, as well as the SGLT-2 inhibitor Dapagliflozin, on vascular reactivity in human saphenous vein (SV) and internal mammary artery (IMA) grafts used as coronary artery bypass material.

Methods: This study was approved by the Institutional Review Boards of Haydarpasa Numune Training and Research Hospital (Protocol No: HNEAH-KAEK 2020/KK/224). Bypass grafts isolated from diabetic and nondiabetic patients (n=6-9 for each experimental group) were incubated for 16 hours in a normoglycemic (5.5 mM glucose) and/or hyperglycemic (25 mM glucose) medium with 0.5 µM Dapagliflozin. After incubation, the rings were placed in an isolated organ bath system. Contractions to potassium chloride (10-100 mM) and phenylephrine (10-8 – 10-4 M) were evaluated, and relaxation responses to acetylcholine (10-9–10-4 M) and sodium nitroprusside (10-8–10-4 M) were assessed to evaluate endothelial and smooth muscle function, respectively.

Results: Hyperglycemic conditions significantly increased contraction responses to potassium chloride but did not result significant changes in phenylephrine-induced contraction responses in IMA grafts isolated from diabetic patients. Dapagliflozin significantly reduced potassium chloride and phenylephrine contractions under hyperglycemic conditions. Hyperglycemic conditions significantly increased phenylephrine contractions in SV grafts isolated from diabetic patients, which were normalized by incubation with Dapagliflozin. In IMA grafts isolated from diabetic patients, hyperglycemic conditions significantly reduced acetylcholine-induced relaxation responses, which is normalized by Dapagliflozin. Hyperglycemic conditions and/or Dapagliflozin incubation did not result significant changes in vascular reactivity in IMA grafts isolated from nondiabetic patients. Hyperglycemic conditions increased potassium chloride and phenylephrine contractions in SV grafts, which were prevented by incubation with Dapagliflozin.

Conclusions: The beneficial effects induced by Dapagliflozin on vascular reactivity of human bypass grafts, are believed to contribute to the pleiotropic effects of SGLT-2 inhibitors.

- [1] He G, Yang G, Huang X, Luo D, Tang C, Zhang Z. SGLT2 inhibitors for prevention of primary and secondary cardiovascular outcomes: A meta-analysis of randomized controlled trials. Heart Lung. 2023 May-Jun;59:109-116.

PP003. Influence of Mediterranean diet and intermittent Fasting on Type II diabetes mellitus among Adults: A systematic review from ClinicalTrials.org

Ashour A¹

¹*Pharmacology and Toxicology Department, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia*

Background: Type 2 diabetes mellitus (T2DM) represents a growing global health challenge (1). Lifestyle modifications, particularly dietary interventions, are pivotal in its management (2). Intermittent fasting (IF) has emerged as a dietary strategy with the potential to impact glucose regulation and insulin sensitivity in adults (3). The Mediterranean diet (MTD), rich in fruits, vegetables, whole grains, and healthy fats, is renowned for its potential benefits on heart health and diabetes prevention (4). Therefore, this paper provides an overview of the influence of MTD and IF on T2DM in adults, with a focus on insights from clinical trials registered on ClinicalTrials.gov.

Methods: On October 25th, 2023, we performed a search within the ClinicalTrials.gov database using the query "Type 2 diabetes mellitus, Mediterranean diet and Intermittent fasting". The inclusion criteria was designed to encompass clinical trials associated with T2DM that employed MTD and IF as dietary approaches. Trials that did not meet these criteria were excluded from the analysis. We compiled various data points, including the trial's title, status, study type, details of the interventions, and recorded outcomes.

Results: Out of the 54 clinical trials identified, only 10 were specifically oriented towards investigating the impact of MTD and IF on T2DM among adult population. Comprehensive tables have been created, detailing the study titles, trial statuses, medical conditions, types of interventions, outcome measures, and the number of participants. The data extracted from these trials enhanced our comprehension of the potential advantages and disadvantages associated with the application of MTD and IF in the context of T2DM.

Conclusion: There is a growing interest in the potential of MTD and IF as a dietary strategy for T2DM management in adults. While preliminary results are promising, further comprehensive research is needed to establish the effectiveness, safety, and practicality of MTD and IF in the context of T2DM.

- [1] Zheng, Y., Ley, S. H. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature reviews endocrinology*, 14(2), 88-98.
- [2] Uusitupa, M., Khan, T. (2019). Prevention of type 2 diabetes by lifestyle changes: a systematic review and meta-analysis. *Nutrients*, 11(11), 2611.
- [3] Yuan, X., Wang, J. (2022). Effect of intermittent fasting diet on glucose and lipid metabolism and insulin resistance in patients with impaired glucose and lipid metabolism: A Systematic Review and Meta-Analysis. *International journal of endocrinology*, 2022.
- [4] Tuttolomondo, A. (2019). Metabolic and vascular effect of the Mediterranean diet. *International journal of molecular sciences*, 20(19), 4716.

PP004. Pharmaco-proteomic profiling of direct oral anticoagulants on endothelial cells

Atzemian N¹, Portokallidou K¹, Leonidis G¹, Pallikarou M¹, Ragia G¹, Manolopoulos V¹

¹Laboratory of Pharmacology, Medical School, Democritus University of Thrace, Individualised Medicine & Pharmacological Research Solutions Center (IMPReS), Alexandroupolis, Greece

Introduction: Direct Oral Anticoagulants (DOACs) are the first-line treatment for thromboembolism prevention in patients with atrial fibrillation. Clinical and preclinical data suggest that beyond their anticoagulant properties, DOACs exhibit pleiotropic effects, such as anti-inflammatory, antioxidant, and anti-senescence effects [1]. Identifying the full range of the beneficial pleiotropic effects of DOACs treatment may enable more effective utilization of these compounds and promote their earlier application in cardiovascular conditions beyond anticoagulation. The objective of this study was to explore the pharmacoproteomic profiles of all four DOACs on ECs in the absence of external stimulants.

Methods: Cultured HUVECs were exposed for 6h to 2 doses of related plasma levels of Dabigatran, Rivaroxaban, Apixaban, and Edoxaban. All experiments were carried out in serum-free media, after a starvation period of up to 18 h. After protein extraction using the Trizol Reagent (Invitrogen), label-free-based quantitative proteomic analysis was performed using the nanoLC-MS/MS system, followed by bioinformatics analysis.

Results: A total of 1919 proteins were identified, including 1357 that were quantifiable. Proteins with FDR < 0.1 and Abundance ratio > 2 or < 0.5 were considered significant, which led to the identification of 669 differential expressed proteins (420 upregulated and 249 downregulated). Analysis of the pharmacoproteomic profiles at low DOACs doses revealed 16 commonly upregulated proteins and 7 downregulated proteins across all four DOACs. Notably, three proteins-IRS2, LMLN, and SPATA20-exhibited exclusive upregulation by FXa inhibitors and not dabigatran, linked to ERK1/2-ET-1 activation, cell migration, and TNF signaling, respectively. In contrast, the comparison at high DOAC doses demonstrated no common upregulated proteins among the four DOACs and identified 10 downregulated proteins. Furthermore, APLNR, AQR, BCAR3, CPAMD8, EXOSC2, and PCDHGA6 were exclusively downregulated by FXa inhibitors. APLNR and BCAR3 play roles in angiogenesis, CPAMD8 is associated with the immune system and inflammation, and PCDHGA6 is implicated in vascular integrity.

Conclusions: Herein, we show the pharmacoproteomic profiles of DOACs on endothelial cells, revealing novel regulatory effects. This study emphasizes the commonalities and distinctions among DOACs that are influencing endothelial functions. Notably, varying DOACs doses and the comparison of FXa to thrombin inhibitors show diverse protein effects, highlighting the unique characteristics of each DOAC. In summary, DOACs present distinct protein effects on endothelium regardless of PAR signaling, showcasing their pleiotropism beyond the mechanism of action.

- [1] Atzemian, N., et al., Distinct pleiotropic effects of direct oral anticoagulants on cultured endothelial cells: a comprehensive review. *Frontiers in Pharmacology*, 2023. 14.

PP005. Antimicrobial stewardship and clinical pharmacist interventions in intensive care unit: a pre and post-interventional study

Pehlivani A¹, Yanık Yalçın T², İrem Yeşiler F³, Şahintürk H³, Kurt Azap Ö², Zeyneloğlu P³, **Basgut B¹**

¹*Department of Pharmacology, Faculty of Pharmacy, Baskent University, Ankara, Turkey,*

²*Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Baskent University, Ankara, Turkey,* ³*Department of Anesthesiology and Critical Care Unit, Faculty of Medicine, Baskent University, Ankara, Turkey*

Background: The implementation of both comprehensive Antimicrobial Stewardship (AMS) program and clinical pharmacists (CP) into the healthcare system is still in its nascent stages in Turkey. Therefore, this study aimed to assess the impact of CP in the implementation of an AMS program in an ICU setting.

Methods: This was a single-center interventional study conducted between November 2021, and August 2023, at the ICU of Baskent University Ankara Hospital. In November 2022, a CP joined the AMS team of intensivists and infectious diseases physicians. To evaluate the impact of CP on the AMS program, the same date ranges from one year ago were evaluated (pre-interventional period). The study has two main outcomes: i. The prevalence of antimicrobial drug-related problems (DRPs) by using the Pharmaceutical Care Network Europe (PCNE) V9.1 classification. ii. The change in the frequency of antibiotic usage via the "Reserve" group of the World Health Organization's AWaRe classification. To determine the amount of "Reserve" group antibiotic use, days of therapy (DOT) were calculated per 1000 patient days (PDs).

Results: The interventional period included 173 patients, while the pre-interventional period included 169 patients. A total of 175 DRPs were found in 116 (31.4%) patients by CP in the interventional period. The most common medication class in DRPs was antimicrobial drugs (70.0%). The AMS team accepted 90.1% of the interventions. The total number of "Reserve" group antibiotic DOTs per 1000 PDs decreased from 40.6 in the pre-interventional period to 27.7 in the interventional period, but not significantly ($p > 0.05$). When the pre-interventional and interventional periods were compared, the DOTs of ceftazidime/avibactam, colistin, daptomycin, fosfomycin, linezolid, polymyxin B, and tigecycline were 5.4 vs 3.1 ($p=0.707$), 13.4 vs 8.0 ($p=0.525$), 3.6 vs 0.0 ($p=0.319$), 4.9 vs 1.8 ($p=0.540$), 13.4 vs 4.9 ($p=0.215$), 0.0 vs 6.2 ($p=0.319$), 0.0 vs 6.2 ($p=0.132$) days, respectively.

Conclusions: This study shows that antimicrobial DRPs are common in the ICU. Multidisciplinary, front-line provider-focused AMS programs may reduce "reserve" group antibiotic usage in the ICU by providing 13% reductions in DOT. Thus, the CP in a multidisciplinary team may improve the therapeutic outcomes of critically ill patients.

PP006. Methylene blue as a potential intervention in sepsis: effects on survival and microcirculation in rat models

Becari C¹, Mestriner F², Bruch Dantas P², Flora Dugaich V², Michelon-Barbosa J², R. Barbosa Evora P², Ribeiro M²

¹School of Dentistry of Bauru, University of São Paulo, Bauru, Bauru, Brazil, ²Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

Introduction: Sepsis mortality is high, and new and old drugs are being studied [1] to help maintain hemodynamic stability [2] as methylene blue (MB). MB has been used to increase blood pressure in sepsis, efficiently rebalancing circulatory integrity in physiological patterns. We sought to investigate methylene blue treatment in the sepsis survival rate and mesenteric microcirculation preservation in rats model.

Methods: Adult male Hannover rats (200-250g) were treated by intravenous bolus with MB (4mg/kg) or saline. Mild sepsis was induced by CLp [2] with 4 holes, moderate sepsis with 10 holes, and severe sepsis with 20-holes in the cecum using a 16-gauge needle. The rats groups: Mild-Sepsis+Saline (n=21), Mild-sepsis+MB (n=21), Moderated-sepsis+saline (n=26), Moderated-sepsis+MB (n=28), Severe-sepsis+saline, Severe-sepsis+MB. The rats' survival was evaluated 10 days after surgery and analyzed by the log-rank test (X², chi-square). Intravital microscopy was performed to evaluate the mesenteric microcirculation integrity, analyzing the leukocyte rolling and adhesion by lipopolysaccharide (LPS) sepsis model. LPS was administered intraperitoneal, and MB was treated intravenously. The leukocyte rolling and adhesion by intravital microscopy in rats received Saline (n=5), LPS+Saline (n=6), MB+Saline (n=5), LPS+MB (n=7), MB+LPS (n=5).

Results: MB treatment significantly improved survival rate in severe sepsis. Severe sepsis groups treated with MB had a survival rate of 30% after 9 days ($p=0.02$). Severe sepsis rats groups that received saline died after 10 days. Interestingly, MB treatment did not change the survival rate in the mild and moderate sepsis compared with saline groups. The rats that received LPS showed an accentuated improvement of leukocyte adhesion and rolling in mesenteric vessels compared with the saline group. Rats that received only MB had similar results to the control group. However, when rats received LPS and were treated with MB, the leukocytes adhered and rolled in the mesentery diminished significantly ($p<0.001$), similar to the control group.

Conclusion: Our data suggest that MB was able to increase survival in severe sepsis and might be beneficial drug for the protection of microcirculation and sepsis.

Funding: FAPESP (# 2017/21539-6; 2018/23718-8, 2019/11485-1), CAPES, FAEPA.

- [1] Ibarra-Estrada M et al. Early adjunctive methylene blue in patients with septic shock: a randomized controlled trial. Crit Care. 2023;27(1):110. doi:10.1186/s13054-023-04397-7
- [2] Luis-Silva et al. Effect of methylene blue on hemodynamic response in the early phase of septic shock: A case series. Medicine. 2023;102(4):e32743. doi:10.1097/MD.0000000000032743
- [3] Siempos II et al.. Cecal Ligation and Puncture-induced Sepsis as a Model To Study Autophagy in Mice. JoVE. 2014;(84):51066. doi:10.3791/51066

PP007. The Impact of Student-designed Clinical Simulations in OSCE scenarios on the assessment score of Medicine Degree's Anaesthesia students and Podiatry Degree's Pharmacology students

Bellido Estevez I^{1,2}, Blanco Reina E^{1,2}, Bellido Estevez M³, Barroso González A^{1,3}, Guerrero Orriach J^{1,4}, Raigon Ponferrada A^{1,4}, Gomez Luque A^{1,4}

¹*University of Malaga, Malaga, España*, ²*IBIMA, Malaga, España*, ³*Service of Anaesthesia, Recovery care and Medicine of Pain, Regional-Materno-Infantil University Hospital. Andalucia Health Service, Malaga, España*, ⁴*Service of Anaesthesia, Recovery care and Medicine of Pain, Virgen de la Victoria University Hospital. Andalucia Health Service, Malaga, España*

Introduction: Training based on Clinical Simulation (SC) in a Structured Objective Clinical Evaluation (OSCE) scenario is a strategy to improve the quality of learning of Medicine and Podiatry students, other Health Sciences disciplines, Residents and for the constant updating of knowledge of the professionals in health care. Involving students in the design of their own class tasks, explaining to them why they need to acquire their skills and how they can do it, could improve their learning and stimulate their desire to learn and participate.

Objective: Determine the impact of Student-Designed Clinical Simulations in OSCE scenarios on the learning and assessment score of Anaesthesia students from Medicine Degree and Pharmacology students from Podiatry Degree.

Method: Five-year study in which a cohort of medicine and podiatry undergraduate students from a single institution was recruited for undergraduate students were trained using SC-ECOE that included: 1) Medical students: endotracheal intubation, assisted ventilation, peripherally inserted central catheter, and drug administration through various routes. 2) Podiatry students: locoregional anesthesia and cures with administration of topical drugs to the foot. Medicine and Podiatry students were involved in the design of clinical simulation scenarios for OSCE assessments. They collaborated with faculty members to develop realistic scenarios based on course objectives and clinical guidelines. These scenarios were then implemented in the OSCE assessments. The results obtained in the Student-Designed Clinical Simulations in OSCE scenarios were compared with data obtained from courses prior to the development of the study.

Results: 633 students were included, 78% female, 21 ± 2.6 years old. The average time spent by the students in completing the designed and training in SC-OSCE was 10.2 ± 3.6 h in Anaesthesia-Medicine and 10.5 ± 2.5 h in Pharmacology-Podiatry student. The percentage of students who were satisfied with this form of learning was 89.5%. The group of SC-OSCE designed by students showed a greater number of correct answers to the evaluation questions compared to students which no collaborate in the clinical simulation in OSCE scenario design, +16.8% correct answers ($P < 0.05$). Even more students in the experimental group showed improvements in clinical reasoning, decision-making, communication skills and team work.

Conclusion: Incorporating student to the design of clinical simulations into OSCE scenarios positively impacts their assessment scores, promotes their active learning, and fosters the development of clinical and communication skills.

- [1] Brentnall J. Evaluating the Clinical Reasoning of Student Health Professionals in Placement and Simulation Settings. Int J Environ Res Public Health. 2022;19(2):936.

PP008. Impact of weight and gender on the elimination of continuously infused 5-fluorouracil in colorectal cancer patients: A prospective population pharmacokinetic study

Bouchenak F¹, Bouchene S², Sobhi K¹, Mansouri K³, Bouzid K⁴

¹Toxicology Laboratory, Central Army Hospital Dr. Mohammed Seghir Nekkache, Algiers, Algeria,
²Pumas-AI, Inc, Kamputa Dr Centreville, USA, ³Pharmacology Laboratory, Faculty of Pharmacy of Algiers, Algiers, Algeria, ⁴Medical Oncology Department. Pierre and Marie Curie Center, Algiers, Algeria

Introduction: 5 Fluorouracil (5-FU) is an anticancer agent largely used in oncology, notably in the therapy of colorectal cancers. 5-FU is a good candidate for therapeutic drug monitoring due to its narrow therapeutic index and significant inter-individual variability pharmacokinetics (PK). The aim of this study is to assess the impact of anthropometrical parameters and dihydropyridine dehydrogenase (DPD) phenotype on 5-FU PK.

Methods: Seventy-five patients undergoing 46-hour continuous infusion of 5-FU were included in the study. PK samples were collected at the baseline, 10 minutes, 20 minutes, 2h, 22h, at the end of infusion, as well as 15, 40, 90 and 120 minutes after the end of infusion during the first cycle. Plasma levels of uracil, dihydrouracil, 5-FU and fluorodihydrouracil (FUH2) were determined by LC-MS/MS and used for the PK population (PK-pop) study via a non-linear mixed-effects modeling method implemented by the NL MIXr package in the open-source R software. DPD activity and anthropometric parameters were tested as potential covariates.

Results: A PK-pop model “parent-metabolite” were used, composed by a bicompartimental model with mixed elimination for 5-FU and mono-compartmental with linear elimination for FUH2. Two covariates were reported, gender on central volume with a significantly smaller volume in female patients and therefore a higher concentration of 5-FU (P-value<10-3). This could explain the more frequent occurrence of 5-FU toxicity in female patients. Other studies have reported similar findings [1, 2]. We also found a significant impact of weight on 5-FU clearance which increases with weight (P-value<10-3). Supratherapeutic 5-FU Area under the concentration-time curve (AUC5-FU>30 mg.h.L-1) [3] were more frequent in female than in male patients (17.2% vs 8.9% respectively). The dihydrouracil/uracil ratio correlated significantly with the 5-FU clearance.

Conclusion: Gender-specific distribution and elimination of 5-FU is supported by available data and may, in part, explain the associations between sex and 5-FU specific toxicity.

- [1] Bressolle F, Joulia JM, Pinguet F, et al. Circadian rhythm of 5-fluorouracil population pharmacokinetics in patients with metastatic colorectal cancer. *Cancer Chemother Pharmacol.* 1999;44(4):295-302. doi:10.1007/s002800050980
- [2] Mueller F, Büchel B, Köberle D, et al. Gender-specific elimination of continuous-infusional 5-fluorouracil in patients with gastrointestinal malignancies: results from a prospective population pharmacokinetic study. *Cancer Chemother Pharmacol.* 2013;71(2):361-370. doi:10.1007/s00280-012-2018-4
- [3] Kaldare RR, Haregewoin A, Grier CE, Hamilton SA, McLeod HL. Modeling the 5-fluorouracil area under the curve versus dose relationship to develop a pharmacokinetic dosing algorithm for colorectal cancer patients receiving FOLFOX6. *The Oncologist.* 2012;17(3):296-302. doi:10.1634/theoncologist.2011-0357

PP009. Securing Fluoropyrimidine-based chemotherapy: Comparison of three methods of screening for dihydropyrimidine dehydrogenase deficiency

Bouchenak F¹, Laoufi N¹, Sobhi K¹, Mansouri K², Djidjik R³, Bouzid K⁴

¹Toxicology Laboratory, Central Army Hospital Dr. Mohhamed Seghir Nekkache, Algiers, Algeria,

²Pharmacology Laboratory, Faculty of Pharmacy of Algiers, Algiers, Algeria, ³Immunology Laboratory, Issad Hassani University Hospital, Algiers, Algeria, ⁴Medical Oncology Department, Pierre and Marie Curie Centre, Algiers, Algeria

Introduction: 5-Fluorouracil (5-FU) is a fluoropyrimidine widely used in oncology. It can cause severe toxicity, particularly in cases of dihydropyrimidine dehydrogenase (DPD) deficiency, the enzyme responsible for inactivating over 80% of the 5-FU dose administered. DPD also catabolizes uracil (U) to dihydrouracil (UH2). A plasma U level >16 ng.mL⁻¹ and a UH2/U (R) <6 are in favor of DPD deficiency [1]. The European Medicines Agency (EMA) and the French Haute Autorité de Santé (HAS) recommend screening for DPD deficiency by determining plasma U levels [2,3]. The aim of this study was to compare the performances of three screening methods for DPD deficiency.

Methods: One hundred and eighty-eight (188) patients treated with continuous infusion of 5-FU for colorectal cancers, according to protocols LV5FU2, FOLFOX, FOLFIRI, FOLFOXIRI, were screened for DPD deficiency by: Genotyping (Gx) of the DPYD gene by rt-PCR for mutations including c.1905+ 1G>A (2A*), c.2846 A>T (p.D949V) and c.1679 T>G (DPYD*13). Phenotyping using U or R, by the determination of U and UH2 pretherapeutic plasma levels with an UPLC/MS-MS validated method. A Multi-Parametric Method (MPM = U ± R). The performances of the methods were compared based on the positive and negative likelihood ratio (RV+>10, RV-<0.1). Moderate to severe toxicities were investigated in the 4 first cycles according to the NCI-CTCAE V5.0.

Results: No mutations was found in our cohort except a heterozygous c.1905+ 1G>A (2A*) mutation in one patient, Gx was therefore excluded from the screening. U levels fluctuated between [3.52 - 18.63] ng.mL⁻¹. The R was distributed between [4.33 - 30.74]. The RV+/RV- were 4.2/0.6, 32.0/0.05 and 39.9/0.05 for U, R and MPM respectively. Gender, weight, U and R were significantly correlated with the toxicity ($p=0.019$, $p=0.0165$, $p<0.001$, $p<0.001$ respectively).

Conclusion: R as well as MPM are better at predicting moderate to severe toxicities to fluoropyrimidines compared to the U as recommended by the HAS and EMA.

[1] Boisdron-Celle M, Remaud G, Traore S, et al. 5-Fluorouracil-related severe toxicity: A comparison of different methods for the pretherapeutic detection of DPD deficiency. *Cancer Lett.* 2007;249(2):271-282. doi:10.1016/j.canlet.2006.09.006

[2] EMA recommendations on DPD testing prior to treatment with fluorouracil, capecitabine, tegafur and flucytosine. EMA. Published April 30, 2020. Accessed June 14, 2022. <https://www.ema.europa.eu/en/news/ema-recommendations-dpd-testing-prior-treatment-fluorouracil-capecitabine-tegafur-flucytosine>

[3] Screening for DPD deficiency to decrease the risk of severe toxicities related to fluoropyrimidines (5-fluorouracil or capecitabine) - INAHTA Brief. Haute Autorité de Santé. Published September 2023. Accessed December 4, 2023. https://www.has-sante.fr/jcms/c_2891090/en/screening-for-dihydropyrimidine-dehydrogenase-deficiency-to-decrease-the-risk-of-severe-toxicities-related-to-fluoropyrimidines-5-fluorouracil-or-capecitabine-inahta-brief

PP010. Endothelial Transcription Factor EB Protects Against Hypoxic Pulmonary Hypertension

Chen Q¹, He L¹, Wang L¹, Zhang C², Huang Y¹

¹City University of Hong Kong, Hong Kong, China, ²Shenzhen University, Shenzhen, China

Background: Pulmonary hypertension (PH) is a life-threatening disease characterized by a sustained increase in pulmonary artery (PA) resistance and vascular remodeling, leading to right heart failure and death. The transcription factor EB (TFEB) is known as the master regulator of autophagy and lysosomal biogenesis. Our previous study showed that TFEB was abundantly expressed in endothelial cells (ECs) and that reduced TFEB plays a crucial role in regulating endothelial dysfunction in diabetic mice. However, whether altered endothelial TFEB in PA is also associated with the pathogenesis of PH remains to be investigated.

Methods: To obtain EC-specific TFEB knockdown mice, EC-enhanced AAV9-mediated Tfeb-sgRNA was used in Cdh5Cre Cas9fl/fl mice by tail vein injection. Overexpression of TFEB was achieved by injection of adenovirus in C57BL/6 mice. These mice administrated with SU5416 (20 mg/kg/week) were kept under a hypoxic condition (10% O₂) for 1 or 3 weeks to induce PH model. Haemodynamics and histological staining were conducted to identify PH phenotypes and vascular remodelling. Endothelium-dependent relaxations (EDRs) were measured by wire myography. Human pulmonary artery endothelial cells (PAECs) were incubated in hypoxia incubator (1% O₂) to mimic hypoxic situation in vitro. The mRNA and protein levels of TFEB and its target genes have been determined by Western blot and qPCR. In vitro, overexpression of TFEB was achieved using adenovirus and lentivirus, respectively.

Results: TFEB is mainly expressed in ECs of the pulmonary arteries. The TFEB protein level was reduced by hypoxia exposure in PAECs. In vitro, overexpression of TFEB significantly reduced the expression of ET-1 and endothelial-mesenchymal transition markers while it increased the expression and activity of eNOS, in contrast to the effect of TFEB knockdown. In vivo, TFEB overexpression ameliorated the development of PH in mice by reducing right ventricular systolic pressure (RVSP) and right ventricular hypertrophy index, as well as pulmonary vascular remodeling. Furthermore, overexpression of TFEB ameliorated endothelial dysfunction in PA as demonstrated by improved acetylcholine (ACh)-induced EDRs without affecting sodium nitroprusside-induced endothelium-independent relaxations. By contrast, EC-specific TFEB knockdown mice exhibited an exaggerated PH phenotypes as reflected by the increased of RVSP and further blunted ACh-induced PA relaxations.

Conclusion: The present study demonstrates the importance of suppressed endothelial TFEB in PH pathogenesis. TFEB may be a potential therapeutic target for drug development in the treatment of PH (supported by GRF 14100121 and SRFS2021-4S04).

PP011. A network meta-analysis on the risk of incident dementia associated with antidiabetic medications: clinical significance of high hypoglycemic activity on elevated dementia risk

Sunwoo Y¹, Park J¹, Choi C², Shin S², **Choi Y¹**

¹Kyung Hee University, Seoul, Republic of Korea, ²Ajou University Hospital, Suwon, Republic of Korea,

³Ajou University, Suwon, Republic of Korea

Introduction: The risk of dementia is significantly higher in diabetes patients [1], and insulin resistance, which subsequently induce chronic hyperglycemia and hyperinsulinemia is considered most integral pathophysiological contributor for T2DM-related neurodegeneration and cognitive impairment [2, 3]. However, obscurity on the clinical significance of dementia risk from hypoglycemic therapy still remains. Hence, the aim of this study is to investigate the incident dementia risk among commonly prescribed hypoglycemic agents.

Methods: Keyword searches of databases, including Cochrane Central, Embase and MEDLINE (PubMed), were conducted in accordance with Preferred Reporting Items for Systematic Reviews-Network Meta-Analysis (PRISMA-NMA) guidelines. Frequentist network meta-analysis was performed and the risk of dementia was estimated as odds ratio with 95 confidence intervals. The protocol of this study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD 42022365927).

Results: Total of 531,699 patients from 10 clinical trials were included in the analyses. Glycemic control with dipeptidyl peptidase (DPP)-4 inhibitors (OR 0.63, 95 % CI 0.43- 0.91, P<0.05, glucagon-like peptide (GLP)-1 analogs (OR 0.18; 95% CI 0.12-0.28, P<0.05), metformin (OR 0.75, 95% CI 0.63-0.89, P<0.05), sodium glucose co-transporter (SGLT)-2 inhibitors (OR 0.15; 95% CI 0.10-0.24, P<0.05) and thiazolidinediones (OR 0.49, 95 % CI 0.37-0.66, P<0.05) substantially decreased the risk of incident dementia in diabetic patients when compared to no treatment. However, sulfonylurea substantially increased incident dementia risk than no treatment (OR 1.34, 95% CI 1.06-1.69, P<0.05). All hypoglycemic agents except α -glucosidase inhibitors (OR 0.75, 95 % CI 0.50-1.11), insulin (OR 0.55; 95% CI 0.30-1.00), and meglitinides (OR 0.82, 95 % CI 0.42-1.60) had significantly reduced incident dementia risk than sulfonylurea.

Conclusions: Optimal glycemic control is critical to prevent neurodegeneration. This study revealed significance of drug-induced hypoglycemia on neurodegeneration and incident dementia.

- [1] Zilliox LA, Chadrasekaran K, Kwan JY, Russell JW. Diabetes and cognitive impairment. Curr Diab Rep. 2016; 16:87
- [2] Sim AY, Barua S, Kim JY, Lee YH, Lee JE. Role of DPP-4 and SGLT2 inhibitors connected to Alzheimer disease in type 2 diabetes mellitus. Front Neurosci. 2021;15:708547
- [3] Hanyu H. Diabetes-related dementia. Adv Exp Med Biol. 2019; 1128:147-160

PP012. Real-World Data (RWD) derived pharmacovigilance assessment on medications with strong abuse potentials: focusing on opioids and stimulants

Kim Y¹, Choi C², **Choi Y¹**

¹Kyung Hee University, Seoul, Republic of Korea, ²Ajou University Hospital, Suwon, Republic of Korea

Introduction: Both opioids and stimulants (amphetamine) have strong abuse potentials [1]. The incidences of opioid & stimulant-related overdose and death are skyrocketing each year [1]. Moreover, the substantial increased prescriptions of opioids not only increased adverse drug events (ADE) incidences but also resulted in escalating number of patients with opioid use disorder (OUD) in Korea [2]. Hence, the aim of this study is to investigate ADEs associated with opioids and amphetamine and to identify agents with ADE cases pertaining to drug abuse, misuse, and dependency.

Methods: This was a cross-sectional study performed in accordance with STROBE guidelines. ADE cases reported to the Korean Institute of Drug Safety & Risk Management-Korean Adverse Event Reporting System Database (KAERS DB) from January 2013 to December 2022 were included. Opioid and stimulant related ADE reports with causality assessment of “certain”, “probable/likely” and “possible” per World Health Organization-Uppsala Monitoring Centre criteria were included in the analysis. Reported ADEs were classified into system organ class (SOC). The protocol was approved by the Korea Institute of Drug Safety & Risk Management (Ministry of Food and Drug Safety) (No.2308A0004) and institutional review board of Kyung Hee University (KHSIRB 23-535)

Results: Total of 427,491 ADE cases were included in the analysis. The most etiologic agent was tramadol (n=192,860, 45.11%), followed by fentanyl (n=125,852, 29.4%). The risks of reporting serious adverse events were higher in SOCs involving cardiovascular disorders (ROR 36.55), respiratory system disorders (ROR 15.967), heart rate and rhythm disorders (ROR 6.487) and liver and biliary system disorders (ROR 4.302). Total of 212 ADEs related to medication abuse, misuse and dependence were reported. Fentanyl (ROR 8.032) and tramadol (ROR 6.936) were more likely to report ADEs related to abuse and misuse, respectively. Dependence is more likely to be reported with oxycodone (ROR 11.670).

Conclusions: The most causative agent for opioid & stimulant-related ADEs was tramadol. Tramadol was more likely to be reported drug misuse cases, whereas fentanyl and oxycodone were more likely to be reported with drug abuse and dependency, respectively. Hence, optimal monitoring guidelines on preventing opioid & stimulant related ADEs including abuse/dependency are recommended.

[1] Maguire DR, Minervini V. Interactions between opioids and stimulants: Behavior pharmacology of abuse-related effects. *Adv Pharmacol.* 2022; 93:1-33

[2] Kim EJ, Hwang EJ, Yoo YM, Kim KH. Prevention, diagnosis, and treatment of opioid use disorder under the supervision of opioid stewardship programs: It's time to act now. *Korean J Pain.* 2022;35:361-382

PP013. Real-world data (RWD) derived pharmacovigilance investigation on drug-induced cognitive dysfunction

Sunwoo Y¹, Eom S¹, Yun J¹, Go C¹, Kim S¹, Kim Y¹, **Choi Y¹**

¹Kyung Hee University, Seoul, Republic of Korea

Introduction: Cognitive dysfunction, defined as deteriorated function related to memory, attention, language, and judgement, is an important healthcare problem with potentially serious consequences for the health and well-being of patients [1]. Drug-induced cognitive dysfunction is relatively common in the elderly and plays as common cause for delirium [2]. Thus, the aim of this study is to analyze the etiologic medications associated with drug-induced cognitive dysfunction and identify predictors associated with serious cognitive dysfunction.

Methods: This was a cross-sectional study utilizing adverse drug event (ADE) cases spontaneously reported to Korea Institute of Drug Safety & Risk Management - Korean Adverse Event Reporting System database (KAERS DB) from January 2012 to December 2021. The study was performed in accordance with STROBE guidelines. Any drug-induced cognitive impairment with “certain”, “probable/likely” and “possible” causality assessment per World Health Organization- Uppsala Monitoring Centre criteria were included in the analysis. The prespecified Medical Dictionary for Regulatory Activities (MedDRA) term include cognitive impairment, major/minor neurocognitive disorder, cognitive deterioration, cognitive disturbance, and abnormal cognitive function. The protocol was approved by the Korea Institute of Drug Safety & Risk Management (Ministry of Food and Drug Safety) (No.2212A0073) and institutional review board of Kyung Hee University (KHSIRB 23-124).

Results: A total of 254 ADE reports were analyzed in this study. The prevalence of serious cognitive dysfunction was 13.4 %. The majority of the ADEs were reported in the elderly population aged 60 years or older. The most etiologic agents were analgesics (n=45, 17.7%), followed by sedative-hypnotics (n=29; 13.0%) and antidepressants (n=29; 11.4%). However, the risk of reporting serious ADEs were higher with anti-Parkinson agents (ROR 4.057, 95% CI 1.121-14.688). Male sex and increasing age are most contributing predictors associated with higher risk of SAEs related to hospitalizations.

Conclusions: Although analgesics were most etiologic agents for drug-induced cognitive dysfunction, agents used for Parkinson’s disease are more likely to report serious cognitive dysfunction. Male sex and aging are contributing factors increasing risk of develop serious cognitive dysfunction requiring hospitalization. Nonetheless, more follow-up studies on the occurrence of serious cognitive dysfunction and the impact of medications for chronic disease management are needed. drugs

[1] Lam RW, Kennedy SH, McIntyre RS, Khuyllar A. Cognitive dysfunction in major depressive disorder: effects on psychosocial functioning and implications for treatment. Can J Psychiatry. 2014;59:649-654

[2] Bowen JD, Larson EB. Drug-induced cognitive impairment. Defining the problem and finding solutions. Drugs Aging 1993; 3:349-57.

PP014. Determination of the chemo-protective effects of specific Phyto-compounds in normal cells against the adverse effects of Breast Cancer chemotherapy

Christodoulou P^{1,2}, Boutsikos P¹, Neophytou C³, Kyriakou T^{1,3}, Christodoulou M², Papageorgis P³, Stephanou A¹, Patrikios I¹

¹*School of Medicine, European University Cyprus, Nicosia, Cyprus*, ²*Tumor Immunology and Biomarkers Laboratory, Basic and Translational Cancer Research Center, Department of Life Sciences, European University Cyprus, Nicosia, Cyprus*, ³*Tumor Microenvironment, Metastasis and Experimental Therapeutics Laboratory, Basic and Translational Cancer Research Center, Department of Life Sciences, European University Cyprus, Nicosia, Cyprus*

Introduction: Amygdalin is a naturally occurring glycoside known to have anti-cancer properties. Even though the anti-cancer properties of amygdalin are well known, its effect on normal cells has not been investigated [1]. The aim of this study was to investigate a possible chemo-protective role of amygdalin against the cytotoxic effects of the strong chemotherapeutic drug cisplatin.

Methods: Human non-tumorigenic MCF12F epithelial cell line, human fibroblasts cells, human breast cancer MCF7 and MDA-MB-231 cells (n=3/experiment) were treated with cisplatin in a dose- and time-depended manner in the absence or presence of amygdalin.

Results: When MCF12F cells and fibroblasts underwent pre-treatment with amygdalin followed by cisplatin treatment (24 h amygdalin + 24 h cisplatin), the cell viability was increased (22%, p < 0.001) as indicated using MTT assay. As attested by flow cytometry, combination treatment was associated with a decrease in late apoptotic cells compared with monotherapy (fold-change of decrease = 1.6 and 4.5 for 15 and 20 µM, respectively). Also, PUMA, p53, phospho-p53 and Bax expression decreased, when a combination treatment was used vs. cisplatin alone, while the proapoptotic proteins Bcl-2 and Bcl-xL exhibited an increased tendency in the presence of amygdalin. Moreover, the levels of pro-apoptotic genes PUMA, p53, and BAX mRNA were significantly downregulated (~83%, ~66%, and ~44%, respectively), while of anti-apoptotic genes BCL-2 and Bcl-XL mRNA were upregulated (~44.5% and ~51%, respectively), vs. cisplatin alone after 24 h of combination treatment. A combination index assay indicated that amygdalin could be considered as an antagonist to cisplatin (2.2 and 2.3) for MCF12F and fibroblast cells, respectively. In contrast, for the breast cancer MCF7 and MDA-MB-231 cells, amygdalin and cisplatin indicated a synergistic effect (0.8 and 0.65), respectively. The differential effects of amygdalin on healthy and cancer cells may result from differences in the metabolism of the compound. Future studies will involve investigation of differential expression of metabolic enzymes between healthy and cancer cells.

Conclusion: Amygdalin has chemo-modulatory effect when used in co-treatment with cisplatin and is able to protect normal breast cells as well as fibroblasts during chemotherapy treatment, indicating a strong selective chemoprotective ability and may contribute to a better quality of life for cancer patients.

- [1] Saleem M, Asif J, Asif M, Saleem U. Amygdalin from apricot kernels induces apoptosis and causes cell cycle arrest in cancer cells: An updated review. Anti-Cancer Agents in Medicinal Chemistry. 2019;18(12):1650-1655.

PP015. Resolvin-D1 Mitigates LPS-Induced Senescence in Undifferentiated Cardiac Fibroblasts: The Autocrine Influence of TGF- β 1

Díaz Araya G¹

¹Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile, Santiago De Chile, Chile

Introduction: Cellular senescence has been identified as a relevant component in several cardiovascular pathologies, where the abnormal accumulation of senescent cardiac fibroblasts (FC) has been observed, especially in contexts of cardiac fibrosis. The cellular senescence is characterized by a stable arrest of the cell cycle and associated with a complex pro-inflammatory secretome. CF in culture are self-differentiated to Cardiac Myofibroblast (CMF) by secretion of TGF- β 1, and an increase in senescence has been observed. The activation of the TLR4 receptor has been observed to induce FC senescence; and previous results from our laboratory showed that Resolvin D1 (RvD1) has a remarkable anti-inflammatory effect on CF treated with LPS, reducing cytokines secretion. However, whether RvD1 is capable of preventing the induction of senescence in CF by LPS is unknown.

Methods: CF from adult Sprague-Dawley rat were incubated in presence of 10% FSB and SB-43152 to maintain cell viability and to prevent CF self-differentiation. CF were treated with LPS (1 μ g/mL) for 3 or 7 days, and then were treated with Resolvin-D1 for additional 2 days. Protein levels of p16, p21, p53, p-Rb, γ H2A.X, were analyzed by WB. Also, SA- β -gal activity, cell size, were quantified. Secretome were measured by Milliplex.

The results indicate that FBS 10% serum maintains cell viability over extended periods of time, and SB-431542 prevents TGF- β autocrine-induced self-differentiation. LPS induces senescence markers at 3 days (pRb and SA- β -gal activity) and at 7 days (p16, pRb, increased size, and SA- β -gal activity). LPS induces an increase in SASP at 3 days, while at 7 days no difference were observed. Pre-treatment with RvD1 prevents the increase in SA- β -gal activity by LPS in FC cultured in the presence of SB-431542 for 3 and 7 days.

In conclusion, LPS induce CF senescence at 3 and 7 days in 10% FBS (highly myogenic medium), and a senescence-associated secretome was evident at 3 days. Resolvin D1 demonstrated a preventive effect on the senescent effects of LPS.

PP016. Endothelial cysteinolysis protects against age-related cardiac hypertrophy

Drekolia M^{1,2}, Mogler C⁴, Wittig J^{1,2}, Guenther S⁵, Fleming I^{2,3}, Kojonazarov B⁶, Bibli S^{1,2,3}

¹Department of Vascular Dysfunction, European Centre for Angioscience (ECAS), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany, ²Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany, ³German Centre for Cardiovascular Research (DZHK), Germany, ⁴Institute of Pathology Technical University, München, Germany, ⁵Max Planck Institute for Heart and Lung Research, Department I Cardiac Development and Remodeling, Bad Nauheim, Germany, ⁶Institute for Lung Health (ILH), University Hospital Giessen and Marburg, Medical Clinic II, Giessen, Germany

Introduction: Cysteine catabolism in the endothelium is regulated by cystathionine gamma lyase (CSE) and is essential for proper endothelial cell function [1], [2]. This project set out to characterize the effects of reduced endothelial cell cysteine catabolism (inducible endothelial cell-specific CSE knockout mice/ CSEiEC mice) on endothelial angiocrine effects and its impact on cardiomyocytes.

Methods: H2S levels in endothelial cells were evaluated with HPLC-MS/MS in 1, 3, 6 and 18 month old mice. Cardiac function was evaluated with cardiac echocardiography and histopathological analysis in CSEiEC and wild type littermates, with or without supplementation of sodium polysulfthionate (SG1002, 40mg/kg/day) in the chow diet starting from 9 months. Cardiomyocyte transcriptome was determined with bulk RNA sequencing in isolated with a modified Langendorff method cardiomyocytes.

Results: Analysis of CSE activity and H2S levels in endothelial cells revealed an age related gradual decrease in H2S production. Endothelial specific deletion of CSE resulted in an age dependent systolic and diastolic dysfunction, which was initiated at the 6 months and progressively enhanced in 18 month old mice. In addition, CSEiEC mice showed severe right ventricular dysfunction, with significantly shorter pulmonary acceleration time and tricuspid annular plane systolic excursion measures. Severe hypertrophy without signs of inflammation was also observed in the hearts from CSEiEC mice. Interestingly, supplementation of SG1002 prevented the progression of hypertrophy and improved the cardiac function. Mechanistically, endothelial CSE deletion influenced the cardiomyocyte transcriptional program in a hydrogen sulfide dependent manner. Bioinformatic approaches were able to identify that H2S derived- Krüppel-like factor 6 (KLF6) activation in the cardiomyocytes could be responsible for this transcriptional shift.

Conclusions: Age-related alterations in the endothelial cysteine catabolism impact on cardiac function through KLF6 mediated control of the cardiomyocyte transcriptional profile. Polysulfide supplementation might serve as a novel therapeutic strategy to maintain cardiac function.

[1] Bibli SI, Fleming I. Oxidative Post-Translational Modifications: A Focus on Cysteine S-Sulphydrylation and the Regulation of Endothelial Fitness. *Antioxid Redox Signal*. 2021;35(18):1494-1514. doi:10.1089/ars.2021.0162

[2] Hu J, Leisegang MS, Looso M, et al. Disrupted Binding of Cystathionine γ -Lyase to p53 Promotes Endothelial Senescence. *Circ Res*. 2023;133(10):842-857. doi:10.1161/CIRCRESAHA.123.323084

PP017. Macrophage polarization as a novel target of cardioprotection. Implication of accelerated myocardial repair after myocardial infarction

Efentakis P¹, Founta K¹, Kostopoulos I², Chania C¹, Kostomitsopoulos N³, Tasouli A⁴, Perdikaris S⁵, Tsitsilonis O², Roussis V⁵, Ioannou E⁵, Andreadou I¹

¹Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece, ²Flow Cytometry Unit, Section of Animal and Human Physiology, Department of Biology, National and Kapodistrian University of Athens, Athens, Greece, ³Biomedical Research Foundation of the Academy of Athens, Athens, Greece, ⁴Onassis Cardiac Surgery Center, Athens, Greece, ⁵Section of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Myocardial infarction (MI) is the primary cause of death, while myocardial inflammation orchestrates subsequent ischemic heart failure. Intracardiac M1 inflammatory macrophage recruitment occurs acutely post-MI. However, sustained M1-macrophages presence in the post-MI myocardium induces extensive cardiac inflammatory damage. Macrophage polarization (MPo) towards their M2 anti-inflammatory phenotype limits M1-mediated damage, mediating post-MI myocardial repair. Considering that increased anti-inflammatory capacity and reparative fibrosis increases myocardial resistance against rupture and reverse-remodeling post-MI, we herein sought to investigate the cardioprotective potential of M2 MPo by pharmacological agents of marine origin.

Methods: RAW 264.7 murine macrophages underwent 24h hypoxia (H) and cell viability was assessed. MPo markers were addressed via Real-Time PCR. Four metabolites of marine macroalgal origin were tested in vitro and their effect on macrophages' viability and polarization were assessed. Among them, the acetogenin derivative (AD), exerting the best M2-polarizing effect, was selected and mitochondrial membrane potential in presence of Complexes V and II inhibitors was investigated by Tetramethylrhodamine. Male C57BL6 mice underwent 30min ischemia (I) and 24h reperfusion (R) and received AD (12.5mg/kg) at the 20th min of I (n=6/group). Infarct Size (IS) was quantified by Triphenyl-Tetrazolium Chloride (TTC)-EVAN's Blue staining. In vivo experiments were repeated and whole blood, spleen and myocardium were obtained at 3 and 7 days of R for flow-cytometry analysis (n=5/group). M2 polarization markers were assessed at 7 days by immunoblotting. Collagen deposition at 7 days was evaluated by Picrosirius-red staining.

Results: H reduced RAW 264.7 macrophages viability and polarized macrophages to a mixed M1/M2 phenotype, as shown by M1 markers Nos2 and Tlr4 and M2 markers Mrc1, Arg1 and Tgfb upregulation. AD mitigated H-induced cytotoxicity and induced M2 polarization, as shown by decreased HIF-1 α expression and NF κ B phosphorylation. Moreover, it inhibited Complex II-related aerobic glycolysis in vitro, as confirmed by increased ACC and AMPK α phosphorylation and Tetramethylrhodamine studies. AD limits IS compared to controls (24.06 \pm 1.82 vs 39.19 \pm 2.03, respectively) and leads to M2 polarization in the blood and spleen at 3 days and in the blood, spleen and heart at 7 days, as shown by increased M2 (F4/80+/Ly6Clow macrophages)/M1 ratio (F4/80+/Ly6Chigh macrophages). Additionally, AD increased Arginase-1 and decreased TNF α and IL6 expression, confirming M2 MPo at 7 days. Increased collagen deposition in the AD group was observed at 7 days.

Conclusions: M2 MPo confers to IS reduction, and induces an early, possibly reparative, fibrosis at 7 days. Timely macrophage polarization appears as a novel target of cardioprotection.

PP018. Microvascular coronary endothelial dysfunction drives Pembrolizumab-induced cardiotoxicity. Prophylactic potential of Atorvastatin

Efentakis P¹, Choustoulaki A¹, Kwiatkowski G², Varela A³, Kostopoulos I⁴, Tsekenis G³, Ntanasis-Stathopoulos I⁵, Georgoulis A¹, Gakiopoulou H⁶, Briasoulis A⁵, Davos C³, Kostomitsopoulos N³, Tsitsilonis O⁴, Dimopoulos M⁵, Terpos E⁵, Chłopicki S², Gavriatopoulou M⁵, Andreadou I¹

¹Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece, ²Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland,

³Biomedical Research Foundation of the Academy of Athens, Athens, Greece, ⁴Flow Cytometry Unit, Section of Animal and Human Physiology, Department of Biology, National and Kapodistrian University of Athens, Athens, Greece, ⁵Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, ⁶1st Department of Pathology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Immune-checkpoint inhibitors (ICIs) exhibit outstanding anti-tumor activity, which is accompanied by immune-related cardiotoxicity of unknown pathomechanism. Herein, we investigated ICIs class-dependent cardiotoxicity in vitro, in primary murine cardiomyocytes and splenocytes and Pembrolizumab (Pem)-induced cardiotoxicity in vivo, establishing a novel murine model of Pem-mediated cardiac dysfunction. Additionally, we challenged translational prevention therapies against Pem-induced cardiotoxicity in vitro and in vivo.

Methods: Direct and immune-mediated cytotoxicity were investigated in primary cardiomyocytes and splenocytes, incubated with Ipilimumab (Anti-CTLA-4 antibody), Pem (Anti-PD-1 antibody) and Avelumab (Anti-PD-L1 antibody). Viability was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Pem's cross-reactivity with the murine PD-1 was assessed by circular dichroism (CD) on biotechnologically-produced human and murine PD-1 extracellular domains (PD-1-EDs) and by in silico docking. For the establishment of the murine model of Pem-induced cardiotoxicity, C57BL6/J male mice received IgG4 or Pem (2mg/kg, weekly) for 2 and 5 weeks (n=5-9/group). Echocardiography, histology, and molecular analyses were performed. Coronary blood flow velocity mapping and cardiac magnetic resonance imaging were conducted at 2 weeks (n=6/group). Human EA.hy926 endothelial cells were treated with Pem-conditioned media from human peripheral blood mononuclear cells, in presence and absence of Atorvastatin or Pravastatin (5, 10µM) and viability and molecular signaling were assessed. Finally, Atorvastatin (20mg/kg, daily) was co-administered in vivo with IgG4 or Pem (n=6/group) and its prophylactic potential was investigated by all the aforementioned functional and molecular analyses.

Results: Only Pem exerted immune-related cytotoxicity in vitro in primary murine cardiomyocytes, while it induced a Th17-like phenotype in the primary splenocytes. Pem's cross-reactivity with the murine PD-1-ED was confirmed by CD and in silico docking. In vivo, Pem initiated coronary endothelial and diastolic dysfunction at 2 weeks and systolic dysfunction at 5 weeks. At 2 weeks, Pem induced endothelial activation, as shown by ICAM-1 and iNOS upregulation and intra-cardiac leukocyte infiltration. At 5 weeks, Pem exacerbated endothelial activation and triggered cardiac inflammation. Additionally, Pem led to an acute circulatory Th17-like cytokine storm after the 1st week of administrations. Pem led to immune-related cytotoxicity in EA.hy926 cells, which was prevented only by Atorvastatin. Atorvastatin mitigated functional and molecular deficits, as well as Th17 cytokine storm by inhibiting endothelial activation and dysfunction in vivo.

Conclusions: Herein we report for the first time, the cross-reactivity of Pem with the murine PD-1-ED. Additionally, we established a novel in vivo model of Pem-induced cardiotoxicity. Coronary endothelial dysfunction precedes Pem-induced cardiotoxicity, whereas Atorvastatin emerges as a novel prophylactic therapy.

PP019. Dimethyl fumarate improves erectile function in streptozocin-induced diabetic rats by attenuating oxidative stress via the activation of Nrf2 antioxidant pathway

Engin S¹, Barut E¹, Kaya Yaşar Y¹, Ay İ², Sezen S^{1,2}

¹*Department of Pharmacology, Faculty of Pharmacy, Karadeniz Technical university, Trabzon, Türkiye,* ²*Drug and Pharmaceutical Technology Application & Research Center, Karadeniz Technical University, Trabzon, Türkiye*

Introduction: Diabetic erectile dysfunction (DMED) is a prevalent complication of diabetes. Oxidative stress plays a major role in diabetic complications, including DMED, although underlying mechanism is not completely defined and lacks an effective treatment [1]. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcription factor that ameliorates oxidative damage via enhancing antioxidant enzymes such as heme oxygenase-1 (HO-1), superoxide dismutase (SOD) and catalase (CAT) [2]. In this study, we aimed to investigate the therapeutic effect of dimethyl fumarate (DMF), a Nrf2 activator approved for multiple sclerosis, on DMED in streptozocin (STZ)-induced diabetic rats.

Methods: To induce diabetes, male Sprague-Dawley rats were intraperitoneally injected with a single intraperitoneal dose of STZ (60 mg/kg). 8 weeks after STZ, diabetic and nondiabetic rats were orally treated with DMF (25 or 100 mg/kg) or vehicle (2.5% sodium carboxymethyl cellulose) for 4 weeks (n=8-10/group). At week 12, erectile function was determined as the maximum intracavernosal pressure (mICP)/mean arterial pressure (MAP) and total ICP/MAP ratios by cavernous nerve electrostimulation at 8 volts [3]. The levels of Nrf2, malondialdehyde (MDA), HO-1, and the activities of SOD and CAT enzymes were detected by commercial kits in the penile tissues.

Results: mICP/MAP and total ICP/MAP values were lower in diabetic rats (0.41 ± 0.03 and 6.60 ± 0.85 , respectively) than control (0.78 ± 0.06 and 11.92 ± 0.79 , respectively). DMF (100 mg/kg) treatment for 4 weeks significantly ($p<0.01$) increased these functional parameters (0.68 ± 0.06 and 11.85 ± 1.23 , respectively). Moreover, markedly increased MDA level along with decreased Nrf2 and HO-1 levels, reduced SOD and CAT enzymes activities in the penile tissues of diabetic rats were partially restored by DMF (100 mg/kg) treatment ($p<0.05$). DMF did not alter either blood glucose level or body weight of rats.

Conclusions: DMF improved erectile dysfunction in diabetic rats by suppressing oxidative stress via the enhancement of Nrf2-mediated antioxidant response.

- [1] Agarwal A, Nandipati KC, Sharma RK, Zippe CD, Raina R. Role of oxidative stress in the pathophysiological mechanism of erectile dysfunction. J Androl. 2006;27(3):335-347.
- [2] He F, Ru X, Wen T. NRF2, a transcription factor for stress response and beyond. Int J Mol Sci. 2020;21(13):4777.
- [3] Sezen SF, Lagoda G, Musicki B, Burnett AL. Hydroxyl fasudil, an inhibitor of Rho signaling, improves erectile function in diabetic rats: a role for neuronal ROCK. J Sex Med. 2014;11(9):2164-2171.

PP020. PDE-4 inhibition with Roflumilast does not improve cardiovascular abnormalities and survival of septic rats

Ferreira Alves G^{1,2}, Delfrate G¹, Garcia de Oliveira J¹, de Almeida Nakashima M¹, Sordi R¹, Assreuy J¹, da Silva-Santos J¹, Collino M², Fernandes D¹

¹*Federal University of Santa Catarina, Florianópolis, Brazil*, ²*University of Turin, Turin, Italy*

Introduction: cAMP is one of the most potent signaling molecules to stabilize the endothelial barrier, modulate inflammatory pathways and increase cardiac contractility. Phosphodiesterase-4 (PDE-4) is an enzyme highly expressed in several tissues that suffer damage during sepsis, such as kidney, lung, and heart, and is responsible for cAMP hydrolysis, regulating its intracellular levels [1,2]. Therefore, this study aimed to evaluate the effect of roflumilast (RFM), a clinically approved PDE-4 inhibitor on cardiovascular collapse and survival in experimental sepsis.

Methods: Sepsis was induced by cecal ligation and puncture (CLP) in male rats. Six hours after the CLP/Sham procedure, animals were randomly assigned to receive RFM (0.3 mg/kg, s.c) or vehicle and 24h after surgery, cardiovascular parameters were recorded, and organ/plasma samples were collected for analyses. Statistical significance ($p<0.05$) was determined using two-way ANOVA followed by Bonferroni's post-hoc test. Data are expressed as mean±S.E.M.

Results: Sepsis triggered cardiovascular impairments, such as hypotension (BP, 82.7 ± 2.6), reduced renal blood flow (RBF, 264.7 ± 26.3) and hyporeactivity to vasoconstrictor when compared to Sham-rats (BP, 129.9 ± 5.1 ; RBF, 354 ± 18.5). RFM treatment effectively increased systemic cAMP levels in a disease-dependent manner (CLP-vehicle 9.64 ± 0.89 vs CLP-RFM 20.57 ± 5.23) and this effect was associated with the restoration of the RBF impaired by CLP-induced sepsis (RBF, 333.6 ± 10.5). On the other hand, treatment with RFM induced a further reduction in blood pressure (BP, 68.8 ± 2.9) and did not improve vascular hyporeactivity. Lastly, septic rats had a mortality of 70% (without antibiotic therapy), which was reduced to 20% with antibiotic treatment. Treatment with roflumilast did not exhibit any effect on mortality in the absence of antibiotic therapy, however it showed to be slightly protective when associated with antibiotic therapy. Septic rats receiving antibiotic and RFM treatment had higher survival rate (83%) at 24h compared to the CLP+vehicle group (69%), although the difference did not achieve statistical significance.

Conclusions: Our study has shed light on the effects of roflumilast, revealing some positive impacts but ultimately falling short in enhancing most hemodynamic parameters. Besides, we have not discerned any significant alterations in mortality rates. Hence, despite prior research suggesting the potential therapeutic value of roflumilast in sepsis management, our findings diverge from this perspective.

[1] Feng H, Chen J, Wang H, et al. Roflumilast reverses polymicrobial sepsis-induced liver damage by inhibiting inflammation in mice. *Lab Invest.* 2017;97(9):1008-1019. doi:10.1038/labinvest.2017.59

[2] Schick MA, Wunder C, Wollborn J, et al. Phosphodiesterase-4 inhibition as a therapeutic approach to treat capillary leakage in systemic inflammation. *J Physiol.* 2012;590(11):2693-2708. doi:10.1113/jphysiol.2012.232116

PP021. Ghrelin as a new therapeutic approach to mitigate aging phenotype in Hutchinson-Gilford progeria syndrome

Ferreira-Marques M^{1,2,5}, Carvalho A¹, Franco A^{1,2,5}, Leal A¹, Botelho M¹, Carmo-Silva S^{1,2}, Águas R¹, Cortes L^{1,2}, Lucas V¹, Real A¹, López-Otín C³, Nissan X⁴, Pereira de Almeida L^{1,2,5}, Cavadas C^{1,2,5}, Aveleira C^{1,2,6}

¹CNC-UC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, ²CIBB – Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal,

³Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Instituto Universitario de Oncología, Universidad de Oviedo, Oviedo, Spain, ⁴CECS, I-Stem and INSERM, Corbeil-Essonnes, France, ⁵Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal, ⁶MIA-Portugal – Multidisciplinar Institute of Ageing, University of Coimbra, Coimbra, Portugal

Introduction: Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic condition caused by a single nucleotide alteration in the LMNA gene, leading to the production of defective lamin A protein (progerin). This results in a premature aging phenotype in affected children, including low body weight, lipodystrophy, and metabolic dysfunction. The lack of effective treatments accentuates the demand for novel therapeutic strategies.

The primary objective is to investigate the role of ghrelin as a novel therapeutic strategy to counteract premature aging in HGPS.

Methods: Ghrelin effectiveness was assessed through two approaches: 1) Reversing the HGPS cellular phenotype using human dermal fibroblasts from HGPS patients, exposing cells to ghrelin (1 nM), assessing its impact on several cellular aging hallmarks. 2) Studying the impact of peripheral ghrelin administration in blocking or delaying the HGPS phenotype and extending lifespan, utilizing a HGPS mouse model (LmnaG609G/G609G mice). Mice received daily subcutaneous administration of ghrelin (50 µg/Kg) or saline. Body weight, food intake, lifespan, and age-related alterations and cellular hallmarks of aging were assessed. Results, presented as mean±SEM, with statistical analyses conducted using unpaired Student's t test, one-way and two-way ANOVA followed by Dunnet's or Bonferroni's multiple comparisons tests.

Results: Ghrelin effectively rescued several hallmarks of cellular aging of HGPS fibroblasts promoting autophagy, progerin clearance, reducing dysmorphic nuclei, DNA damage, and enhancing cell proliferative capacity, delaying cellular senescence.

In HGPS mice, ghrelin administration prevented progressive weight loss in later stages (14.8 ± 0.3 g in vehicle-treated vs 17.5 ± 0.6 g in ghrelin-treated group, $p < 0.0001$, $N=6-19$ per group) without changes in food intake and reversing the lipodystrophic phenotype by restoring white adipose tissue (WAT) characteristics. This included an increase in gonadal WAT weight, adipocyte size, and structural improvements. Ghrelin also reduced progerin levels in WAT. The altered expression of key adipogenic and metabolic genes was normalized with ghrelin treatment. Additionally, ghrelin countered reduced hormone levels (leptin, adiponectin, resistin), indicating improved WAT function. These findings suggest the potential of ghrelin as a therapeutic strategy for LMNA mutation-associated lipodystrophy. The mean survival time of ghrelin-treated LmnaG609G/G609G mice increased from 148 to 173 days and the maximum survival time increased from 177 to 198 days ($p < 0.05$, $N=5-8$ per group), representing a ~22 % increase in the Kaplan-Meier area under the curve.

Conclusions: Our findings uncover the potential of modulating ghrelin signalling as a promising therapeutic approach for HGPS and other age-related pathologies, offering new treatment targets to enhance patient outcomes and quality of life.

PP022. Influence of anticoagulants in platelet-monocyte aggregates (PMA) occurrence in metabolic diseases patients

Gunaseelan C¹, Konečný L¹, Fadraersada J¹, Alva-Gallegos R¹, Gunaseelan C¹, Skořepa P^{2,3}, Matoušová K⁴, Kujovská Krčmová L^{4,5}, Paclíková M², Blaha M², Blaha V², Mladěnka P¹

¹*Charles University, Faculty of Pharmacy, Hradec Králové, Czech Republic*, ²*3rd Department of Internal Medicine-Metabolic Care and Gerontology, University Hospital, Hradec Králové, Czech Republic*, ³*Department of Military Internal Medicine and Military Hygiene, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic*, ⁴*Department of Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové, Hradec Králové, Czech Republic*, ⁵*Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic*

Background and aims: Platelet-monocyte aggregates (PMA) are possible novel biomarkers of early onset of cardiovascular diseases. For PMAs formation, circulating platelets need to be activated. However, the extent of PMAs generation varies probably among particular diseases and other characteristics of individual patients. Additionally, pharmacotherapy might affect their formation.

Aims: The aims of the study was to: 1) analyse the occurrence of PMA and activated platelets in different groups of patients and compare them to that of healthy donors; 2) evaluate the effect of direct anticoagulants on PMA occurrence and 3) study correlations between PMA and biochemical parameters.

Methods: Individuals presenting different metabolic diseases (15 patients with familial hypercholesterolemia - FH, 50 with DM type I – DMT1, and 50 with metabolic syndrome - MS) were enrolled in this study. As control group, 50 healthy volunteers were recruited. PMAs were labelled in freshly withdrawn blood (CD41 for platelets, CD14 for monocytes and CD45 as a pan-leukocyte marker) and analyzed by flow cytometry. Activated platelets were labelled with CD41 and CD62P. In a separate set of experiments, blood was also treated with anticoagulants.

Results: PMA occurrence in enrolled patients (20% for FH; 48% for DM T1; 40% for MS) was higher than in healthy volunteers (15%). Regarding activated platelets, as expected, the result mostly correlated with that of PMAs except for those of FH, in which the number of activated platelets was even lower than in healthy donors. This result is probably due to the intensive pharmacological treatment these patients undergo. After in vitro anticoagulant treatment, the mean percentage of PMAs was decreased in all groups. However, the reduction was variable depending on the disease. Additionally, differences among anticoagulants groups (antiFII or anti-FXa) were also observed.

Conclusion: PMA occurrence is significantly increased in patients suffering from metabolic disorders compared to healthy volunteers. Modern anticoagulants might decrease the occurrence of these biomarkers.

This research was possible thanks to the financial support of the Czech Health Research Council, project no.: NU21J-02-00021

PP023. Intrinsic redox balance and glutathione oxidation preserve endothelial cell sprouting capacity and promote vessel growth

Karantanou C^{1,2}, Wittig J^{1,2}, Drekolia M^{1,2}, Wang D³, Xu C^{1,2}, Mettner J^{1,2}, Weigert A⁴, Hu J^{1,3}, Bibli S^{1,2,5}

¹*Department of Vascular Dysfunction, European Center for Angioscience (ECAS), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany*, ²*Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany*, ³*Department of Histology and Embryology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, China*, ⁴*Institute of Biochemistry I, Faculty of Medicine, Goethe-University Frankfurt, Frankfurt am Main, Germany*, ⁵*German Centre for Cardiovascular Research (DZHK), Germany*

Introduction: Glutathione serves as the primary antioxidant defense amino acid in the human body. Once generated intracellularly, it undergoes rapid oxidation with the aid of glutathione peroxidase 4 (GPX4) to protect against lipid peroxidation. Given that endothelial cells (ECs), lining the vessel wall, are tightly regulating oxidative balance to control angiogenesis and blood vessel growth [1], here we aim to explore the role of GPX4 in the regulation of vascular growth.

Methods: GPX4 activity was evaluated in both proliferating and quiescent human umbilical vein endothelial cells using ¹³C cysteine metabolic flux analysis to assess the flux from reduced to oxidized glutathione. GPX4 silencing was achieved through RNAi technologies. In vitro angiogenesis was studied through proliferation and spheroid-based sprouting assays. Mitochondrial reactive oxygen species and lipid peroxidation were measured using a mitochondrial superoxide indicator and a fluorescent mitochondria-targeted lipid peroxidation probe, respectively. Levels of oxidative protein damage were assessed by 3-nitrotyrosine. Limited proteolysis mapping was employed to investigate the metabolite-protein interactions in relation to oxidized and reduced glutathione. For in vivo angiogenesis phenotyping, a tamoxifen inducible EC specific (GPX4 fl/fl Cdh5 Cre+/-) mouse model was utilized.

Results: Proliferating ECs exhibited increased GPX4 activity, as well as enhanced incorporation of ¹³C cysteine to the oxidized glutathione, an effect that coincided with protection against mitochondrial lipid peroxidation and protein oxidation. In contrast, deletion of GPX4 in proliferating ECs, increased lipid peroxidation as well as oxidative protein damage. Phenotypically, ECs lacking GPX4 showed reduced proliferative and sprouting capacity. In vivo EC specific inducible deletion of GPX4 in embryonic days E7.5-E10.5 led to vessel leakage and disrupted vessel growth in E13.5 embryo skin as well as reduced vascular growth on the developing retina at postnatal day 6. Mechanistically, oxidized glutathione did not only protect against mitochondrial lipid peroxidation, but also interacted with proteins responsible to maintain endothelial growth and interfered with their stability.

Conclusions: GPX4 activity promotes EC antioxidant defense and maintains the stability of proteins responsible for EC proliferation making it indispensable for vascular growth. Targeting oxidized glutathione levels emerges as a promising strategy for vessel regeneration.

- [1] Schröder K. Redox control of Angiogenesis. *Antioxidants & redox signaling*. 2019; 30(7):960-971

PP024. Non-psychotropic Drug Interactions among Psychiatric Patients in Bahrain

Tayem Y¹, Al-Ghadani M, Jahrami H, Ali M

¹Arabian Gulf University, Manama, Bahrain

Background: In psychiatric patients, interactions among non-psychotropic drugs may be unintentionally overlooked. **Aims:** The aim of this study was to investigate the rate and degree of interactions among non-psychotropic drugs in patients suffering from mental illnesses in Bahrain.

Methods: This was a retrospective cross-sectional study. A random sample of prescriptions ordered by the outpatient clinics of the psychiatry hospital from the 1st of January until the 31st of December 2017 was selected. The orders, which were issued for patients diagnosed with schizophrenia, depression, anxiety disorder, bipolar disorder, and schizoaffective disorder were included in this study. The quantity and grade of drug interactions were measured by using Medscape drug interaction checker. The factors associated with those interactions were also examined. Data analysis was performed by using t-test, Chi-Square test, one-way and two-way ANOVA.

Results: 995 prescriptions were included (55.4% males and 44.5% females) were included. The psychiatric diagnoses of the subjects were schizophrenia (39.1%), depression (23.1%), bipolar disorder (22.4%), schizoaffective disorder (11.2%) and anxiety disorders (4.1%). Polypharmacy was observed in 33.3% of the patients. Drug interactions were detected in 22.7% of the prescriptions. The grade of interaction was minor in 4.6%, significant in 15.6%, and serious in 2.5%. There was a positive correlation between the total number of interactions and polypharmacy ($p < 0.001$), and age over 35 years ($p < 0.001$). Moreover, interaction between those two risk factors was observed and resulted in a statistically significant increase in the total number of drug interactions ($F = 6.286$, $p = 0.002$).

Conclusions: a relatively high rate of drug interactions was observed, associated with polypharmacy. There is a need to raise awareness among psychiatrists to check for non-psychotropic drug interactions in their patients.

PP025. An investigation of the protective effect of berberine on vancomycin-induced nephrotoxicity in rats

Yildirim E¹, Al Homsi T¹, Burukoğlu Dönmez D²

¹Eskisehir Osmangazi University, Eskisehir, Türkiye, ²Eskisehir Osmangazi University, Eskisehir, Türkiye

Introduction: One limitation of vancomycin (VCM) use is its nephrotoxicity due to the high doses of vancomycin used to treat resistant infections. Berberine (BBR) is a natural product with a broad pharmacological activity including anti-inflammatory and antioxidant effects. In this study the reno-protective effect of BBR has been investigated against nephrotoxicity induced by VCM in wistar albino rats.

Biomarkers including creatinine (Cr), blood urea nitrogen (BUN), kidney injury molecule (KIM-1), superoxide dismutase (SOD), malondialdehyde (MDA), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), B-cell lymphoma 2 (BCL-2) and caspase-9 (CAS-9) have been evaluated. Additionally we have aimed to study the potential reno-protective effect of BBR against nephrotoxicity by using histological imaging methods.

Material and methods: 35 male Wistar Albino rats were randomly assigned into five groups (n:7). The control group received oral saline (SF) for eight days and intraperitoneally (i.p) SF for seven days. The other groups were administered i.p. VCM dissolved in SF for seven days. Respectively BBR (50mg/kg, 100mg/kg, and 200mg/kg) was given by oral gavage for eight days, starting one day before the VCM administration.

Results: The administration of VCM has led to nephrotoxicity, as evidenced by elevated levels of inflammation and oxidative stress biomarkers, as well as significant histological damage. In addition, a minor increase of apoptosis biomarkers has been noted. However, VCM has showed insignificant apoptotic related kidney damage. The administration of BBR has shown the potential improvement of the oxidative stress and inflammation significantly. The reduction of apoptosis has not been significant.

Conclusion: Our results have showed that BBR might has the potential to reduce the nephrotoxicity induced by the intraperitoneal administration of VCM at the dose of 400 mg/kg for seven days in male rats.

'This work has been supported by Eskisehir Osmangazi University Scientific Research" Projects Coordination Unit under grand number TYL-2022-2352.

- [1] Adil, M., Kandhare, A. D., Dalvi, G., Ghosh, P., Venkata, S., Raygude, K. S., & Bodhankar, S. L. (2016). Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. *Renal Failure*, 38(6), 996–1006.
- [2] Ahmad, S., Hussain, A., Hussain, A., Abdullah, I., Ali, M. S., Froeyen, M., & Mirza, M. U. (2019). Quantification of berberine in berberis vulgaris L. Root extract and its curative and prophylactic role in cisplatin-induced in vivo toxicity and in vitro cytotoxicity. *Antioxidants*, 19:8(6).

PP026. Physical Exercise Effects on a Mouse Model of Depression: Mind the Proteomics

Reis J^{1,2,3}, Soares E^{1,2,3}, Azkargorta M⁴, Elortza F⁴, Caramelo F^{2,3,5}, Fontes Ribeiro C^{1,2,3}, Pereira F^{1,2,3}
¹*University of Coimbra, Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine, Coimbra, Portugal*, ²*University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal*, ³*University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal*, ⁴*CIC bioGUNE, Bizkaia Science and Technology Park, Derio, Spain*, ⁵*University of Coimbra, Laboratory of Biostatistics and Medical Informatics (LBIM), Faculty of Medicine, Coimbra, Portugal*

Introduction: Depression, affecting nearly 300 million worldwide, lacks effective treatments, prompting exploration of non-pharmacological approaches like physical exercise (PE). This study aimed to study PE's impact on depression/anxiety-like phenotypes recapitulated by a chronic social defeat stress (CSDS) mouse model.

Methods: Young-adult male C57BL/6 mice (7-8 weeks old) underwent a four-week incremental treadmill PE program (moderate running) prior to be subjected to social defeat (SD) protocol. Mice faced bouts of social defeat by aggressive male CD-1 mice for 10 minutes over ten consecutive days. Two clusters emerged from behavioral categorization: SD1 (social avoidance/anxiety) and SD2 (stress resilience). Seric and neural cytokines and neurochemical markers, including BDNF, were assessed in the frontal cortex across Control, SD1, SD2, PE+SD, and PE groups (Multiplex Eve Technologies Discovery Assays). Western Blotting evaluated neurochemical profiles, including TH, GFAP, GR, METRNL, Iba-1, PSD-95, iNOS, IL-6, TrkB, MAPK, CREB, and NF-KB p65 biomarkers. Groups (N=2-8) were compared using ANOVA or Kruskal-Wallis followed by posthoc multiple comparison tests ($p<0.5$). Additionally, serum proteins were extracted for proteomic analysis. Following a FASP protocol with trypsin, peptides were processed and analyzed using a timsTOF Pro with PASEF coupled to an Evosep ONE liquid chromatograph. PEAKS X software aided protein identification and quantification against Mus musculus entries from Uniprot Swissprot. Perseus platform facilitated data processing (log2 transformation, imputation) and statistical analysis (N=5, ANOVA, Tukey's test and Benjamini-Hochberg procedure to control the FDR at 0.5).

Results: PE did not prevent stress-induced behavioral alterations. Moreover, BDNF significantly increased in the frontal cortex of SD2 versus Control, SD1, and PE+SD groups ($p<0.05$). However, TRKB, MAPK, and CREB densities remained unchanged (NS). No significant alterations occurred in other brain and seric markers. Importantly, proteomic analysis unveiled distinct protein cluster profiles between groups: comparative analysis between SD1 and SD2 highlighted variations in proteasome core complex, proteasome-related processes (Psma1, Psma5, Psma2), endopeptidase/peptidase activities (Mug1, Serpina1a, Serpinf2). Notably, PE+SD exhibited downregulation in cytokine response/endopeptidase regulation proteins (Serpina1d, Serpina1a, Serpina3m, Timp2) and alterations in extracellular matrix (Col3a1, Vtn, Postn), phagocytosis, and immune-related proteins (Il1rap, Sirpa, CFI) when compared to control.

Conclusion: PE did not prevent stress-induced behaviors in mice. Moreover, this study disclosed robust proteomics alterations underpinning stress/resilience mechanisms. This highlights possible diagnostics/therapeutics avenues in mental disorders. Finally, PE needs to be tailored to properly fit brain health.

This work was supported by “CHAngeing – Connected Hubs in Ageing: Healthy Living to Protect Cerebrovascular Function” funded by the European Union’s Horizon Europe program (Excellence Hubs - HORIZON-WIDERA-2022-ACCESS-04-01) under grant agreement No. 101087071 and by Foundation for Science and Technology (FCT, Portugal) for the financial support to CIBB (UIDB/04539/2020 and UIDP/04539/2020) research unit through COMPETE-FEDER (POCI-01-0145-FEDER-007440).

PP027. Integration of Pharmacological Principles and AI into GI physiology

Reuben J¹, Warner A¹, Logan S¹

¹Texas A&M University School of Dentistry, Dallas, United States

Although Artificial Intelligence (AI) is already being used in health care there are few published standards about its incorporation into dental physiology and pharmacology education. This study was conducted to assess dental students' use of AI as a study tool and their use of AI to integrate pharmacological principles in a first-year dental physiology course.

Methods: One hundred first-year dental students enrolled in a dental physiology course were surveyed before the start of GI physiology instruction using the polleverywhere platform. The survey included a 5-point Likert scale and an option for open-ended comments. Survey questions were divided into two categories to assess student attitudes concerning the use of AI for studying and their use of AI for integrating pharmacological principles into their study of gastrointestinal physiological concepts. Descriptive statistics were computed to provide a summary of their responses.

Results: Ninety-five students participated in the survey. A majority (72%) of the students surveyed agreed that AI could positively supplement traditional educational methods and that they were interested in exploring new AI tools to enhance their studying (73%). However, only 32% of the students indicated that they preferred courses that integrated AI-based resources for a more interactive learning experience with the majority (59%) stating that it depended on the course. The majority of students polled (62%) did not think about any pharmacological concepts in their preparations despite having been introduced to general pharmacological principles and autonomic pharmacology before the first physiology class. However, those students who did reflect on pharmacological concepts primarily focused on pharmacokinetic principles and autonomic receptor pharmacology.

Conclusion: While dental students thought that AI can be a beneficial educational tool and were interested in its use, the data suggests that more opportunities need to be provided to educate them about the different AI platforms available and their use. Furthermore, additional effort can be focused on teaching students how to integrate pharmacology and other biomedical sciences earlier in their dental education using AI.

PP028. The impact of 1-week empagliflozin treatment on cardiac mitochondrial respiration and ROS production in healthy rats

Mornar M¹, Grahovac M¹, Marinović Ljubković J¹, Boban M¹, Ljubković M¹

¹*University of Split School of Medicine, Split, Croatia*

Introduction: Empagliflozin (EMPA), a sodium-glucose co-transporter 2 (SGLT2) inhibitor, is being increasingly used for treatment of heart failure. Although the complete mechanism(s) underlying its cardioprotective effect is yet to be identified, studies suggest that EMPA has pleiotropic effects, including directly on the myocardium [1]. Mitochondrial dysfunction and oxidative stress were reported to play a major role in progression of heart failure and were recently studied as potential targets of EMPA. Majority of research was performed in animal heart failure models and reported improved mitochondrial respiratory function and reduced reactive oxygen species (ROS) production in diseased myocardium [2]. Here, we examined the effects of EMPA on cardiac mitochondria in healthy animals.

Methods: Male Sprague Dawley rats (n=16) consumed EMPA (30 mg/kg/day) for 7 days, via drinking water (EMPA previously dissolved in DMSO). The Control group (littermates) was exposed to the equivalent amount of DMSO (0.7%). Afterwards, mitochondrial function was evaluated in homogenized samples of left ventricular myocardium. Mitochondrial respiration was measured as tissue oxygen consumption rate (expressed in pmolO₂/s/mg) and ROS production was monitored using Amplex UltraRed detection system (in pmolH₂O₂/s/mg). The study was approved by the institutional Ethics committee.

Results: All animals completed 1-week treatment with no apparent side effects. Mitochondrial respiration fueled by NADH-producing substrates (Complex I-linked; pyruvate, malate and glutamate, 5/0.5/5 mM, respectively) and stimulated by ADP (2.5 mM) was not different between EMPA and Control rats (188.3±21.5 vs. 193.1±14.77). The same was detected when Complex II substrate (succinate, 10 mM) was used (131.0±18.7 vs. 130.3±22.8). Also, when respiration was fueled by all substrates simultaneously (OxPhos), no difference was observed (287.2±35.4 vs. 296.4±34.4). Finally, stimulation of maximal electron transfer system activity by an uncoupler (FCCP, 1µM) showed no difference (293.4±40.4 vs. 301.0±43.8). Exposure to EMPA did not result in altered ROS production (H₂O₂) during OxPhos (0.27±0.08 vs. 0.36±0.13). Finally, when ROS was measured under conditions of simulated reperfusion (high protonmotive force and reduced Q pool) no effect of EMPA treatment was observed (1.19±0.27 vs. 1.00±0.25).

Conclusion: A 1-week empagliflozin consumption did not affect mitochondrial respiratory activity and ROS production during OxPhos and simulated reperfusion in healthy animals.

- [1] Kowalska K, et al. Empagliflozin-A New Chance for Patients with Chronic Heart Failure. Pharmaceuticals (Basel). 2021;15(1):47.
- [2] Li X, et al. Direct Cardiac Actions of Sodium-Glucose Cotransporter 2 Inhibition Improve Mitochondrial Function and Attenuate Oxidative Stress in Pressure Overload-Induced Heart Failure. Front Cardiovasc Med. 2022;9:859253.

PP029. Could H₂S be a new mechanism for the vascular effects of sildenafil in healthy and oxidative stress conditions?

Özbek E¹, Bozkurt A¹, Anacak G²

¹Ege University, Faculty of Pharmacy, Dept. of Pharmacology, Izmir, Turkiye, ²Acibadem Mehmet Ali Aydinlar University, Faculty of Pharmacy, Dept. of Pharmacology, Istanbul, Turkiye

Introduction: Hydrogen sulfide(H₂S) is a gasotransmitter, regulates various functions in the vascular system, such as vascular tone, blood pressure, endothelial and smooth muscle cells proliferation.H₂S induces a biphasic vascular response; it causes vasoconstriction at low doses, while vasodilation at higher doses. Mechanisms of H₂S-induced relaxation consist of several pathways, including regulation of ion channels, increase in cAMP and cGMP production by inhibition of phosphodiesterase(PDE)[1]. Sildenafil, similar to H₂S, inhibits the PDE, which breaks down intracellular cGMP in vascular smooth muscles. Although it has been reported that sildenafil causes relaxation in aorta through both endothelium-dependent and -independent mechanisms[2], however exact signaling pathway causing endothelium-independent relaxation induced by sildenafil has not yet been fully elucidated. Our previous study showed that H₂S contributes to the relaxant effects of sildenafil on penile[3]. Thus, in the current study, we investigated the role of H₂S in the effects of sildenafil on vascular tonus and oxidative stress in mice aorta under healthy and Pyrogallol-induced oxidative stress conditions.

Methods: Male Swiss-albino mice were obtained from Ege University Animal Centre(2022-016).The effect of sildenafil on endogenous H₂S formation in mice aorta was investigated by methyleneblue assay, the effect on vascular tonus was evaluated by myograph studies in healthy and Pyrogallol-induced (0.1mM) oxidative stress conditions. Production of reactive oxygen species measured by chemiluminescence. For the statistical analyses ANOVA and Bonferroni tests were used.

Results: Sildenafil stimulated endothelium-dependent ACh-induced relaxations as expected($p<0.001, n=6$). Sildenafil increased endogenous H₂S production both in healthy and pyrogallol-induced oxidative stress conditions in mice aorta($p<0.001, n=5$). Sildenafil increased endogenous H₂S-dependent L-cysteine-induced relaxation and exogenous Na₂S-induced relaxations responses significantly($p<0.001, n=6-7$). In addition, both the direct relaxant effect of sildenafil and the increase in endogenous H₂S-dependent relaxation responses by sildenafil were inhibited in the presence of the H₂S synthesis inhibitor aminoxyaceticacid(AOAA,2mM,30 min, $p<0.05, n=5-6$). Sildenafil also exerted antioxidant effects through H₂S($p<0.05, n=5$).

Conclusion: The H₂S-mediated vascular effects of sildenafil may trigger the investigation of new therapeutic uses of sildenafil in pathologies accompanied by vascular oxidative stress such as atherosclerosis, myocardial infarction and pulmonary hypertension. Combination therapy of sildenafil with H₂S substrate-L-cysteine may be a new therapeutic approach in vascular pathologies accompanied by oxidative stress.

- [1] Bucci M, Papapetropoulos A, Vellecco V, et al. Hydrogen sulfide is an endogenous inhibitor of phosphodiesterase activity. *Arterioscler Thromb Vasc Biol.* 2010;30(10):1998-2004.doi:10.1161/ATVBAHA.110.209783.
- [2] Sharabi FM, Daabees TT, El-Metwally MA, Senbel A. Effect of sildenafil on the isolated rat aortic rings. *Fundamental & Clinical Pharmacology.* 2005;19(4):449-456.doi:10.1111/j.1472-8206.2005.00345.x1.
- [3] Dikmen A, Di Villa Bianca RD, Mitidieri E, et al. P10 New mechanism for the beneficial effect of sildenafil on erectile function:H₂S.Nitric Oxide.2013;31:S38.doi:10.1016/j.niox.2013.06.072.

PP030. The Role of Hydrogen Sulfide in Ischemic Priapism

Kaya N¹, **Ozbek E²**, Ersoy N³, Yetik-Anacak G⁴, Cilaker Mıçılı S⁵, Bozkurt O⁶, Durmuş N⁷

¹Dokuz Eylül University, Health Sciences Institute, Izmir, Turkey, ²Ege University, Faculty of Pharmacy, Department of Pharmacology, Izmir, Turkiye, ³Izmir Tinaztepe University, Faculty of Medicine, Department of Histology & Embryology, Izmir, Turkiye, ⁴Acibadem Mehmet Ali Aydinlar University, Faculty of Pharmacy, Department of Pharmacology, Istanbul, Turkiye, ⁵Dokuz Eylül University, Faculty of Medicine, Department of Histology & Embryology, Izmir, Turkiye, ⁶Dokuz Eylül University, Faculty of Medicine, Department of Urology, Izmir, Turkiye, ⁷Dokuz Eylül University, Faculty of Medicine, Department of Medical Pharmacology, Izmir, Turkiye

Introduction: Ischemic priapism (IP) is a persistent and painful erection lasting more than 4 hours that requires urgent treatment to prevent corporal fibrosis and erectile dysfunction [1] and treatment aims to ensure reperfusion of the tissue. Dysfunctional nitric oxide (NO) signaling is shown as one of the pathophysiological mechanisms of Sickle Cell Disease-Associated Recurrent Ischemic Priapism [2]. Another gasotransmitter hydrogen sulfide (H2S), is known to prevent ischemia/reperfusion (IR) damage by reducing free radical-induced stress, promoting mitochondrial function, activating vascularization pathways and reducing apoptosis in different tissues [3]. The present study aimed to evaluate the role of H2S in IP.

Methods: IP model was conducted by vacuum method under anesthesia. Wistar male rats were divided into three groups: In group 1 (control group) only penectomy was performed. In group 2, penectomy was performed after 4 hours of IP. In group 3, penectomy was performed after 4 hours of IP and 1 hour of reperfusion. The endogenous H2S levels and hypoxia-induced factor 1-alpha (HIF-1 α) levels were measured by methylene blue assay and immunohistochemistry, respectively. Histopathological changes were also examined in the penile tissue.

Results: Penile edema, inflammation, desquamation, vasocongestion, and increased collagen levels were observed in the priapism group. These findings were similar in the group that underwent reperfusion following priapism. The endogenous H2S levels decreased ($p<0.05$), while HIF-1 α levels increased ($p<0.0001$) in the priapism and priapism-reperfusion groups compared to the control group.

Conclusion: In conclusion, we demonstrated a decrease in H2S levels during the 4-hour ischemia and subsequent reperfusion period in parallel with tissue damage in penile which indicates the role of H2S in the pathophysiology of IP. Further studies are required to investigate the mechanism of the decrease in H2S levels due to priapism.

- [1] Halls JE, Patel DV, Walkden M, Patel U. Priapism: pathophysiology and the role of the radiologist. Br J Radiol. 2012 Nov;85 Spec No 1(Spec Iss 1):S79-85.doi:10.1259/bjr/62360925.
- [2] Anele UA, Burnett AL. Nitrergic Mechanisms for Management of Recurrent Priapism. Sex Med Rev. 2015 Jul 1;3(3):160-68.doi:10.1002/smrr.56.
- [3] Wu D, Wang J, Li H, Xue M, Ji A, Li Y. Role of Hydrogen Sulfide in Ischemia-Reperfusion Injury. Oxid Med Cell Longev. 2015;2015:186908.doi:10.1155/2015/186908.

PP031. Adverse events associated with the use radio-pharmaceuticals: A prospective study from a tertiary care institute of national importance

Meher B¹, Kanhaiyalal K¹, Kumar N¹, Baranwal A¹

¹All India Institute of Medical Sciences, Bhubaneswar, India

Introduction: Radio-pharmaceuticals (RPs) are used in the diagnosis and management of various cancer and non-cancerous conditions. [1] Like those of conventional drugs use of RPs may also be associated with development of various adverse events (AEs). [2] The information obtained from patients about these AEs may empower medical professionals to detect, assess, and manage them more efficiently and ensure their safe use. The objectives of our study was to assess the type, timing, and frequency of reported AEs and determine their causal association with RPs as well as evaluation of the outcome and follow-up of those AEs from the perspective of patients.

Methods: This study was a prospective cohort study conducted in 312 patients who underwent nuclear medicine examination for various indications in a tertiary care center. Relevant data was collected from study participants regarding the suspected AEs associated with use of various RPs. Collected data were then objectively analysed and assessed.

Results: Out of 312 study participants 41 reported 59 AEs. Reported AEs were proportionally more among female than male (17.7% vs 9.6%, p=0.02). Most commonly reported AEs were pruritus, giddiness, headache, dyspnea, vomiting, and diarrhea. All reported AEs were mild in nature and did not require hospitalization or death of any participants. Of the patient-reported adverse events, 13 % had causal association (possible or probable) whereas 87% did not have causal association with RPs. Frequency of patient-reported adverse drug reactions(ADRs) was found to be 1.2%. 37% AEs occurred within 1 hour of administration of the RPs whereas 67% developed between 1 to 24 hours. All reactions spontaneously resolved within a few hours and did not require any kind of intervention.

Conclusion: Adverse reactions to RPs can occur though the prevalence is lower than that of conventional drugs. We hope this study will increase the awareness about adverse reactions associated with use of RPs among medical professionals and patients and encourage them to report it.

[1] Kennedy-Dixon TG, Gossell-Williams M, Cooper M, Trabelsi M, Vinjamuri S. Evaluation of radiopharmaceutical adverse reaction reports to the British Nuclear Medicine Society from 2007 to 2016. J Nucl Med. 2017; 58:2010–2

[2] Schreuder N, Jacobs NA, Jager PL, Kosterink JGW, van Puijenbroek EP. Patient-Reported Adverse Events of Radiopharmaceuticals: A Prospective Study of 1002 Patients. Drug Saf. 2021 Feb;44(2):211-222

PP032. Effect of adrenalin alpha-2 agonists on thrombogenesis: risk in animal anesthesia with medetomidine, but safety in clinical sedation with dexmedetomidine

Kondo K¹, Kano T¹, Suganuma Y¹, Ikemoto K¹, Sumi-Ichunose C¹, Mochizuki T²

¹*Department of Pharmacology, Fujita Health University, Toyoake-city, Japan*, ²*Department of Anesthesiology and Resuscitology, Fujita Health University, Okazaki, Japan*

Introduction: Adrenalin α -2 agonists are known to stimulate platelets and enhance their aggregation. We have investigated prothrombotic potency of practically used two agonists, medetomidine and dexmedetomidine. The former is used for experimental animal anesthesia, and the latter is adopted to clinical sedation in the Intensive Care Unit for example.

Method: Experimental protocol was approved by the Committee on Animal Experiments of Fujita Health University (AP19135). ICR male mice of 6 months old were anesthetized with pentobarbital 80mg/kg-i.p. (1) Blood was collected by venipuncture of inferior vena cava with sodium citrate. Platelet rich plasma (PRP) was separated by centrifugating the obtained blood and cells count was adjusted to 25x10⁴/uL. Platelet aggregation induced by collagen (0.5-1.2 ug/mL) addition was measured by light transmission method using HEMA TRACER (MC-Medical). Medetomidine (1~1,000 ng/mL) or dexmedetomidine (1~100 ng/mL) was added in-vitro to PRP samples 30 minutes before agonist stimulation. (2) Mice were intraperitoneally injected with either dexmedetomidine 10 ug/kg (initial dose in clinical use) or medetomidine 0.75 mg/kg (dose in MMB-mixed animal anesthesia) before pentobarbital 80 mg/kg anesthesia. Platelet aggregation was evaluated similarly. (3) Mice were similarly anesthetized and femoral artery was exposed by inguinal incision. Small paper tip containing 10% FeCl₃ was placed over femoral artery and blood flow was monitored for 30 minutes to detect thrombotic vessel occlusion.

Results: (1) The higher concentration of dexmedetomidine 100 ng/mL significantly enhanced collagen 0.6 mg/mL-induced platelet aggregation from 14.8±1.5 % to 71.4±9.8 %, while 10 ng/mL which assumes clinical dosage did not (33.8±14.5 %, each n=5). On the other hand, medetomidine 100 ng/mL which assumes animal anesthesia significantly enhanced aggregation (from 15.8±4.1 % to 79.0±3.0 %). (2) In an ex-vivo situation, dexmedetomidine 10 ug/kg-i.p. at clinical dosage did not reveal significant aggregation enhancement (from 14.0±2.0 % to 20.6±12.5 % by collagen 0.5 ug/mL-stimulation) while medetomidine 0.75 mg/kg for animal anesthesia did enhance one (60.7±15.1 %). (3) Thrombotic occlusion times were 1,219±147, 1,214±155 (n.s.) and 724±46 (P<0.01) seconds in vehicle-, dexmedetomidine 10 ug/kg- and medetomidine 0.75 mg/kg-treated animals, respectively.

Conclusions: Dexmedetomidine at clinical use seems not prone to be prothrombotic, while medetomidine in MMB mixed anesthesia for animal experiment enhances platelet aggregation, in comparison with classical pentobarbital anesthesia. Researchers should pay attention when evaluating platelet aggregation data obtained under MMB-anesthesia.

Financially supported by a Grant-in-Aid from Fujita Health University

PP033. Cardioprotective MicroRNAs (ProtectomiRs) in a Pig Model of Acute Myocardial Infarction and Cardioprotection by Ischemic Conditioning: MiR-450a and MiR-451

Nagy R¹, Makkos A¹, Baranyai T¹, Giricz Z^{1,6}, Szabó M¹, Kravcsenko-Kiss B¹, Puskás L², Faragó N², Schulz R³, Gyöngyösi M⁴, Lukovic D⁴, Varga Z^{1,5}, Görbe A^{1,6}, Ferdinandy P^{1,6}

¹MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, ²Laboratory of Functional Genomics, Biological Research Centre, Szeged, Hungary, ³Institute of Physiology, Justus-Liebig University of Giessen, Giessen, Germany, ⁴Division of Cardiology, Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria, ⁵HCEMM-SU Cardiometabolic Immunology Research Group, Semmelweis University, Budapest, Hungary, ⁶Pharmahungary Group, Hungary

Introduction: Cardioprotective miRNAs (protectomiRs) are promising therapeutic tools [1]. Here, we aimed to identify protectomiRs in a translational porcine model of acute myocardial infarction (AMI) and cardioprotection by ischemic conditioning and to validate their cardiocytoprotective effect.

Methods: We used tissue samples from our previous study from the infarcted region of left ventricles in a closed-chest AMI model in domestic pigs subjected to sham operation, ischemia-reperfusion to induce AMI (AMI), and ischemic preconditioning (IPreC), postconditioning (IPostC), or remote preconditioning (RIPerC) [2]. ProtectomiR candidates were selected by systematic comparison of miRNA expression changes due to different conditioning stimuli versus AMI following high-throughput qPCR analysis. Rat orthologues of the protectomiR candidates were identified by cross-species sequence comparison. To validate their cardiocytoprotective effect, 25, 50, or 100 nM of mimics or inhibitors (antagomiRs) of the selected protectomiRs or negative control miRNAs were transfected into isolated rat cardiomyocytes using DharmaFECT1 transfection reagent, and cell survival was assessed following 6 hours of simulated ischemia and 2 hours of reperfusion (sI/R).

Results: Of the 220 detected miRNAs, 57 miRNAs were altered by IPreC, 54 by IPostC, and 68 by RIPerC compared to AMI. Four miRNAs were upregulated and ten were downregulated due to all three conditionings vs. AMI. 12 of the protectomiR candidates showed 100% sequence orthology in rats with the identified pig miRNAs. MiR-451 and miR-450a mimics at 25 nM significantly improved the survival of rat cardiomyocytes following sI/R compared to negative control miRNA transfection ($93.71 \pm 3.47\%$ vs. $76.57 \pm 3.57\%$ and $84.84 \pm 2.61\%$ vs. 70.70 ± 2.20 , respectively, $n=5$). Other tested protectomiR candidates did not demonstrate cardiocytoprotection.

Conclusions: This is the first demonstration that miR-450a and miR-451 are associated with cardioprotection by ischemic conditioning in a clinically relevant porcine model, and show cardiocytoprotective effect. These protectomiRs are potential therapeutics for cardioprotection.

PP034. Blood levels of serotonin in fairly controlled and poorly controlled type 2 diabetes mellitus patients: An observational cross-sectional study

Mohanty R¹, Srinivasan A, Rajkumar P

¹AIIMS, Bhubaneswar, Bhubaneswar, India

Introduction: Type 2 diabetes resulting from insulin resistance and relative insulin deficiency accounts for 90% of total diabetic population. Uncontrolled diabetes account for 15 % of overall human mortality. Literature review over last 10 years exposed a promising role of serotonin in insulin secretion and glucose metabolism in animal models. Animal models showed that loss of serotonin in beta-cells of transgenic mice leads to decreased insulin production and that pharmaceutical restoration of serotonin improves insulin secretion. Serotonin also found to enhance glucose metabolism by serotonylating the small cell GTPase, which promotes GLUT 4 translocation to the cell membrane. Hence we conducted a study to find any relation of serotonin level in diabetes control.

Objectives: 1. To study the difference in blood levels of serotonin in patients with fairly controlled and poorly controlled diabetes mellitus. 2. To assess the relationship between Body Mass Index (BMI) and blood level of serotonin. 3. To assess the relationship between insulin resistance and blood level of serotonin.

Methodology: An observational cross sectional study was conducted among diabetic population. A total of 140 patients were recruited into the study and categorized into two groups based on glycemic status (HbA1C) – fairly controlled (HbA1C <7.5) and poorly controlled diabetes (HbA1C > 7.5). Serum serotonin, Insulin Resistance, demographic details, treatment and complication distribution and their relation with serotonin were analysed in both groups.

Results: Diabetic population with fairly controlled (HbA1c<7.5) glycemic status found to have higher serotonin level compared with poorly controlled group ($330.83 \pm 283.34\text{ng/ml}$ vs $182.26 \pm 276.94\text{ng/ml}$) with high statistical significance $p <0.001$. Relation of Body mass index with serotonin revealed a significant negative correlation ($r_s - 0.27$, $p <0.001$). However, relation of insulin resistance & type of complications with serotonin revealed no significant difference.

Conclusion: A statistically significant difference is found in serotonin level among the fairly and poorly controlled diabetes. As well as, significant negative correlation is established between BMI and serotonin levels.

- [1] Paulmann N, Grohmann M, Voigt J-P, et al. Intracellular serotonin modulates insulin secretion from pancreatic β -cells by protein serotonylation. PLoS Biol. 2009;7(10):e1000229.
- [2] Al-Zoairy R, Pedrini MT, Khan MI, et al. Serotonin improves glucose metabolism by Serotonylation of the small GTPase Rab4 in L6 skeletal muscle cells. Diabetol Metab Syndr. 2017;9:1

PP035. Repurposing Colchicine for the Management of COVID-19: A Systematic Review and Meta-analysis

Mohanty R¹, Meher B¹, Padhy B¹

¹AIIMS, Bhubaneswar, Bhubaneswar, India

Introduction: Coronavirus disease 2019 (COVID-19) has become a pandemic affecting more than 195 countries with significant morbidity and mortality. The major complication of COVID-19 is due to immune activation, hyper-inflammation, and cytokine storm. [1] The inflammatory mediators responsible for cytokine storm like interleukin 1 (IL1), interleukin 6 (IL6), and tumor necrosis factors (TNFs) have been found to increase in severe COVID-19.[2] Colchicine is a lipid-soluble alkaloid which exhibits anti-inflammatory activity by tubulin disruption with subsequent down regulation of inflammatory pathway and inhibition of leucocyte-mediated inflammatory activities like production of superoxide and release of various cytokines.[3] Based on these anti-inflammatory properties, colchicine has been used in the management of COVID-19 with variable success. Hence, we conducted this systematic review and meta-analysis to assess the currently available data to rationalize the repurposing of colchicine for the treatment of COVID-19 as add on therapy.

Method: The PROSPERO registration number is CRD42020209814. All randomized controlled trials (RCTs) comparing the use of colchicine in COVID 19 with usual care were included for the analysis. The primary outcome measure was to access the Clinical deterioration within the follow up time available in each study (Clinical deterioration was defined as the need for oxygen supplementation, ICU care or death). PubMed, EMBASE, the Cochrane Library, SCOPUS, and Web of Science were searched for articles.

Result: A total of 10 studies (RCTs) were included in the meta-analysis. Risk of bias assessment by RoB 2 revealed low to some concern in all the studies. The grade of evidence was moderate for the primary outcome. Out of 9217 COVID-19 patients, 4604 patients received colchicine along with usual care. The random effect model showed the overall pooled OR to be 0.83 (95%CI: 0.61 to 1.12) for the primary outcome (Clinical deterioration) which was statistically insignificant ($p = 0.22$).

Conclusion:

This meta-analysis showed statistically insignificant reduction in clinical deterioration in COVID-19 with the use of colchicine.

- [1] Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol. 2020;39(7):2085-2094. doi:10.1007/s10067-020-05190-5
- [2] Meftahi GH, Jangravi Z, Sahraei H, Bahari Z. The possible pathophysiology mechanism of cytokine storm in elderly adults with COVID-19 infection: the contribution of "inflame-aging". Inflamm Res. 2020;69(9):825-839. doi:10.1007/s00011-020-01372-8
- [3] Leung YY, Yao Hui LL, Kraus VB. Colchicine—Update on mechanisms of action and therapeutic uses. Semin Arthritis Rheum. 2015;45(3):341-350. Doi:10.1016/j.semarthrit.2015.06.013

PP036. Cardiometabolic effect of add-on Sarcosine versus add-on placebo in major depressive disorder: a randomized controlled trial

Jena M¹, Padhan M¹, Mohapatra D²

¹*Department of Pharmacology, AIIMS, Bhubaneswar, Khorda, India*, ²*Department of Psychiatry, AIIMS, Bhubaneswar, Department of Pharmacology, AIIMS, Bhubaneswar, India*

Background and aim: Selective serotonin reuptake inhibitors (SSRIs) used in major depressive disorders (MDD) may increase the risk of cardiometabolic derangements.[1,2] The present study was conducted to determine whether add-on sarcosine with SSRIs in patients with major depressive disorder changes the cardiometabolic parameters over eight weeks.

Methods: The present study is a randomized, double-blind, parallel-designed, placebo-controlled clinical trial (NCT04975100). After recruitment and randomization of 60 MDD patients (a total sample size of 60), a baseline assessment of MADRS, BP, Lipid profile, FBS, serum insulin, and adiponectin was done. HOMA-IR, AI, CRI, and CVRI were calculated as derived parameters. Patients received sertraline 50mg or other SSRIs in equivalent doses along with either Sarcosine 500mg (n=30) or similar looking placebo (n=30) as an add-on and followed after eight weeks.

Result: There was a significant decrease in MADRS score in both groups, but the mean change in the sarcosine group was significant over the placebo group (mean change =4.53, p=0.002). There was a significant increase in fasting blood sugar (FBS) (<0.001), serum insulin (<0.001), and HOMA IR (<0.001) after eight weeks of therapy in both the groups but the difference between the groups was not significant (FBS: Mean=0.02, p=0.86; Serum insulin: Mean=0.18, p=0.10; HOMA IR: mean=0.17, p=0.19). The improvement in LDL (P<0.001) and TG (P=0.02) in the sarcosine group was significant compared to the placebo group, whereas changes in HDL, VLDL, and total cholesterol were insignificant. There was a significant difference in BMI in both the groups (p<0.01) but no significant difference between the groups at follow-up (p= 0.98). Total body fat(p=0.02), and visceral fat(p=0.002) were decreased significantly in the sarcosine group.

Conclusion: In this study, add-on sarcosine to SSRIs improved the symptom severity of MDD. Sarcosine had a favorable effect on cardiometabolic parameters by decreasing body fat composition and decreasing LDL and TG.

- [1] Olguner Eker O, Ozsoy S, Eker B, Dogan H. Metabolic Effects of Antidepressant Treatment. Noro Psikiyatration 2017; 54(1): 49-56.
- [2] Tovilla-Zarate CA, Perez-Mandujano A, Ramirez-Gonzalez IR, et al. Vortioxetine versus sertraline in metabolic control, distress and depression in Mexican patients with type 2 diabetes. Ann Transl Med 2019; 7(22): 656.

PP037. Modelling of a precision oncology program on a breast cancer cell line panel in vitro

Makkos A, Takács Á, Somogyi O, Peták I, Görbe A, Dóczi R

¹Semmelweis University, Budapest, Hungary

Introduction: Optimal cancer treatment selection can be challenging due to the complex pathogenetics of tumors. Precision oncology aims to personalize the treatment based on the molecular profile of the tumor. Digital drug assignment (DDA) systems can help to select the appropriate tumor therapy by analyzing the molecular profile of the tumor. The complexity of these systems requires preclinical testing of their performance.

Objective: Here we aim to model a precision oncology program incorporating a DDA system on breast cancer cell lines with known molecular patterns.

Method: 8 widely used breast cancer cell lines were involved in our study. The molecular profile of the cell lines was determined by gene sequencing and receptor expression measurements. Subsequently, the molecular profile was used to score and rank oncological agents by the DDA system. From a list of treatment options, 10 agents (afatinib, neratinib, olaparib, talazoparib, rucaparib, niraparib, crizotinib, palbociclib, tamoxifen, vorinostat) were selected for further in vitro testing representing various mechanisms of action and DDA score values. The inhibitory concentration 50 (IC50) was determined for all cell lines for these agents. Finally, the relationship between IC50 values and DDA score was analyzed.

Results: The molecular profiling determined the mutation and copy number alterations of 591 genes and the expression of hormone receptors. The selected 8 cell lines were classified into three main groups: BRCA mutant (CAL-85-1, MDA-MB- 436), HER-2 overexpressing (HCC-1954, SKBR3, MDA-MB-361), and BRCA mutant and HER-2 protein overexpressing cell lines (JIMT-1, BT-474, HCC-1569). Subsequently, based on the molecular profile of the cell lines, DDA scores of the selected drugs were calculated and IC50 values were determined. Correlation of the measured IC50 values and DDA scores showed a weak correlation for all IC50-DDA score pairs all together. However, for DDA scores with absolute values above 500, the correlation was above 0.6, whereas, for DDA scores with absolute values above 1000, the correlation was above 0.8.

Conclusions: In our work, we demonstrate for the first time the in vitro modelling of a DDA-based precision oncology program on a breast cancer cell line panel. The correlation between the scores calculated with DDA and the measured IC50 values underscores that the DDA system can support precision oncology decision-making.

PP038. Potential inappropriate prescribing in elderly patients with various degrees of kidney failure

Nedin Rankovic G¹, Krtinic D^{1,2}, Stokanovic D¹, Trajkovic H¹, Jovanovic H¹, Jankovic S³

¹*Department of Pharmacology with toxicology, Medical Faculty, University of Nis, Nis, Serbia*, ²*Clinic for Oncology, Clinical center Nis, Nis, Serbia*, ³*Department of Pharmacology with toxicology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*

Inappropriate prescribing of drugs in the elderly patients is an important clinical and economic issue, but still little is known about prescribing of medicines in elderly patients with CKF. The aims of this study were to compare the prevalence of potential inappropriate drug prescribing (PIP) in hemodialysis patients and patients with kidney failure who did not require renal replacement therapy (RRT), as well as to determine factors that are most associated with the occurrence of PIP. The study was designed as a cross-sectional study conducted at the Department of Nephrology, Clinical Center in Nis, Serbia. The approval of the study by Ethics Committee of the Faculty of Medicine, University of Nis was obtained prior to the study commencement. The patients were divided into two groups: (1) patients on hemodialysis treatment and (2) patients with various degrees of CKF and without RRT. In both groups, we had cases and controls, where cases were patients with PIPs, and control patients without PIPs. The presence or absence of PIP was determined using Beers criteria [2]. The data were collected from the interviews with patients, as well as from medical files. The study included a total of 218 patients aged 65 years and over. Out of 83 patients in the first group, according to the Beers criteria, inadequate prescribing was found in 27 (32.5%) patients and from a total of 135 patients in the second group, 44 patients (32.6%) with potentially inappropriately prescribed drugs were detected. In both studies group (hemodialysis patients/ patients without renal replacement therapy) PIP was more common in patients who took more drugs ($t=5.612$, $p<0.001$)/($t=5.748$, $p<0.001$) and had more comorbidities ($t=3.504$, $p <0.001$)/ ($t=2.094$, $p<0.001$). Our study showed that PIP was frequent phenomenon in both hemodialysis patients and those without RRT and that rates of PIPs were not significantly different, present in about a third of patients from both groups. The most important factors associated with rate of PIPs in both groups were number of prescribed drugs and number of comorbidities.

[1] Rankin A, Cadogan CA, Patterson SM, et al. Interventions to improve the appropriate use of polypharmacy for older people. Cochrane Database Syst Rev. 2018;9(9):CD008165. Published 2018 Sep 3. doi:10.1002/14651858.CD008165.pub4

[2] By the American Geriatrics Society 2015 Beers Criteria Update Expert Panel. American Geriatrics Society 2015 Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults. J Am Geriatr Soc. 2015;63(11):2227-2246. Doi:10.1111/jgs.13702

PP039. Efficacy and safety of adipose-derived mesenchymal stem cell therapy for Osteoarthritis: A systematic review and Meta-analysis

Singh S¹, Anil A¹, Shamim M¹, Saravanan A¹, Yadav I¹, Dodiya R¹, Jalan D², Varthya S¹, Dwivedi P¹, Chugh A¹, Chugh V¹

¹All India Institute of Medical Sciences Jodhpur, Jodhpur, India, ²Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India

Introduction: Stem cell therapy, gaining attention for musculoskeletal regeneration, esp, osteoarthritis. Adipose Derived Mesenchymal Stem Cells (AD-MSC), renowned for their regenerative abilities, exhibit potential in preclinical studies for easing osteoarthritic symptoms and fostering cartilage repair. This systematic review and meta-analysis assess the efficacy and safety of AD-MSC in treating osteoarthritis.

Method: We systematically searched four databases and grey literature from inception to October 2023 for randomised controlled trials (RCT) assessing the efficacy and safety of AD-MSC therapy in people with osteoarthritis. Data synthesis was done with Cochrane review manager 5 (RevMan) 5.3 version. Cochrane risk of bias V.2.0 tool was used for methodological quality assessment [1]. The GRADEpro GDT was applied for overall quality of evidence[2]. Primary efficacy objective was the change in the visual analogue scale (VAS) in AD-MSC group versus control group. Secondary outcomes were change in WOMAC (Western Ontario and McMaster Universities) overall score, change in KOOS (Knee injury and Osteoarthritis Outcome Score) pain score, adverse events, and serious adverse events.

Results: A total of 2159 studies were screened, of which 11 RCTs with 440 patients were included. Risk of bias (ROB) was concluded as low in 9 out of 11 RCTs, while 2 studies had high ROB. The change in VAS score from baseline was comparable between the AD-MSC and control group (Mean difference(MD)= -0.43[95%CI: -1.33 to 0.47], p=0.34, I²=0%, 2 studies, 131 participants, high certainty of evidence). No significant difference was found in WOMAC overall score, and KOOS pain score (Standardized mean difference(SMD)=0.03[-0.76 to 0.82],p=0.94, I²=71%, 3 studies, 96 participants, high certainty of evidence), and (MD= 2.14[-3.80 to 8.08], p=0.48, I²=0%, 2 studies, 134 participants, high certainty of evidence), respectively. Adverse events (Risk ratio(RR)= 1.27[0.96 to 1.68], p=0.10, I²=0%, 3 studies, 102 participants, moderate certainty of evidence) and serious adverse events (RR=0.33[0.01 to 7.82],p=0.50, I²=0%, 2 studies, 116 participants, moderate certainty of evidence) also showed no significant difference between two groups.

Conclusion: Meta-analysis revealed no discernible benefits in subjective outcomes, such as visual analogue scale, WOMAC overall, or KOOS pain, when utilizing adipose-derived mesenchymal stem cells for osteoarthritis treatment. Subsequent research could significantly influence effect estimates, potentially altering our conclusion.

- [1] The Cochrane Collaboration.. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, 2020.
- [2] Schünemann H, Brożek J, Guyatt G. Grade Handbook for grading quality of evidence and strength of recommendations. Updated October 2013. The grade Working group, 2013. Available: guidelinedevelopment.org/handbook.

PP040. Romosozumab in Osteoporosis: A Systematic Review and Meta-analysis

Singh S¹, Anil A¹, Saravanan A¹, Shamim M¹, Dwivedi P¹, Varthya S¹, Dutta S², Chugh A¹, Khera D¹, Chugh V¹

¹All India Institute of Medical Sciences Jodhpur, Jodhpur, India, ²All India Institute of Medical Sciences Rajkot, Rajkot, India

Introduction: Studies showed the benefit of Romosozumab, an anti-sclerostin antibody in post-menopausal osteoporosis patients. We plan to evaluate the overall effectiveness and safety of Romosozumab in post-menopausal osteoporosis.

Method: Data synthesis was done with Cochrane Review Manager 5 (RevMan) V.5.3. Cochrane risk of bias (ROB) V.2.0 tool was used for methodological quality assessment.[1, 2] The GRADE pro-GDT was applied for the overall quality of evidence.[3] The primary efficacy objective was an incidence of vertebral fractures. Secondary outcomes were non-vertebral and clinical fractures, bone mineral density (BMD) and adverse events.

Results: One hundred seventy-nine studies were screened, and 10 eligible studies were included in the analysis. The ROB of included studies was low. Romosozumab significantly reduced the incidence of vertebral fractures [OR = 0.43 (95%CI = 0.35–0.52), High-quality evidence], nonvertebral fractures [OR = 0.78 (95%CI = 0.66–0.92), High quality], and clinical fractures [OR = 0.70 (95%CI = 0.60– 0.82), High quality] at 24 months. Bone mineral density was significantly increased with Romosozumab at lumbar spine [MD = 12.66 (95%CI = 12.66–12.67), High quality], total hip [MD = 5.69 (95%CI = 5.68 – 5.69), Moderate quality], and femoral neck [MD = 5.18 (95%CI = 5.18–5.19), Moderate quality] at 12 months. The total adverse events [RR = 0.98(95%CI = 0.96–1.01), Moderate quality] and serious adverse events [RR = 0.98(95%CI = 0.88–1.08), Moderate quality] were comparable in two groups.

Conclusions: Meta-analysis of studies showed effectiveness (decreased incidence of vertebral, non-vertebral and clinical fractures) and safety of Romosozumab in post-menopausal osteoporosis. Hence, authors recommended the use of Romosozumab for treatment of post-menopausal osteoporosis.

[1] The Cochrane Collaboration.. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, 2020.

[2] McGuinness LA, Higgins JPT. Risk-of-bias visualization (robvis): an R package and shiny web APP for visualizing risk-of-bias assessments. Res Synth Methods 2021;12:55–61.

[3] Schünemann H, Brožek J, Guyatt G. Grade Handbook for grading quality of evidence and strength of recommendations. Updated October 2013. The grade Working group, 2013. Available: guidelinedevelopment.org/handbook.

PP041. Knowledge and attitude regarding anabolic androgen steroids in athletes: Correlation with sociodemographic characteristics

Srb N¹, Petrovic A¹, Hefer M¹, Klaric F², Kralik K³, Smolic R¹, Vcev A¹, **Hefer M¹**

¹*Faculty of Dental Medicine and Health Osijek, J. J. Strossmayer of Osijek, Croatia, Osijek, Croatia,*

²*Faculty of Kinesiology Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia,* ³*Faculty of Medicine Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia*

Introduction: Anabolic androgenic steroids (AASs), are frequently abused due to their ability to enhance performance and increase muscle strength. The misuse of AASs has become a significant public health issue. AASs can have detrimental adverse effects, leading to cardiovascular, neuropsychiatric, gastrointestinal, renal, musculoskeletal, dermatological, immune, and hematological disorders 1. Conducting prospective studies to investigate the mechanisms and health consequences of AAS abuse is ethically challenging. Thus, researchers have relied on survey-based studies. Our study aimed to assess the knowledge and attitudes of athletes towards AASs and explore the correlation with sociodemographic characteristics and whether there is a need for additional measures and pharmacovigilance.

Methods: The study was designed as a cross-sectional study. Participants were 107 athletes, both male and female, average age of 21 from professional and amateur sports clubs in Croatia. Data was collected using an anonymous questionnaire, which included questions regarding their knowledge and attitudes towards AASs, as well as their sociodemographic characteristics. Statistical analysis was performed accordingly.

Results: 76,6 % of our participants live in the urban area and 66.4 % have secondary vocational education. 85 % are mostly amateur athletes, of the average age of 21. Regarding knowledge about AASs, only 44% of our participants are aware that nutritional supplements can also contain prohibited AASs, while 41% are aware of AASs' negative effects (impotence, acne, gynecomastia). Retired examinees showed significantly lower results in questions regarding knowledge compared to other occupations ($P = 0.04$). Results of the questionnaire regarding attitude show there is a significantly pronounced positive attitude towards AASs among respondents who are employed compared to the unemployed or retired ($P = 0.02$). A more positive attitude towards doping is also more pronounced among respondents with a monthly income of more than higher wages ($P = 0.02$).

Conclusions: Considering that 76,6 % of our examinees live in urban areas and their average age is 21, these results show unsatisfactory knowledge regarding adverse effects of AASs and possible unintentional intake of AASs through nutritional supplements. Also, with higher wages, athletes' attitude towards AASs are more positive, which is a concerning result, since elite athletes are among highest paid occupations. Our results highlight a need for better education and pharmacovigilance among athletes.

- [1] Albano GD, Amico F, Cocimano G, et al. Adverse Effects of Anabolic-Androgenic Steroids: A Literature Review. Healthcare. 2021;9(1):97. doi:10.3390/healthcare9010097

PP042. Effects of SGLT2 Inhibitors on SERCA2a/ Sarcolipin, which are New Therapeutic Targets in Diabetic Cardiomyopathy

Esgi S¹, **Özşarlak-Sözer G¹**, Kizrak U¹, Shamamedov K¹, Kerry Z¹

¹Ege University Faculty of Pharmacy Department of Pharmacology, Izmir, Turkey

Introduction: Sodium Glucose Co-Transporter-2 Inhibitors (SGLT2 Inhibitors) are a relatively new and effective group of drugs used in the treatment of Diabetes Mellitus. SGLT2 Inhibitors have recently been observed to have favorable effects on cardiomyopathy and cardiovascular dysfunction, which are macrovascular complications associated with T II DM. In this study, we aimed to investigate the favorable effect of Empagliflozin (EMPA) on cardiomyopathy mediated through SERCA2a/ Sarcolipin and Endothelin B (ETB) Receptor pathways.

Methods: Rats (n=30) were divided into 3 groups: control (n=10), diabetic (n=10) and diabetes+EMPA treatment (n=10). The type 2 diabetes model was established by feeding a high-fat diet (HFD, 35% kcal) for 4 weeks followed by low-dose streptozotocin (STZ) (35 mg/kg, i.p.). Empagliflozin (10 mg/kg/day) was administered to the treatment group via drinking water for 12 weeks. Blood glucose levels of the rats were measured weekly during the experiment (18 weeks). Heart tissues were isolated and the protein expression levels of SERCA2a and ETB Receptor were determined by Western-Blot and sarcolipin levels were determined by ELISA method.

Results: When evaluated in terms of target protein expressions, SERCA2a expression level and EndothelinB expression level were highest in the Control group and decreased in the Diabetes group and approached the Control group in the Treatment group. However, this was not statistically significant for EndothelinB expression level. For SERCA2a Expression Level, the comparisons of Control vs. Diabetes ($p^{***}= 0.0008$) and Diabetes vs. Diabetes+Empa ($p^*= 0.0129$) were statistically significant. We then extended our study to cover sarcolipin and sarcolipin analyses strengthened our SERCA2a results.

Conclusion: Our study suggests that SERCA2a and sarcolipin might have a significant role in cardioprotective effect of empagliflozin in diabetes.

- [1] Demir, S., Nawroth, P. P., Herzog, S., & Ekim Üstünel, B. (2021). Emerging targets in type 2 diabetes and diabetic complications. *Advanced Science*, 8(18), 2100275. <https://onlinelibrary.wiley.com/doi/epdf/10.1002/advs.202100275>
- [2] Park, K., Mima, A., Li, Q., Rask-Madsen, C., He, P., Mizutani, K., Katagiri, S., Maeda, Y., Wu, I.-H., Khamaisi, M., Preil, S. R., Maddaloni, E., Sørensen, D., Rasmussen, L. M., Huang, P. L., & King, G. L. (2016). Insulin decreases atherosclerosis by inducing endothelin receptor B expression. *JCI Insight*, 1(6), e86574. <https://doi.org/10.1172/jci.insight.86574>
- [3] Tentolouris, A., Vlachakis, P., Tzeravini, E., Eleftheriadou, I., & Tentolouris, N. (2019). SGLT2 Inhibitors: A Review of Their Antidiabetic and Cardioprotective Effects. *International Journal of Environmental Research and Public Health*, 16(16), Article 16. <https://doi.org/10.3390/ijerph16162965>

*This study was supported by TUBİTAK 2209 project to Simge Esgi.

PP043. Empagliflozin exerts no-mediated vascular beneficial effects in diabetic rats

Kizrak U¹, **Özşarlak-Sözer G¹**, Shamamedov K¹, Alan-Albayrak E¹, Cilaker-Micili S², Ural C³, Cavdar Z³, Cetinkalp S⁴, Bilici-Guler G², Kerry Z¹

¹Ege University Faculty of Pharmacy Department of Pharmacology, Izmir, Turkey, ²Dokuz Eylul University, Faculty of Medicine, Department of Histology and Embryology, Izmir, Turkey, ³Dokuz Eylul University, Faculty of Medicine, Department of Molecular Medicine, Izmir, Turkey, ⁴Ege University, Faculty of Medicine, Department of Endocrinology and Metabolic Diseases, Izmir, Turkey

Introduction: Sodium glucose cotransporter 2 inhibitors (SGLT2i) are a group of antihyperglycemic drugs that have recently been used for the treatment of T2DM by inhibiting glucose reabsorption from the kidneys [1]. Vascular and endothelial dysfunction in diabetes occurs as a result of impaired NO release and/or impaired responses to NO [2]. The aim of this study is to investigate the possible effects of empagliflozin (EMPA) against vascular damage in diabetic rats.

Method: Rats (n=30) were divided into three groups: control (n=10), diabetic (n=10), and diabetes+EMPA treatment (n=10). The Type 2 diabetes model was induced by feeding the rats a high-fat diet (HFD, 35% kcal) for 4 weeks, followed by administration of a low dose streptozotocin (STZ) (35 mg/kg, i.p.). The treatment group received EMPA (10 mg/kg/day) through drinking water for 12 weeks. Aortic rings isolated in rats were suspended, mounted in a 10 mL organ bath for isometric force recording (Panlab, Spain) coupled to a PowerLab data acquisition system (AD Instruments, USA), and bathed in carboxygenated (95% O₂; 5% CO₂) Krebs solution at 37°C. Cumulative ACh (10⁻⁹ -10⁻⁴ M) after pre-contraction with phen (10⁻⁶ M) in the presence/absence of the NO inhibitor L-NAME (10⁻⁴ M). Salbutamol (SLB) (10⁻⁹ -10⁻⁵ M) relaxation responses and cumulative 5-HT (10⁻⁹ -3x10⁻⁵ M) contraction responses were taken.

Results: Ach-induced relaxations ($E_{max} = 78,66 \pm 4,5$, $p < 0,001$) were inhibited in diabetic rats ($E_{max} = 50,95 \pm 6,09$, $p < 0,001$) and EMPA ($E_{max} = 85,54 \pm 4,181$, $p < 0,001$) prevented this inhibition in relaxation responses. SLB relaxations ($E_{max} = 72,45 \pm 6,13$, $p < 0,001$) were diminished in diabetic rats ($E_{max} = 48,48 \pm 5,09$, $p < 0,001$) and EMPA ($E_{max} = 69,31 \pm 3,735$, $p < 0,001$) restored this relaxation. In the absence of L-NAME, cumulative 5-HT (10⁻⁹ -3x10⁻⁵ M) sensitivity to contraction response (pD_2) ($5,40 \pm 0,07$) were increased in diabetic rats ($pD_2 = 5,99 \pm 0,17$, $p < 0,05$) and EMPA ($5,22 \pm 0,31$, $p < 0,05$) restored this contraction.

Conclusion: Diabetes impairs Ach- 5-HT, and SLB responses in rat aorta. EMPA protects NO-mediated responses against diabetes, restores SLB responses and 5-HT sensitivity.

[1] da Silva PN, da Conceição RA, do Couto Maia R, de Castro Barbosa ML. Sodium-glucose cotransporter 2 (SGLT-2) inhibitors: a new antidiabetic drug class. *Medchemcomm*. 2018;9(8):1273-1281. Published 2018 Jun 6. doi:10.1039/c8md00183a

[2] Huszka M, Kaplar M, Rejto L, et al. The association of reduced endothelium derived relaxing factor-NO production with endothelial damage and increased in vivo platelet activation in patients with diabetes mellitus. *Thromb Res*. 1997;86(2):173-180. Doi:10.1016/s0049-3848(97)00060-1

PP044. New Approach to Evaluate Adherence to Therapy Using Comparison of Single Measured Level with Population Pharmacokinetic Model: Study with Abiraterone in Prostate Cancer Patients

Merdita S¹, Šíma M¹, Dvořák J², Matějů M³, Richter I^{4,5}, Kozlík P⁶, Křížek T⁶, Královičová J¹, Bosák J⁷, Petruželka L³, Slanář O¹

¹Institute of Pharmacology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic, ²Department of Oncology, Third Faculty of Medicine, Charles University and Královské Vinohrady University Hospital, Prague, Czech Republic,

³Department of Oncology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic, ⁴Department of Oncology, Krajská Nemocnice Liberec, a.s., Liberec, Czech Republic, ⁵Department of Oncology, First Faculty of Medicine, Charles University and Thomayer Hospital, Prague, Czech Republic, ⁶Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic, ⁷Zentiva, k.s., Prague, Czech Republic

Introduction: Because of the significant food effect, abiraterone treatment requires regular intake of the drug under fasting conditions, which may be a barrier to adherence to the treatment. The aim of this prospective study was to develop a population pharmacokinetic model for abiraterone and assess adherence to treatment in prostate cancer patients by comparing a single measured abiraterone level with model-based prediction.

Methods: Data from the bioequivalence study, totalling 1469 plasma abiraterone concentrations from 83 healthy volunteers, were analyzed using nonlinear mixed-effects modelling. Based on final abiraterone pharmacokinetic model, theoretical distribution of concentration-time profiles at dosage of 1000 mg once daily was simulated using the Monte Carlo method. Subsequently, adherence of 36 prostate cancer patients undergoing abiraterone treatment was evaluated by comparing the real abiraterone concentrations measured in each patient during follow-up visits with simulations. Patients whose abiraterone levels fell below the 5th or exceeded the 95th percentile of the simulated profiles were categorized as non-adherent.

Results: Following this evaluation, 13 patients (36%) were categorized as non-adherent. A significant association was identified ($P=0.0361$) between richness of breakfast and incidence of non-adherence. Adherent patients reported significantly better overall condition self-assessment ($P=0.0384$). Additionally, a tendency towards a higher prevalence of adverse effects was observed in non-adherent patients.

Conclusions: In this study we successfully established a population pharmacokinetic model of abiraterone and introduced an advanced approach to evaluating medical adherence. The challenge of administering abiraterone under fasting conditions contributes to a relatively elevated rate of non-adherence. Our findings underscore the importance of personalized interventions to enhance adherence and minimize risks in prostate cancer patients treated with abiraterone.

This study was sponsored by Zentiva, k.s., Prague, Czech Republic and supported by the Charles University projects Cooperatio (research area PHAR) and SVV 260523. MŠ and OS was supported by project MH CZ – DRO – VFN00064165. JK wishes to acknowledge the support provided by Pharmaceutical Applied Research Centre (The Parc) for her scientific work.

PP045. Liver X-receptor (LXR) agonist T0901317 modulates hepatic steatosis, fibrosis and leukocyte populations in a diet-induced NAFLD mouse model

Aparicio-Collado J¹, Marques P^{1,2,3}, Descalzo I¹, Gómez Martín Á¹, Domingo E^{1,2}, Real J^{1,2,4,5}, **Francisco V^{1,4}**, Sanz M^{1,2,5}

¹Institute of Health Research INCLIVA, Valencia, Spain, ²Faculty of Medicine and Odontology, University of Valencia, Valencia, Spain, ³CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Spain, ⁴Endocrinology and Nutrition Service, University Clinic Hospital of Valencia, Valencia, Spain, ⁵ CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Spain

Introduction: Non-alcoholic fatty liver disease (NAFLD) is an unmet medical need due to its increasingly high incidence, high mortality associated with cardiovascular diseases and absence of effective drugs. The liver x receptors (LXR) are masters regulators of lipid homeostasis, inflammation and vascular function¹. Therefore, we have explored the effects of synthetic LXR agonist T0901317 on hepatic steatosis and fibrosis, as well as on systemic and hepatic immunophenotype, in a diet-induced NAFLD mouse model.

Methods: Male C57BL/6 mice were fed with a NAFLD-inducing diet or with a control diet as previously². Eight weeks after diet initiation, NAFLD group was randomly treated for further 28 days with T0901317 (25 mg/kg/day) or vehicle (10% DMSO, 88% PEG-300, 2% Tween-80) at a constant rate by subcutaneous osmotic minipumps. Hepatic steatosis and fibrosis were determined by histopathological staining. Leukocyte subsets' (neutrophils, eosinophils, monocytes or macrophages, CD4 and CD8 lymphocytes, and iNKT) levels and activation, were analyzed in peripheral blood and livers by flow cytometry. Data was presented as mean±SEM (n=6 to 9) and statistical significance calculated using unpaired two-tailed Student's t-test.

Results: Decreased hepatic fibrosis was found in T0901317-treated NAFLD mice vs vehicle (by 25%). Moreover, a significant increase on hepatic lipid accumulation was verified (by 28%). Accordingly, liver weight was augmented by T0901317 comparing to vehicle (8.6 ± 0.8 vs $14.5 \pm 0.7\%$ of body weight, p<0.001), while epididymal white adipose tissue (eWAT) weight was reduced (1.2 ± 0.06 vs $0.7 \pm 0.08\%$ of body weight, p<0.001). Notably, T0901317 increased both circulating ($1.65 \pm 0.37 \times 10^9$ vs $2.21 \pm 0.49 \times 10^9$ cells/L) and hepatic (2.49 ± 0.68 vs $5.33 \pm 0.78\%$ of viable hepatic cells) levels of neutrophils in NAFLD mice.

Conclusions: LXR agonist T0901317 seems to exert hepatic anti-fibrotic effects while increasing liver steatosis in a diet-induced NAFLD mouse model, likely through mobilization of lipids from eWAT to the liver. Moreover, T0901317 activity may be correlated with an increased infiltration of peripheral neutrophils into the liver. Therefore, LXR agonist T0901317 might be useful in the prevention of NAFLD progression through modulation of liver immune environment.

Spanish Ministry of Science and Innovation: [PID2020-120336RB-I00]; Carlos III Health Institute (ISCIII), European Regional Development Fund (FEDER) and European Social Fund Plus (ESF+) [CP21-00025, PI21-00220]; Generalitat Valenciana [APOTIP/2020/011, CIPROM/2022/45] and European Union-Next Generation EU [INVEST/2022/470]

- [1] Endo-Umeda K, Makishima M. Liver X receptors regulate cholesterol metabolism and immunity in hepatic nonparenchymal cells. *Int J Mol Sci.* 2019;20(20).doi:10.3390/ijms20205045
- [2] Matsumoto M, et al. An improved mouse model that rapidly develops fibrosis in non-alcoholic steatohepatitis. *Int J Exp Pathol.* 2013;94(2):93-103.doi:10.1111/iep.12008

PP046. Positive vascular effects of phylloquinone - anti-senescence and anti-inflammatory activity in endothelial cells and vascular smooth muscle cells

Kieronska-Rudek A^{1,2,3}, Kij A¹, Bar A¹, Mohaissen T¹, Grosicki M¹, Stojak M¹, Kurpinska A¹, Buczek E¹, Proniewski B¹, Kus K¹, Panek A⁴, Pietrowska M⁵, Zapotoczny S⁶, Shanahan C⁷, Szabo C³, Chlopicki S^{1,2}

¹Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland,

²Jagiellonian University Medical College, Krakow, Poland, ³University of Fribourg, Chair of Pharmacology, Faculty of Science and Medicine, Fribourg, Switzerland, ⁴Institute of Nuclear Physics Polish Academy of Sciences, Krakow, Poland, ⁵Centre for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland,

⁶Jagiellonian University, Department of Physical Chemistry and Electrochemistry, Faculty of Chemistry, Krakow, Poland, ⁷King's College London, School of Cardiovascular and Metabolic Medicine and Sciences, James Black Centre, London, United Kingdom

Introduction: For many years, the role of vitamin K1 (phylloquinone, PK) was limited to the regulation of coagulation, while vasoprotective activity was attributed to vitamin K2 (menaquinone, MK). However, recent data suggest that the activity of PK may be broader, including i.e., regulation of the inflammatory response. Therefore, the aim of the present study was to verify whether PK, similar as reported earlier for MK, can positively influence vascular function and, if so, to investigate its possible involvement in the regulation of the main pathways of vascular dysfunction: senescence and inflammation.

Methods: The vasoprotective activity of PK in comparison to MK has been studied functionally in two models of endothelial dysfunction: 1/in vivo by MRI, in ApoE/LDLR-/ mice (n=6-10) fed with PK or MK supplemented diet for 8 weeks 2/isolated, TNF-stimulated aorta incubated with PK or MK by myography (n=6). The anti-senescence activity on PK with influence on DNA damage and anti-inflammatory activity with influence on NFκB was analyzed in a replicative and induced model of senescence of endothelial and vascular smooth muscle cells in vitro.

Results: The ApoE/LDLR-/ mice fed with control diet exhibited impairment of acetylcholine (Ach) induced response in vivo in Brachiocephalic artery (BCA) (-9.7±2.9%) and left coronary artery (LCA) (-25.7±4.5%) while 8-weeks diet supplemented with PK or MK resulted in improved of endothelial function in this mice in BCA (4.7±4.0% and 8.0±2.9%, respectively) and LCA (8.0±2.9% and 7.5±1.6%, respectively). Similarly, in isolated aorta, 24h incubation with TNF (10 ng/mL) resulted in impairment of Ach-induced vasorelaxation, which was fully (PK) or partially (MK) when 10μM vitamin was added to incubation with TNF. In vitro studies indicated that both PK and MK significantly decreased expression of senescence-associated markers (p21, p16) and β-galactosidase activity in various models of cellular senescence via inhibition of DNA damage. Additionally, both PK and MK decreased the adhesion of monocytes to endothelial cells and expression of inflammatory markers (ICAM-1, COX-2, PGE2) via NFκB inhibition.

Conclusion: PK similar to MK plays a beneficial role in vasoprotection that can be ascribed among others, to the inhibition of cell senescence and inflammation.

- [1] Bar A, Kus K, Manterys A, et al. Vitamin K2-MK-7 improves nitric oxide-dependent endothelial function in ApoE/LDLR-/ mice. *Vascul Pharmacol*. 2019;122-123:106581.
- [2] Mohaissen T, Proniewski B, Targosz-Korecka M, et al. Temporal relationship between systemic endothelial dysfunction and alterations in erythrocyte function in a murine model of chronic heart failure. *Cardiovasc Res*. 2022 Sep;118(12):2610-2624.

PP047. Adverse events related to etonogestrel implant: an old but still current issue?

Mendes Fernandes J¹, Sousa M², Bhikha J², Silva I³, Moreira P³, Silva M¹

¹*INFARMED I.P., Lisboa, Portugal*, ²*ESTeSL, Lisboa, Portugal*, ³*Department of Clinical Pharmacology, São João University Hospital Center (CHUSJ), Porto, Portugal*

Introduction: In December 2019, in Portugal, a Direct Healthcare Professional Communication (DHPC) was approved regarding the subcutaneous implant of etonogestrel 68 mg. The genesis of this communication was based on reported cases of neurovascular injury and migration of the implant from the insertion site within the arm, or, in rare cases, into the pulmonary artery, possibly associated with deep or incorrect implant insertion.

To minimize risks, the DHPC has introduced updates to the instructions for the insertion and removal of the implant [1]. The aim of our work is to conduct a descriptive analysis of the number of Individual Case Safety Reports (ICSRs) received by the Portuguese National Pharmacovigilance System (SNF) following the DHPC, related to adverse events associated with the insertion and removal of the etonogestrel implant.

Methods: Retrospective analysis of subcutaneous etonogestrel implant ICSRs reported to the Portuguese SNF, between 1 January 2020 and 31 October 2023. ICSRs were screened by 2 pharmacy students, and adverse events potentially associated with the insertion and removal of the etonogestrel implant have been flagged. All suspected migration ADR were clinically reviewed. Descriptive data analysis was performed.

Results: Our research retrieved 451 ICSRs related to the subcutaneous etonogestrel implant. In 32.2% (n = 145) of the total cases, it was reported that the implant was deeply located, with 12.4% (n= 56) specifically mentioning that it was located below the fascia. Around 8% (n = 35) of the cases had sufficient information to identify a case of migration to locations such as the axilla or the pulmonary artery. Ten migrations occurred in cases where the duration of use was between 3 and 5 years, and one migration occurred with usage exceeding 5 years. In cases where there was an incorrect duration of use exceeding 5 years, in 82.4% (n = 28) of them, the implant was found deeply located. Regarding the ICSRs of migration/implant deeply located, 57.8% (n =104) mentioned complications associated with the implant removal.

Conclusions: Our results provide a general overview of adverse events associated with the etonogestrel implant. Despite inherent limitations in our study, it appears that this issue, although recognized, remains current. Further studies are needed to understand both the effectiveness of the additional risk minimization measures implemented and the potential need for new ones.

- [1] MSD (2020), Implanon NXT® - implante para via subcutânea, etonogestrel 68 mg, Paço de Arcos, pp. 1-3.
https://extranet.infarmed.pt/web/fl/matedu/Implanon%20NXT/DHPC_IMPLANON_NXT_versa_o_final_18_12_2019.pdf (acedido 15/12/2023)

PP048. Drug-drug interactions as a public health problem: a retrospective study of adverse drug reaction reports submitted to the national portuguese pharmacovigilance system

Gouveia M¹, **Mendes Fernandes J²**, Queiroz S², Silva M²

¹*Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal*, ²*Infarmed, Lisbon, Portugal*

Introduction: According to the World Health Organisation, above 50% of patient harm is preventable, with half of this harm being attributed to medications. [1] Drug-drug interactions (DDIs) are an important cause of adverse drug reactions (ADRs), which can have a significant impact at the public health level. Our study aims to analyze Individual Case Safety Reports (ICSRs) submitted to the National Portuguese Pharmacovigilance System and identify ADRs that may result from DDIs.

Methods: Retrospective study which analyzed ICSRs received by the Portuguese National Pharmacovigilance System in January 2023. ICSRs with more than one drug (classified as either suspect or concomitant) were selected. In order to identify potential DDIs, the Summary of Product Characteristics for each drug was consulted, as well as the UptoDate database. It was assessed whether the clinical implications of DDIs aligned with the provided description of ADRs in each ICSR.

Results: Our research retrieved a total of 727 ICSRs of which 307 contained more than one drug involved. Almost half of the ICSRs of interest, 44.6% (n= 137), were related to potential drug interactions. On the other hand, 7.2% (n = 22) of the ICSRs contained ADRs that have been described as resulting from a DDI. Approximately 32% (n =7) of the DDIs-related ICSRs were considered serious, 9% (n= 2) of which resulted in hospitalization. Only 1 DDI-related ICSR contained coding associated to drug interaction. Most of the DDIs identified are due to additive effects of pharmacological class or similar indications involving central nervous system depressants, immunosuppressive medications, hypotensive agents, anti-inflammatory drugs, and anticoagulants for example. Other interactions such as cytochrome inhibition have been identified, resulting in increased drug exposure.

Conclusions: Our study highlights the importance that ADRs resulting from DDIs have in Public Health. Healthcare professionals face an important challenge with the increasing prevalence of polypharmacy, particularly in aging populations with multiple comorbidities which accentuates the importance of understanding and managing these interactions and avoiding placing an additional burden on healthcare systems and resources.

- [1] World Health Organisation - Patient Safety Key Facts, 11 Sept, 2023 - Hodkinson, A., Tyler, N., Ashcroft, D.M. et al. Preventable medication harm across health care settings: a systematic review and meta-analysis. BMC Med 18, 313 (2020). <https://doi.org/10.1186/s12916-020-01774-9>

PP049. Cardioprotective effect of oleoylethanolamide in an animal model of early onset obesity

Sepe C¹, Eramo B¹, Friuli M¹, Zahid N¹, Giudetti A², Vari F², Stanca E², Damato M², Tacconi S³, Siculella L², Vergara D², Romano A¹, Gaetani S¹

¹*Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy,* ²*Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy,* ³*CarMeN Laboratory, INSERM 1060-INRAE 1397, University of Lyon, Lyon-Sud Hospital, Lyon, France*

The consumption of high-fat diet (HFD) is associated to obesity, hyperglycemia, myocardial hypertrophy, and fibrosis, leading to metabolic disturbances and cardiac remodeling. Although cardiac alterations have been observed after a prolonged period of obesity induction, how heart morphological and metabolic changes may occur at the onset of obesity remains unclear. The early identification of pathological changes might prevent/delay the establishment of chronic obesity and allow a timelier treatment.

PPAR-alpha ligands such as fibrates exert cardioprotective effects associated with an increased gene expression of PPAR- and its target metabolic genes promoting fatty acids oxidation. The anorexigenic compound oleoylethanolamide (OEA) is the most potent endogenous activator of PPAR-alpha explored so far, able to regulate lipid metabolism and body energy homeostasis [1]. Moreover, as a drug, OEA ameliorate hepatic fibrosis by inhibiting collagen deposition [2]. Whether OEA might induce protective effects also at cardiac level remains unexplored. Therefore, in the present study, by using an animal model of early-onset obesity as model of acquired heart changes we investigated the potential cardioprotective effect of OEA treatment on morphological and metabolic alterations.

We developed an animal model of early-onset obesity by exposing 40 young male Wistar rats to a high fat diet (HFD) only for 7 weeks; control rats received a low-fat diet. After the induction of the phenotype, both groups were treated daily for 2 weeks either with vehicle (saline/polyethylene glycol/ Tween 80; 90/5/5, v/v/v) or with OEA at the dose of 10 mg/kg, intraperitoneally. Food intake and body weight were monitored daily. At the end of the treatment rats were sacrificed and cardiac tissues were collected and processed for different analyses including morphological changes, lipid content and insulin signalling, by using PCR, histological and western blot techniques.

Our results show that 7 weeks of HFD feeding is sufficient to induce morphological and metabolic changes at heart level. We observed an increase of hypertrophy and atrophy parameters and a significant activation of heart insulin signalling. Such alterations are restored by the pharmacological treatment with OEA, which is also able to reduce food intake and body weight gain.

Our results demonstrate that early onset obesity is associated to early metabolic/structural changes at heart level that can be corrected by the sub-chronic pharmacological treatment with OEA.

[1] Fu J., et al. *Neuropharmacology*. 2005. DOI: 10.1016/j.neuropharm.2005.02.013

[2] Chen L., et al. *Oncotarget*. 2015. Doi: 10.18632/oncotarget.6466

PP050. Analysis of the suspected adverse drug reaction reports of drugs used for the treatment of systemic infections in a period of 10 years (2011 – 2020)

Modun D¹, Grgurević D¹, Bukic J¹, Leskur D¹, Seselja Perisin A¹, Rusic D¹

¹*Department of Pharmacy, University of Split School of Medicine, Split, Croatia*

Introduction: Antibiotics have significantly improved the treatment outcomes for various bacterial infections in humans. However, they are overused today, even when signs of illness are not due to a bacterial infection. In addition to being harmful to patients, the irrational use of antibiotics leads to the development of microbial resistance, which has become a major problem. The objective of this study was to investigate the reported adverse drug reactions (ADRs) associated with the use of Anti-infectives for systemic use, (ATC code J) received by the Agency for Medicinal Products and Medical Devices of Croatia (HALMED) from January 1, 2011, to December 31, 2020.

Methods: The data source was VigiBase, the unique global database of the World Health Organization (WHO). The following data were analyzed from suspected adverse drug reaction reports: age and sex of patients, drugs with the highest number of reports, severity of adverse reactions, adverse reaction reporters, most common adverse reactions, and adverse drug reaction classification according to MedDRA system organ classification.

Results: In the observed period of 10 years, a total of 6441 reports of suspected adverse drug reactions (ADRs) were received. People for whom suspected ADRs were reported were female in 57 % of cases, and the largest number of patients were 18 to 44 years old (21.7 %). The most frequent reporters were physicians (55.8 %), followed by pharmacists (26.2 %), and the rest were patients/drug users and other health professionals. 31.3 % of the total reported suspected ADRs were described as serious ADRs, and 64.8 % were not described as serious ADRs. According to the MedDRA classification of adverse drug reactions by organ system, most of the reports of suspected ADRs were recorded in group VIII, General disorders and administration site conditions (40.4 %), followed by group XXIV, Skin and subcutaneous tissue disorders (29 %), and in group VII, Gastrointestinal disorders (28.8 %). The greatest number of ADRs were reported for the drug amoxicillin/clavulanic acid (10.8 %).

Conclusion: Most reports of suspected adverse drug reactions were received from female patients. The reporters were mostly physicians and then pharmacists. Reports of suspected ADRs were mostly related to the group of general disorders and reactions at the site of application, skin and subcutaneous tissue disorders, and gastrointestinal disorders. The drug with the most reports of suspected ADRs was amoxicillin/clavulanic acid.

PP051. CYLD acts as an oncogene in a cellular model of Chronic Lymphocytic Leukemia

Gerousi M², Gavriilidis G², Keisaris S², Kourouni A², Orfanou A², Iatrou A², Pseftogkas A^{3,4}, Mosialos G⁵, Theodosiou E², Chatzidimitriou A², Psomopoulos F², Ghia P^{3,4}, Stamatopoulos K², **Xanthopoulos K¹**

¹School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece, ²Institute of Applied Biosciences, Centre for Research and Technology Hellas, Thessaloniki, Greece, ³Vita-Salute San Raffaele University, Milan, Italy, ⁴Division of Experimental Oncology, B cell neoplasia, IRCCS Ospedale San Raffaele, Milan, Italy, ⁵School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

Introduction: The cylindromatosis protein (CYLD), is a functional deubiquitinase involved in the regulation of critical signaling pathways, including NF-κB. CYLD mutations have been associated with several forms of tumors, including multiple myeloma, however little is known about its involvement in chronic lymphocytic leukemia (CLL). In CLL, reduced expression of CYLD has been correlated with worse clinical prognosis, in line with its postulated role as a tumor suppressor. To better understand the function of CYLD in CLL we used MEC1 cells, a widely used, CLL-derived cell line.

Methods: Cells were genetically engineered by CRISPR/Cas9, to generate stable CYLD-knockout (CYLDko) and control (CYLDwt) lines, which were further characterized at the molecular, transcriptomic and bioenergetic level.

Results: Phenotypic characterization of CYLDko versus CYLDwt MEC1 cells by flow cytometry showed significantly reduced viability (assessed by Annexin V), lower cell proliferation rate (assessed by Ki67 expression), increased apoptosis (determined by active caspase 3 expression) and reduced expression of CD86 and CD40. Western blotting revealed diminished expression of IKKβ, phospho-IκBα and phospho-p105 in CYLDko cells, suggesting down-regulation of the NF-κB pathway.

In agreement with these findings, transcriptome profiling by RNA-seq, showed increased apoptosis and decreased NF-κB signaling in CYLDko versus CYLDwt MEC1 cells. CYLDko cells also showed downregulation of calcium, BcR and PI3K/AKT/mTOR signaling pathways and, in contrast, upregulation of the glutathione pathway, which contributes to antioxidant defense and nutrient metabolism.

To evaluate the impact of CYLD deletion on CLL bioenergetics, Seahorse XF Real-Time ATP Rate assay were performed, to assess ATP production rate. CYLDko MEC1 cells exhibited impaired ATP production, associated with a shift towards utilization of the glycolytic pathway for energy production, compared to control cells which mostly relied on oxidative phosphorylation.

Finally, we explored whether CYLD knockout might impact MEC1 sensitivity to the BTK inhibitor ibrutinib and the BCL2 inhibitor venetoclax, finding that CYLDko MEC1 cells presented increased apoptosis compared to their CYLDwt counterparts, when cultured in the presence of either drug.

Discussion: Our data indicate that CYLD can also act as an oncogene, at least in the context of the MEC1 CLL model, since its elimination leads to lower proliferation and increased apoptosis rates coupled with diminished signaling capacity. Concomitantly, we discovered that CYLD downregulation leads to metabolic rewiring and augmented sensitivity to CLL therapeutic agents. Further studies are required to understand how these findings are translated in patients with CLL and the potential for pharmacological targeting of CYLD in CLL.

PP052. The effect of fluoride type on the formation of KOH-soluble fluoride on calcium pretreated tooth enamel

Kullashi Spahija F¹, Sutej I¹, Basic K¹, Spahija K², **Peros K¹**

¹*University of Zagreb School of Dental Medicine, Zagreb, Croatia*, ²*Dental Polyclinic Center, Peja, Kosova*

Introduction: The use of a calcium pretreatment is able to enhance fluoride reactivity with dental substrates as enamel and dentine, when used prior to sodium fluoride topical treatment [1]. The aim of this study was to evaluate the contribution of type of fluoride in preparation for topical treatment, on the enamel uptake of alkali-soluble (KOH-soluble) fluoride in combination with pretreatment with calcium lactate.

Methods: All experimental procedures were conducted in accordance with the Declaration of Helsinki's recommendations guiding physicians in biomedical research. This blind and randomized in vitro study was approved by The Ethics Committee of the School of Dental Medicine University of Zagreb under the protocol number (05-PA-30-17-4/2023). Thirty non-carious human wisdom teeth, extracted for orthodontic reasons, were included in the study. The teeth were divided into three groups, one for each fluoride type preparation (sodium fluoride- NaF, amine fluoride -AF, monofluorophosphate- MFP group). Each tooth was cut into 4 enamel slabs and randomly allocated into one of the four following treatments subgroups (4x10): calcium lactate (150mM) followed by fluoride (500ppm); fluoride only; calcium lactate only; deionized water (negative control). Fluoride was extracted from enamel slabs using 1M KOH solution for 24h and under agitation of the shaker at the room temperature, by method of Caslavská. The extracts were analyzed using fluoride ion-specific electrode (Orion Research EA 940) by ISO 19448:2018 standard method. Wilcox matched pairs test and Friedman ANOVA were used to analyze the effect of substrate and treatments.

Results: Significantly greater enamel uptake of KOH-soluble fluoride was measured in group NaF pretreated with a calcium lactate solution, when compared to other tested substrates including calcium lactate or NaF alone (3.919 vs. 0.519 mcg F/cm², p < 0.05). No significant difference in enamel uptake of KOH-soluble fluoride was observed among calcium lactate and other fluoride substrates, AF or MFP (0.897 vs. 0.479 mcg F/cm², 0.571 vs. 0.561 mcg F/cm²; respectively, p > 0.05).

Conclusion: The calcium lactate solution pretreatment of dental enamel followed by application of sodium fluoride treatment, improves the enamel uptake of alkali-soluble fluoride. The calcium lactate solution pretreatment of dental enamel has no effect on the enamel uptake of alkali-soluble fluoride when treated with amine fluoride or monofluorophosphate.

- [1] da Silva Spinola M, Tenuta LMA. Calcium pretreatment enhances fluoride reactivity with enamel and dentine. Arch Oral Biol. 2022; 134:105338.

PP053. Hydrogen sulfide (H₂S) dysfunction in Metabolic Syndrome-Associated Vascular Complications involves cGMP regulation through soluble Guanylyl Cyclase persulfidation

Smimmo M¹, Casale V¹, Mitidieri E¹, d'Emmanuele di Villa Bianca R¹, Bello I¹, Panza E¹, Montanaro R², Brancaleone V², Indolfi C³, Cirino G¹, Bucci M¹, Vellecco V¹

¹*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*

²*Department of Science, University of Potenza, Basilicata, Italy*, ³*Department of Molecular Medicine and Medical Biotechnology, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*

Introduction: H₂S is a gasotransmitter endogenously produced within the body by the action of three enzymes: cystathione-γ lyase (CSE), cystathione-β synthase (CBS), and 3-mercaptoproprylate sulfurtransferase (3-MST) [1]. H₂S contributes to vascular homeostasis and its impairment has been demonstrated in several cardiovascular diseases [2].

This study evaluates the possible beneficial effect of Erucin, a natural H₂S donor, in Metabolic Syndrome (MetS)-associated vascular complications.

Methods: In vivo studies were performed on db/db mice (n=6), a genetic model of MetS, and their littermates (WT). Animals were treated with Erucin (3mg/kg) for 4 weeks. At 10 weeks of age, mice were sacrificed, and aortas were harvested and used for ex vivo and molecular studies.

In vitro experiments were performed on Chinese Hamster Ovary cells overexpressing subunit α1β1 of soluble Guanylyl Cyclase (sGC). Cells were treated or not with Erucin (1μM) for 2h and then the persulfidation levels were assessed.

Statistical analysis was evaluated using one or two-way ANOVA.

Results: Ex vivo experiments showed a downregulation of CBS and CSE expression in aorta of db/db mice compared to WT. This event was coupled with a diminished L-cysteine-induced vasorelaxation (EC50 0.0004M vs. 0.001M; ***p<0.001) suggesting a defective H₂S pathway. db/db mice also displayed an impaired vascular function since acetylcholine (Ach)- and isoprenaline (Iso)- induced vasorelaxation resulted strongly impaired (EC50 Ach 310nM vs. 78nM; EC50 Iso 6,3μM vs. 0,93μM; ***p<0.001). The increase in tension due to L-NIO addition (selective eNOS inhibitor) on PE-induced stable tone, was significantly reduced in db/db mice compared to WT (39,0 ± 10,3 vs. 367,8 ± 49,9 dñe/mg, ***p<0.001) indicating a reduced NO basal content.

In vivo treatment with Erucin ameliorates the Ach-induced vasorelaxation (EC50 Ach 49nM vs. 310nM; ○○○p<0.001) without affecting Iso-induced vasorelaxation and L-NIO-induced contraction, highlighting a specific action on smooth muscle component rather than the endothelium. The molecular mechanism underlying Erucin's beneficial effect involves sGC persulfidation leading to an increased cGMP production.

Conclusions: Our study demonstrates an impairment of H₂S pathway in MetS-associated vascular complications. Moreover, by taking advantage from Erucin, our study suggests a potential use of H₂S donors, as a promising alternative/additive approach, in the complex management of MetS therapy.

[1] Cirino G, Szabo C, Papapetropoulos A. Physiological roles of hydrogen sulfide in mammalian cells, tissues, and organs. *Physiol Rev.* 2023 Jan 1;103(1):31-276.

[2] Cirino G, Vellecco V, Bucci M. Nitric oxide and hydrogen sulfide: the gasotransmitter paradigm of the vascular system. *Br J Pharmacol.* 2017 Nov;174(22):4021-4031.

PP054. Pharmacogenetics of atorvastatin - the role of CYP3A4, CYP3A5 and SLCO1B1 gene variants in the development of myotoxicity and statin therapy switching

Ganoci L¹, Palić J², Šimičević L^{1,2}, Karačić E³, Leskovar D⁴, Pećin I⁴, Mucalo I⁵, Božina N⁶, Božina T²

¹*Division for Pharmacogenomics and Therapy Individualization, Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia*, ²*Department of Medical Chemistry, Biochemistry and Clinical Chemistry, School of Medicine University of Zagreb, Zagreb, Croatia*,

³*Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*, ⁴*Division for Metabolic Diseases, Department of Internal Medicine, University Hospital Centre Zagreb, School of Medicine University of Zagreb, Zagreb, Croatia*, ⁵*Centre for Applied Pharmacy, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*, ⁶*Department of Pharmacology, School of Medicine University of Zagreb, Zagreb, Croatia*

Introduction: Atorvastatin is metabolised by CYP3A4/5 enzymes, and is a substrate of SLCO1B1 and ABCG2 transporters. ABCG2 c.421C>A and SLCO1B1 c.521T>C polymorphisms are associated with a reduced transporter function and higher exposure to statins, and SLCO1B1 increased function variants with lower exposure to some statins. Pharmacogenetic guidelines recommend testing of SLCO1B1 c.521T>C variant for genotype-based atorvastatin dosing. We analysed ABCG2, CYP3A4, CYP3A5 and SLCO1B1 variants and phenotypes and atorvastatin-related adverse drug reactions (ADRs).

Methods: This case-control study recruited cardiovascular and metabolic disease patients of Caucasian origin treated with atorvastatin. Cases were subjects that developed myotoxicity, hepatotoxicity and other (allergic reactions, diarrhoea, headache, insomnia, memory problems, subjective statin intolerance, weakness) atorvastatin-related ADRs, and control subjects were patients free of ADRs. All patients were genotyped for ABCG2 c.421C>A, SLCO1B1 c.388A>G, c.463C>A, c.521T>C and c.1929A>C, CYP3A4*22 and CYP3A5*3 was by TaqMan real-time PCR. Drug-drug interactions (DDIs) were analysed by Lexicomp®. These are data from the "Pharmacogenomics in prediction of cardiovascular drugs adverse reaction – PGx CardioDrug" project funded by the Croatian Science Foundation.

Results: A total of 413 subjects (age M=63 yrs. (18-96); f=185, m=228), cases (n=90) and controls (n=323) were recruited. Developed ADRs or statin therapy switching: myotoxicity n=59 (14.3%), hepatotoxicity n=18 (4.4%), other ADRs n=31 (7.5%), switching n=72 (17.4%). Genotype-phenotype distribution was for ABCG2 phenotype (function): normal n=322 (78.0%), decreased n=75 (18.2%), poor n=3 (0.8%); SLCO1B1 phenotype (function): normal n=138 (33.4%), decreased n=114 (27.6%), poor n=12 (2.9%), increased n=109 (26.4%), highly increased n=25 (6.1%); CYP3A cluster phenotype (CYP3A4*22 and CYP3A5*3 combination): extensive n=51 (12.4%), intermediate n=330 (79.9%), poor n=32 (7.7%) metabolizers.

SLCO1B1 c.521TCC variant carriers had 3.1-times greater odds (OR=3.09, 95%CI: 1.01 9.44; $\chi^2=4.297$, p=0.038) for developing atorvastatin-related ADRs, compared to non-carriers. The ABCG2 c.421C>A variant was not associated with atorvastatin-related ADRs.

CYP3A4*22 variant carriers had greater odds of developing myotoxicity (OR=2.27, 95%CI:1.01 5.13; $\chi^2=4.08$, p=0.043), compared to non-carriers. CYP3A poor metabolisers had greater odds for developing myotoxicity (OR=2.59, 95%CI:1.13 4.66; $\chi^2=5.92$, p=0.020) or switching of statin therapy and developing myotoxicity (OR=2.19, 95%CI:1.03 4.66; $\chi^2=4.29$, p=0.038), compared to normal/intermediate metabolisers.

Conclusions: In this study, the CYP3A4*22 variant was associated with greater odds of developing atorvastatin-related myotoxicity, and CYP3A poor metabolisers were more likely to experience statin therapy switching and myotoxicity. The SLCO1B1 c.521T>C polymorphism was associated with a higher risk of developing atorvastatin-related myotoxicity. This study also provides information on SLCO1B1 increased function variant and haplotype frequencies in the Caucasian population.

PP055. Osimertinib Intensive Monitoring: Hospital Study in Patients with Non-Small Cell Lung Cancer (NSCLC) EGFR positive – Preliminary Analysis

Jordão I^{1,2}, Figueiredo A¹, Parente F¹, Barata F¹

¹*Centro Hospitalar Universitário De Coimbra, Coimbra, Portugal*, ²*Faculty of Medicine of University of Coimbra, Coimbra, Portugal*

Introduction: Osimertinib is a potent oral and irreversible 3rd generation epidermal growth factor receptor tyrosine kinase inhibitor that targets EGFR mutations. Considering its efficacy, it has been recommended as first-line treatment for adult patients with locally advanced or metastatic NSCLC with activating mutations of the epidermal growth factor receptor (EGFR).[1] But their safety and tolerability profiles still require careful evaluation. The systematic collection and analysis of adverse events (AE) plays a crucial role in understanding safety profile. Baseline patient characteristics and clinical information provide important context for evaluating potential influencing factors[2].

Methods: We carried out a retrospective evaluation of all patients undergoing treatment with Osimertinib in the Pulmonary Oncology department at the Centro Hospitalar Universitário de Coimbra. Preliminary data for analysis include demographic data, concomitant medication, neoplasm staging based on TNM and biomarkers, lifestyle habits and survival at 18 months.

Results: The study included a cohort of 66 patients with NSCLC, with a mean age of 70.5 ± 12.0 years (36-87 years), 68.2% were female. TNM staging analysis revealed advanced tumor progression, with Stage IV being the most prevalent (97%). The EGFR mutation in exon 19 was found in 56%, in exon 21 in 42.5% and in exon 20 in 1.5%. PDL1 status was 24.4% strong positive PDL1 (TPS > 50%), 21.2% positive PD-L1 (TPS 1-49%) and the remain negative. Concomitant medications were classified by the ATC system, with the most prevalent groups being related to the cardiovascular system, alimentary tract and metabolism and nervous system, comprising 26, 22 and 16 medications, respectively. Forty-three patients were identified as non-smokers, four as smokers, one as a passive smoker and 15 as ex-smokers. Medical history identified 23 distinct medical conditions. The most prevalent were hypertension, dyslipidemia and depressive disorders affecting 28.8%, 16.7% and 11.1% of patients, respectively. The survival rate at 18 months was 75.8% (95% IC 70-81).

Conclusion: This analysis provides valuable information about baseline characteristics, clinical information, and lifestyle factors of NSCLC patients receiving Osimertinib therapy. These findings contribute to the understanding of AEs and possible factors that affect treatment outcomes.

[1] Malik PS, et al. Safety of osimertinib in adult patients with metastatic epidermal growth factor receptor T790M mutation-positive non-small cell lung cancer: Results from a Phase IV study in India. Indian J Cancer. 2022 Mar;59:S1-S10.

[2] King-Kallimanis BL, et al. Timing is everything: The importance of patient-reported outcome assessment frequency when characterizing symptomatic adverse events. Clin Trials. 2022 Jun;19(3):267-273.

PP056. Relationship of H2S Responses with the Heme Oxygenase Pathway in Mouse Corpus Cavernosum

Shamamedov K¹, Alan-Albayrak E¹, Catakli D², Ozsarlak-Sozer G¹

¹Ege University, Izmir, Turkey, ²Suleyman Demirel University, Faculty of Medicine, Department of Medical Pharmacology, Isparta, Turkey

Introduction: Erectile dysfunction is considered as an early symptom of cardiovascular diseases such as hypertension and diabetes and there is a strong connection between erectile dysfunction and endothelial dysfunction. NO, H2S and CO are the main gasotransmitters in endothelial function. Although H2S and CO are widely studied in corpus cavernosum, the interaction between H2S and CO pathways remain to be elucidated. The aim of this study is to investigate the effect of heme oxygenase pathway which is important for CO production in H2S responses in Mouse corpus cavernosum.

Methods: In order to investigate the relationship between CO and H2S, cumulative l-cysteine responses were obtained in the presence of CrMP which is an HO-1 inhibitor in mouse corpus cavernosum tissues. The mouse corpus cavernosum were divided into 2 strips. One of the strips was incubated with 10 mM CrMP for 30 minutes and the second strip served as control. Cumulative l-cysteine (10-6-3.10-4 M) responses was obtained in Mouse corpus cavernosum strips precontracted with phenylephrine (3.10-6-10-5-3.10-5-10-4 M). In the second experimental protocol, the tissues were homogenized and methylene blue assay was performed in the presence and absence of CORM-2 (CO donor) and AOAA (H2S inhibitor).

Results: HO-1 inhibitor, CrMP decreased the l-cysteine relaxation significantly compared to control group ($p<0.01$). In methylene blue assay, H2S levels were higher when the tissues were incubated with CORM-2 ($p<0.01$) and AOAA decreased this elevation ($p<0.001$).

Conclusion: These results suggest a significant role for CO and H2S interaction in erectile function.

This study is supported by TUBİTAK 1002 project (123S228).

PP057. Antioxidant effect of empagliflozin in diabetic rats

Kizrak U¹, **Shamamedov K¹**, Alan-Albayrak E¹, Ozsarlak-Sozer G¹, Cetinkalp S², Cilaker-Micili S³, Ural C⁴, Cavdar Z⁴, Kerry Z¹

¹Ege University Faculty of Pharmacy Department of Pharmacology, Izmir, Turkey, ²Ege University, Faculty of Medicine, Department of Endocrinology and Metabolic Diseases, Izmir, Turkey, ³Dokuz Eylul University, Faculty of Medicine, Department of Histology and Embryology, Izmir, Turkey, ⁴Dokuz Eylul University, Faculty of Medicine, Department of Molecular Medicine, Izmir, Turkey

Introduction: Sodium glucose cotransporter 2 inhibitors (SGLT2i) are a group of antihyperglycemic drugs that reduce blood glucose levels by inhibiting glucose reabsorption from the kidneys in the treatment of T2DM. However, the reason why SGLT2i's are more prominent in diabetes, cardiology and nephrology guidelines than their antihyperglycemic activity is that they are cardioprotective, especially by significantly reducing the risk of hospitalization and death due to heart failure. They also have a nephroprotective effect by reducing the rate of decline in eGFR and albuminuria. There is an increasing number of studies showing its positive effects on fatty liver and fibrosis. In our study, we aimed to demonstrate an explanatory mechanism for the positive effects of empagliflozin (EMPA), an SGLT2i, on the aorta-heart-kidney and liver in diabetic rats.

Methods: Rats (n=30) were divided into 3 groups: control (n=10), diabetic (n=10) and diabetes+EMPA treatment (n=10). The type 2 diabetes model was established by feeding a high-fat diet (HFD, 35% kcal) for 4 weeks followed by low-dose streptozotocin (STZ) (35 mg/kg, i.p.). Empagliflozin (10 mg/kg/day) was administered to the treatment group via drinking water for 12 weeks. Blood glucose levels of the rats were measured weekly during the experiment (18 weeks). Reactive oxygen species were measured by chemiluminescence method in aorta, heart, kidney and liver tissues taken from rats of all groups after sacrifice. In this method, while superoxide radical (O₂⁻) is detected by lucigenin-mediated measurement, hydroxyl (OH²), hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl) and hydroperoxyl (HO₂) radicals can be detected by luminol-mediated measurement.

Results: Blood glucose level ($E_{max}=252.1\pm6.970$, p<0.001) increased in diabetic rats and EMPA ($E_{max}=116\pm3.741$, p<0.001) reduced this increase. It was determined that in all tissues, both superoxide (O₂⁻) and other ROS increased statistically significantly in the diabetes group compared to the control group. Although the results of luminol and lucigenin vary from tissue to tissue, it has been demonstrated that EMPA treatment reduces reactive oxygen species levels in all tissues in a statistically significant manner (p<0.001).

Conclusion: The increase in reactive oxygen species (ROS) plays a critical role in the emergence/development of macro and micro complications in diabetes. Our results indicate that SGLT2i treatment causes a decrease in the levels of reactive oxygen species in the aorta, heart, kidney and liver tissues, which may be a mechanism explaining its protective effects in preventing advanced complications of diabetes such as vasculopathy, cardiomyopathy, nephropathy and liver diseases.

PP058. CDK9 regulation as a novel pharmacologic approach in sepsis: the role of endothelium

Marzani E¹, Ferreira Alves G², Aimaretti E³, Porchietto E², Einaudi G², Collotta D¹, Mastrocola R³, Aragno M³, Cifani C², Spampinato S⁴, Collino M¹

¹Department of Neuroscience “Rita Levi Montalcini”, University of Turin, Turin, Italy, ²School of Pharmacy, University of Camerino, Camerino, Italy, ³Department of Clinical and Biological Science, University of Turin, Turin, Italy, ⁴Department of Drug Science and Technology, University of Turin, Turin, Italy

Background: Sepsis is a health condition displaying immune system overstimulation and hyperinflammatory state, causing tissues' damages, including impairments of endothelial barrier in the periphery as well as in the central nervous system's integrity. Endothelial disruption contributes to sepsis-associated cardiovascular and neurological dysfunctions [1], and recent findings have demonstrated a pivotal role of cyclin-dependent kinase 9 (CDK9) in promoting vascular dysfunction [2]. Since CDK9 pharmacological modulation on endothelial and sepsis-related cardiovascular injuries have never been investigated, we tested the CDK9 selective inhibitor LDC000067 to prevent sepsis-related cardiovascular and blood brain barrier dysregulation and neuroinflammation in vitro and in vivo.

Methods: In vivo, sepsis was induced by cecal ligation and puncture (CLP) in C57BL/60laHsd mice (n=26). One hour after the CLP/Sham procedure, animals were randomly assigned to receive once either LDC000067 (50mg/kg in DMSO, n=10) or vehicle (n=10), intravenously. Organs were collected at 24h. In vitro, sepsis was induced with a single dose LPS 500ng/ml in DMSO after 6 hours of FBS-deprivation on Human Brain MicroVascular Endothelial Cells (TY-10, grown in MCDB-131 media), in presence or absence of LDC000067 (1-10uM in DMSO). Statistical significance ($p<0.05$) was determined using one-way ANOVA followed by Bonferroni's post-hoc test. Data was expressed as mean±SEM.

Results: Experimental sepsis in vivo evoked massive increases in systemic concentrations of markers of multiple organ dysfunction when compared to Sham-operated animals (AST and LDH arose from $11,095\pm1,066$ U/L and $19,417\pm2,482$ U/L to $97,028\pm11,470$ U/L and $84,634\pm7,570$ U/L respectively). Notably, LDC000067 administration resulted in statistically significant reduction of their blood levels, drastically reduced in presence of CDK9 inhibitor (AST: $44,743\pm5,198$ U/L; LDH: $63,645\pm6,844$ U/L). Sepsis-induced cytokines secretion was counteracted by LDC000067 treatment with CCL-2, IL-6 and IFN- γ displaying the most remarkable improvement (94,08%, 70,56% and 63,48%).

Similarly, LDC000067's anti-inflammatory effects were recorded in vitro. Although CDK9 inhibition did not modify MTT cell viability, western blot analysis showed that LPS-induced overexpression of ICAM-1 adhesion molecule and NLRP3 inflammasome complex were halved by the pharmacological inhibition of CDK9.

Conclusions: Pharmacologically targeting CDK9 may represent an approach to counteract sepsis-induced cardiovascular dysfunction, by blunting the hyperinflammatory response and endothelial dysfunction.

[1] Pan S et al. Sepsis-Induced Brain Dysfunction: Pathogenesis, Diagnosis, and Treatment. *Oxid Med Cell Longev*. 2022 Aug 24;2022:1328729. doi: 10.1155/2022/1328729. PMID: 36062193; PMCID: PMC9433216.

[2] Sundar V, Vimal S, Sai Mithlesh MS, Dutta A, Tamizhselvi R, Manickam V. Transcriptional cyclin-dependent kinases as the mediators of inflammation-a review. *Gene*. 2021 Feb 15;769:145200. doi: 10.1016/j.gene.2020.145200. Epub 2020 Oct 5. PMID: 33031895.

PP059. Treatment of invasive infections caused by *Morganella morganii*

Zivkovic Zaric R¹, Zaric M, Canovic P, Stojadinovic M, Pejcic A, Milosavljevic M, Milovanovic J, Milovanovic D, Djordjevic N, Kostic M, Ruzic Zecevic D, Jankovic S

¹*University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia*

Introduction: There are two subspecies of the facultative anaerobic, gram-negative *Morganella morganii*: *morganii* and *sibonii*. It was once categorized as *Proteus morganii* and is a member of the commensal microbiota in the human stomach. However, it can occasionally result in a potentially fatal systemic infection, particularly in the nosocomial and postoperative environments, in patients with compromised immune systems, and in young children. (1,2)

Our study will identify the types of invasive illnesses in humans that were brought on by *Morganella morganii* and will calculate the results of treating those infections with antibiotics.

Material and method: Prior to the start of the study, this systematic review (registration number CRD42020171919) was registered at the PROSPERO database of systematic reviews and meta-analyses. Including criteria: patients of both sexes and any age carrying only *Morganella morganii* in bodily fluids or tissues. From there, it was isolated and identified using one or more of the following diagnostic techniques: more advanced techniques like Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) and species-specific PCR for *M. morganii*, as well as conventional techniques like colony morphology, Vitek 2, API, or BD Phoenix biochemical systems.

We looked for case reports and case series involving invasive infections caused by *M. morganii* in MEDLINE, EBSCO, SCOPUS, SCINDEX, and Google Scholar.

Results: Patients of any age can develop severe tissue infections from *M. morganii*. The majority of the isolates were amikacin, imipenem, and ceftazidime susceptible. The majority of patients recovered fully from antibiotic therapy; 15% of the patients passed away in spite of the treatment. The antibiotic that was most commonly used to treat *M. morganii* infections was gentamicin.

In conclusion, because of the high rate of death and significant potential for antibiotic resistance, doctors should be aware of *M. morganii* invasive infections, particularly in hospital settings. Gentamycin combined with third generation cephalosporin or another antibiotic that *M. morganii* is tolerant should be used to treat *M. morganii* infections (after testing isolates for third cephalosporin generation for the development of AmpC β-lactamases).

[1] Liu H, Zhu J, Hu Q, Rao X. *Morganella morganii*, a non-negligent opportunistic pathogen. Int. J. Infect. Dis. 2016;50:10–7.

[2] Bandy A. Ringingbells: *Morganella morganii* fights for recognition. Publ. Health 2020;182:45–50

PP060. Pharmacological targeting of RSK as innovative strategy to improve sepsis-induced injury

Rubeo C¹, Ferreira Alves G^{2,3}, Aimaretti E¹, Einaudi G³, Porchietto E³, Collotta D², Marzani E², Mastrocòla R¹, Pagliaro P¹, Aragno M¹, Cifani C³, Collino M²

¹Department of Clinical and Biological Sciences, University of Turin, Turin, Italy, ²Department of Neurosciences "Rita Levi Montalcini", University of Turin, Turin, Italy, ³Pharmacology Unit, School of Pharmacy, University of Camerino, Camerino, Italy

90kDa ribosomal S6 kinase (RSK) is a downstream effector of the mitogen-activated protein kinase (MAPK) pathway. Inappropriate regulation of this pathway is linked to a variety of disorders, including inflammatory diseases. Moreover, aberrant activation of RSK plays a role in the pathogenesis of cardiovascular disorders, such as ischemia/reperfusion injury [1]. Despite sepsis-associated myocardial depression is similar to the response of myocardium to ischemia, so far no experimental data are available on the impact of the pharmacological inhibition of RSK in sepsis.

Male C57BL/6J mice aged 3 months were subjected to cecal ligation and puncture (CLP) to induce sepsis or sham procedure (sham, n=9). 1 h after surgery, they were injected intravenously with 10 mg/kg of the RSK inhibitor BI-D1870 [2] diluted with 80% saline, 10% dimethylacetamide and 10% Cremophor ® RH 40 (CLP+ BI-D1870, n=10) or vehicle (CLP, n=10). 4 h later, they received an additional dose of 5 mg/kg [3]. 24h after surgery, clinical score, temperature and body composition were monitored. The animals were then anesthetized by isoflurane and sacrificed by cardiac exanguination. Plasma and organs were collected for further analyses. Biomarkers of organ damage and inflammatory cytokines were quantified in plasma.

BI-D1870 markedly improved the clinical score of septic mice, which was associated with significant protection against sepsis-induced increase in body temperature (sham=35.63±0.37°C, CLP=30.22±1.45°C, p<0.01 vs sham; CLP+BI-D1870=34.97±0.35°C, p<0.01 vs CLP) and systemic free fluids. Treatment with BI-D1870 improved systemic inflammation, as demonstrated by the levels of pro-inflammatory cytokines, such as IL-1 β (sham=4.75±0.21 pg/mL, CLP=57.74±11.50 pg/mL, p<0.001 vs sham; CLP+BI-D1870=29.92±5.74 pg/mL, p<0.05 vs CLP) and TNF- α (sham=100.5±2.50 pg/mL, CLP=287.5±42.81 pg/mL, p<0.001 vs sham; CLP+BI-D1870=170.3±28.24 pg/mL, p<0.05 vs CLP) among others.

Overall, these results suggest RSK inhibition as a promising pharmacological strategy for counteracting sepsis-induced injury. Ongoing experiments are focusing on the potential impact of RSK inhibition on sepsis-induced myocardial dysfunction.

- [1] Shi X, O'Neill MM, MacDonnell S, Brookes PS, Yan C, Berk BC. The RSK Inhibitor BIX02565 Limits Cardiac Ischemia/Reperfusion Injury. *J Cardiovasc Pharmacol Ther.* 2016;21(2):177-186. doi:10.1177/1074248415591700
- [2] Yang ZH, Wu XN, He P, et al. A Non-canonical PDK1-RSK Signal Diminishes Pro-caspase-8-Mediated Necroptosis Blockade. *Mol Cell.* 2020;80(2):296-310.e6. doi:10.1016/j.molcel.2020.09.004
- [3] Pambid MR, Berns R, Adomat HH, et al. Overcoming resistance to Sonic Hedgehog inhibition by targeting p90 ribosomal S6 kinase in pediatric medulloblastoma. *Pediatr Blood Cancer.* 2014;61(1):107-115. Doi:10.1002/pbc.24675

PP061. Effects of benzamides like naronapride on force of contraction in the isolated human atrium.

Neumann J¹, Hesse C, Hofmann B, Gergs U
¹*University Hospital, Halle (Saale), Germany*

We systematically studied 5-HT4-serotonin receptor agonists that are used to treat gastrointestinal diseases, namely, amisulpride, bromopride, cinitapride, clebopride, naronapride, S-sulpiride, sultopride and t(h)iapride. Chemically, they are benzamide derivatives with affinity for additional receptors besides the 5-HT4-serotonin receptor agonists. These benzamides have been studied in humans for the treatment of gastrointestinal diseases but some are currently only used in neurology like tiapride. Some of these drugs have been approved for use in humans and are on the market (e.g. tiapride, cinitapride) and some are still being studied to obtain regulatory approval (e.g. naronapride). These benzamides may also have affinity for cardiac ion channels like calcium channels or potassium channels. There is concern that some of these drugs also exert cardiovascular effects in humans, but no data are currently available on their inotropic effect in the isolated human atrium. Therefore, we studied the effects of these benzamides in isolated human electrically stimulated (1 Hz) right atrial preparations (obtained during cardiac surgery) under isometric conditions. In the presence of the phosphodiesterase III inhibitor cilostamide, we noted positive inotropic at 10 µM of bromopride, velusetrag, clebopride, naronapride and cinitapride (n=3, p<0.05). We did not measure any positive inotropic effects for up to 10 µM clebopride, sultopride or 10 µM S-sulpiride in the presence of 1 µM cilostamide. The positive inotropic effects of amisulpride, bromopride, cinitapride, naronapride, and tiapride were attenuated by the 5-HT4-serotonin receptor antagonist GR125487 (1 µM). We conclude that some of the studied 5-HT4-serotonin receptor agonists that are used or are studied in patients for non-cardiac diseases can in fact stimulate 5-HT4-serotonin receptor in the isolated human atria. This might predict that these benzamides can also stimulate 5-HT4-serotonin receptors in the sinus node of patients. This might lead to unintended atrial arrhythmias.

PP062. In vivo and in vitro empagliflozin pretreatment does not protect isolated rat cardiomyocytes for oxidative stress-induced damage

Graovac M¹, Mornar M¹, Matijević J¹, Boban M¹, Ljubković M¹, Marinović-Ljubković J¹

¹*University of Split School of Medicine, Split, Croatia*

Introduction: Sodium-glucose cotransporter-2 inhibitors (SGLT2i) initially emerged as a new class of oral antihyperglycemic drugs, however soon they also demonstrated dramatic cardiovascular benefits in diabetic and non-diabetic patients with heart failure [1]. Empagliflozin (EMPA) achieves its cardioprotective effect despite an absence of the SGLT2 on cardiomyocyte membranes, which indicates possible other sites of action of these drugs at the myocardial level [2]. Since oxidative stress plays an important role in acute and chronic myocardial damage during various pathological conditions, from acute myocardial infarction to chronic heart failure, the aim of this study was to investigate whether empagliflozin protects isolated ventricular cardiomyocytes from oxidative stress damage.

Methods: Male Sprague Dawley rats (n=18) consumed EMPA (30 mg/kg/day; dissolved in DMSO) for 7 days, via drinking water. The control group (n=17) was exposed to the equivalent amount of DMSO (0.7%) dissolved in water. Afterwards, the animals were sacrificed, and cardiomyocytes were isolated from their hearts by the usual enzymatic dissociation procedure [3]. Isolated cardiomyocytes were then exposed to oxidative stress (200µM H₂O₂ + 100µM FeSO₄) followed by assessment of cellular survival. Additionally, effects of in vitro empagliflozin exposure for 20 min prior to oxidative stress (1µM and 5 µM) on cardiomyocyte survival was assessed in additional set of experiments. The study was approved by the institutional Ethics committee.

Results: All animals completed 1-week treatment with no apparent side effects. Although there is clearly visible trend, no statistically significant difference in cell survival was found between in vivo EMPA and control rats (62.3±16.1 vs. 48.3±11.9). Additionally, in vitro EMPA pretreatment did not result in better survival rate than untreated cells isolated from the same rat (61.6±26.6 vs. 58.8±23.2 for 1µM; and 62.5±19.3 vs. 62.3±17.7 for 5µM empagliflozin pretreatment).

Conclusion: Neither 1-week empagliflozin in vivo exposure nor acute exposure of cells to empagliflozin did not protect rat cardiomyocytes from in vitro induced oxidative stress damage, suggesting that cardioprotective effects of empagliflozin are mediated by mechanisms other than antioxidant defense.

- [1] Joshi SS, Singh T, Newby DE, Singh J. Sodium-glucose co-transporter 2 inhibitor therapy: mechanisms of action in heart failure. Heart. 2021;107(13):1032-8.
- [2] Filippatos TD, Lontos A, Papakitsou I, Elisaf MS. SGLT2 inhibitors and cardioprotection: a matter of debate and multiple hypotheses. Postgrad Med. 2019;131(2):82-8.
- [3] Louch WE, Sheehan K, Wolska BM. Methods in cardiomyocyte isolation, culture, and gene transfer. J Mol Cell Cardiol. 2011;51(3):288-98.

PP063. Genetic variants of IREB2 are associated with responsiveness to inhaled corticosteroids in patients with chronic obstructive pulmonary disease

Ntenti C¹, Papakonstantinou E², Grize L³, Stoltz D³, Goulas A⁵

¹*First Laboratory of Pharmacology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*, ²*Clinic of Respiratory Medicine, University of Freiburg, Freiburg im Breisgau, Germany*, ³*Clinic of Respiratory Medicine and Pulmonary Cell Research, University Hospital Basel, Basel, Switzerland*, ⁴*Clinic of Respiratory Medicine and Pulmonary Cell Research, University Hospital Basel, Freiburg im Breisgau, Germany*, ⁵*First Laboratory of Pharmacology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Introduction: Inhaled corticosteroids (ICS) have proven efficacy in managing asthma, however, their impact on chronic obstructive pulmonary disease (COPD) varies widely among patients, leading to potential adverse effects and economic burden when improperly administered (1). Hence, there is critical need to comprehend factors that may influence ICS responsiveness in COPD to identify patient subsets that may derive greater benefit. Single nucleotide polymorphisms (SNPs) in various genes, including those coding for Iron Regulatory Protein 2 (IRP2; IREB2) have been associated with lung function characteristics in patients with COPD (2). The aim of this study was to explore the association between common SNPs in IREB2 with COPD progression and responsiveness to treatment with inhaled corticosteroids (ICS).

Methods: To that end, 165 patients, subjects of the HISTORIC study, an investigator-initiated and driven, double-blind, randomized, placebo-controlled trial (ISRCTN11017699), were genotyped with real-time PCR methods, for three IREB2 common SNPs (rs13180, rs2568494 and rs1062980). Associations of SNPs with clinical parameters and response to treatment with ICS were tested by using mixed linear regression models with IBM SPSS Statistics .

Results: Genotype distributions were consistent with the Hardy-Weinberg equilibrium, for all polymorphisms ($p>0.2$). At baseline, CC carriers of rs13180, GG carriers of the rs2568494 and CC carriers of the rs1062980, presented with significantly higher DLCO_SB% of predicted value ($p<0.05$) and significantly lower N2-single breath washout ($p<0.05$).

More importantly, the same genotypes were associated with apparently improved response to ICS as assessed by FEV1 change in one year, as well as clinically significant improvement in quality of life indices based on questionnaire assessments, albeit only that of rs2568494 GG carriers reached statistical significance (adjusted $p=0.028$).

Conclusions: Our results suggest that IREB2 polymorphisms could be promising pharmacogenetic markers, predictive of favorable response of COPD patients to therapy with ICS and warrant further investigation.

- [1] Lu C, Mao X. Risk of adverse reactions associated with inhaled corticosteroids for chronic obstructive pulmonary disease: A meta-analysis. *Medicine (Baltimore)*. 2024 Jan 19;103(3):e36609
- [2] Zhang Y, Qiu J, Zhang P, Zhang J, Jiang M, Ma Z. Genetic variants in FAM13A and IREB2 are associated with the susceptibility to COPD in a Chinese rural population: a case-control study. *Int J Chron Obstruct Pulmon Dis*. 2018;13:1735–45

PP064. Preliminary evidence of association of the AS3MT VNTR with response to antipsychotic treatment

Goulas A¹, Ntenti C¹, Loukaki Gkountara D¹, Filippiadou M², Papazisis G²

¹*First Laboratory of Pharmacology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*, ²*Department of Clinical Pharmacology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Introduction-Aim: A variable number tandem repeat (VNTR) in exon 1 of the gene coding for arsenite methyltransferase (AS3MT), located within the schizophrenia-associated 10q24.32-33 region, directly effects the alternative splicing of, and shows robust expression quantitative locus (eQTL) association with the AS3MTd2d3 isoform, in brain samples. The VNTR was also recently associated with activation of the prefrontal cortex and neuroplasticity during training. The aim of this study was to examine the association of the AS3MT VNTR with response to antipsychotic drug treatment of patients suffering from schizophrenia and other psychotic disorders, in a naturalistic setting.

Materials and Methods: One hundred and sixty Greek patients suffering from schizophrenia and other psychotic disorders were successively enrolled in this observational study. PANSS subscales were used as a measure of symptom intensity for positive, negative, and general psychopathology symptoms, and scores were determined at presentation and after one month of treatment. The Calgary scale was used as a measure of depressive symptoms. VNTR genotyping was accomplished with an established PCR method. Serum folate and vitamin B12 concentrations were determined both before and following treatment.

Results: We have detected a significant association of the AS3MT VNTR with the difference in the positive symptom PANSS subscale values as a result of one month of treatment (3R/3R vs. 2R carriers; $p = 0.019$, ANCOVA).

Conclusions: The AS3MT VNTR appears to affect patients' response to antipsychotic drug treatment, with respect to positive symptoms, with homozygotes for the three-repeat allele (3R/3R) showing better improvement compared to carriers of the two-repeat allele (2R), under the conditions of our study.

PP065. Chicken Ovalbumin Upstream Promoter Transcription Factor II (COUP-TFII aka NR2F2) expression and activity controls endothelial angiogenic and inflammatory responses in HUVECs in vitro

Parianou A¹, Vazoura V¹, Dafni C¹, Paixos C¹, Stratoudaki M¹, Kyrgiridi P¹, Manousaki E¹, Kintos D², Kosma A², Fousteris M², **Topouzis S¹**

¹*University of Patras, Lab of Molecular Pharmacology, Dept. of Pharmacy, Rio/Patras, Greece,*

²*University of Patras, Lab. of Medicinal Chemistry, Dept. of Pharmacy, Rio/Patras, Greece*

Introduction/Aim: The orphan nuclear receptor COUP-TFII/NR2F2 determines venous identity during vasculogenesis, being endogenously expressed in venous but not arterial endothelium. Its expression is modulated in multiple inflammatory and fibroproliferative disorders. Our Aim was to characterize in more depth its role in angiogenesis and vascular inflammation, using primary HUVECs in vitro as a model.

Methods: To reduce NR2F2 activity, we used either a specific siRNA to knock-down NR2F2 (vs a CTL-siRNA) or the selective NR2F2 micromolecular inhibitor CIA2, described elsewhere and synthesized by us.

We investigated the effects of CIA2 on the responses of HUVECs to the archetypal pro-angiogenic polypeptide FGF-2, namely on HUVEC proliferation [by MTT assay], mobilization [by monolayer wound-healing], migration [by Transwell® assays] and pseudo-vessel network formation [in Matrigel®].

We also characterized how NR2F2 modulates the response of HUVEC monolayers to the pro-inflammatory TNF-α and IL-1β in vitro by determining the effect of an NR2F2-specific siRNA on ICAM-1 and P/E Selectin induced expression [by in-situ ELISA], U937 adhesion to HUVECs [fluorescein-loaded U937] and NF-Kb-signaling [p-p65Ser536 by western]. Last, we determined how TNF-α and IL-1β affect the NR2F2 protein levels [by western].

Statistical comparison between treatments was done by Student's t-test, on data from n independent experiments, each done in multiples.

Results: HUVEC treatment with the NR2F2 inhibitor CIA2 almost entirely abrogated (reduction >90%) FGF-2-induced proliferation, Transwell® migration and Matrigel® network formation (all n=3).

Treatment with NR2F2-siRNA reduced protein levels by >95% (n=4, P<0.001). Compared to the Control-siRNA, NR2F2-knock-down resulted in 2.5-fold upregulation of P/E-Selectin and 50% increase in ICAM-1 immunoreactivity (for both: n=3, P<0.01) in response to either IL-1β and TNF-α. In addition, U937 adhesion to cytokine-exposed HUVECs further increased by 3-fold (TNF-α) and 5-fold (IL-1β) [both: n=3, P<0.001], while phosphorylation of p65 at Ser536 was further boosted by 3-fold (in response to TNF-α) and by 4-fold (in response to IL-1β) by the NR2F2-siRNA. Last, 24h-treatment of HUVECs with TNF-α and IL-1β downregulated NR2F2 protein levels by 50% and 60%, respectively (n=3, P<0.01).

Conclusions:

- 1) Endogenous NR2F2 activity in HUVECs is absolutely required for the manifestation of multiple FGF-2 pro-angiogenic effects.
- 2) NR2F2 suppresses endothelial responses to the pro-inflammatory cytokines TNF-α and IL-1β.
- 3) Long-term endothelial exposure to TNF-α and IL-1β downregulates NR2F2 levels, further contributing to the exacerbation of endothelial inflammation.

NR2F2 tightly regulates characteristic homeostatic responses in endothelial cells and modulation of its level or activity may determine endothelial phenotype and pathophysiology.

PP066. Novel 1H-pyrazolo[3,4-c]83yridine-7(6H)-one derivatives act as direct soluble Guanylyl Cyclase (sGC) stimulators and suppress proliferation, migration and cytokinesis induced by PDGF-BB and -DD in vascular smooth muscle cells in vitro

Paixos C¹, Stratoudaki M¹, Chandrinou N¹, Parianou A¹, Laimos-Geranios K², Christopoulou A², Toufas K², Kintos D², Fousteris M², **Topouzis S¹**

¹*University of Patras, Lab of Molecular Pharmacology, Dept. of Pharmacy, Rio/Patras, Greece,*

²*University of Patras, Lab of Medicinal Chemistry, Dept. of Pharmacy, Rio/Patras, Greece*

Introduction/Background: PDGFs are well-known mitogens of vascular smooth muscle cells (VSMC), modulating VSMC differentiation, proliferation and cytokinesis and critically influencing vascular homeostasis and dysfunction. VSMC behavior is also controlled by the NO/sGC/cGMP axis. Therefore, compounds able to directly stimulate VSMC sGC in pathological states characterized by NO paucity, could be promising therapeutics in fibroproliferative diseases implicating PDGFs.

Aim/Methods: The Aims of the present in vitro work, conducted in the A7r5 VSMC line, were: 1) Characterize pharmacologically three novel 1H-pyrazolo[3,4-c]pyridin-7(6H)-one derivatives (DPK-399, KL-67 and KL-69), rationally designed as direct “stimulators” of the reduced, heme-dependent form of sGC, by evaluating their ability to elevate cGMP ± the NO donor SNP [by ELISA kit] ± the heme-oxidant ODQ and to elicit VASP phosphorylation at Ser239 [by western blotting], and 2) Test whether they can modulate functional VSMC responses to PDGF-BB (PDGFR-αβ/-ββ ligand) and PDGF-DD (PDGFR-ββ ligand only), by determining A7r5 proliferation [by a chromogenic MTT assay], migration [using Transwell® chambers], cytokinesis/mobilization [via a wound-closure assay] and ERK1/2Thr202/Tyr204 & srcTyr416 phosphorylation [by western]. Statistical comparison between treatments was done by Student's t-test, on data from n independent experiments, each done in multiples.

Results: DPK-399, KL-67 and KL-69 (3-30μM) did not induce cGMP production by themselves, but all three synergized with 100μM SNP (4.5-fold increases alone) to elevate A7r5 cGMP levels by >40-fold (n=3, P<0.001), comparably with the potent sGC stimulator BAY 41-2272 (10μM). Their effect was abolished by 10μM of the heme-oxidant ODQ, confirming that they act as direct sGC “stimulators”. All three compounds also elicited characteristic cGMP-dependent phosphorylation of VASP at Ser239 by 15-20 fold (n=3, P<0.001) by themselves.

Pre-treatment with 30μM KL-67 reduced PDGF-BB-induced A7r5 proliferation by 24% (n=3, P<0.001), Transwell® migration by 82% (n=3, P<0.01) and wound closure by 60% (n=4, P<0.01). In addition, pretreatment with 30μM DPK-399 inhibited the A7r5 proliferative responses to the PDGFRβ ligand PDGF-DD by 25% (n=9, P<0.01). and suppressed the downstream pERK1/2 phosphorylation in response to PDGF-DD by 75% (n=3, P<0.01), while it entirely abrogated the phosphorylation of scr (n=3).

Conclusions:

A) All three novel, rationally-designed compounds possess the tell-tale features of direct sGC stimulators.

B) DPK-399 and KL-67 can effectively suppress both proximal signaling effects as well as the VSMCs mobilization responses to the (patho)physiologically critical mitogens PDGF-BB and -DD, and finally,

This work further validates the therapeutic usefulness of targeting sGC in vascular fibroproliferative disorders such as (re)stenosis and atherosclerosis.

PP067. The Role of Arachidonic Acid Metabolites in the Vasoconstriction Effect of Tryptamine in the Isolated Perfused Rat Kidney

Iragh D¹, Yousif M¹, Oriowo M¹

¹*Faculty of Medicine Kuwait University, Jabrya, Kuwait*

Introduction: Trace amines such as tryptamine, β -phenylethylamine (PEA), and 3-iodothyronaine (T1AM) are endogenous compounds present in mammalian tissues at very low concentrations. It was believed that trace amines are indirectly acting sympathomimetic. Currently, they activate surface G-protein coupled receptors (GPCRs) specific for them, known as trace amine-associated receptors (TAARs)[1]. The objective of this study was to investigate the possible role of arachidonic acid metabolite behind the vasoconstrictor effect of TAAR agonists in normotensive and hypertensive conditions.

Methods: Male Wistar Kyoto (WKY, n = 69) and Spontaneously Hypertensive Rats (SHR, n = 60) age 12-14 weeks were used in this investigation. Animals were sacrificed on the day of the experiment. The renal artery of the left kidney was isolated and canulated to be placed in a temperature-controlled perfusion chamber and perfused with Krebs' solution using a channel masterflex peristaltic pump. Dose-response curves were established for trace amine agonists in the presence of indomethacin (10 μ M), NS398 (1 μ M), SC560 (1 μ M), and HET0016 (10 μ M). Changes in perfusion pressure were recorded through a transduce connected to a Lectromed. TAARs mRNA expression was assessed using reverse transcription polymerase chain reaction (RT-PCR). Data were statistically analyzed by one-way ANOVA or Student's t-test.

Results: Tryptamine-, PEA-, and T1AM-induced significant increase in perfusion pressure of kidney preparations from normotensive WKY and hypertensive SHR rats ($p < 0.05$). Perfusion of the isolated kidney with indomethacin (10 μ M), significantly reduced the vasoconstriction response to tryptamine, PEA, and T1AM in the perfused kidney in preparations from SHRs compared to WKY rats. Similar results were also seen with tryptamine in the presence of NS398 (1 μ M), SC560 (1 μ M), and HET0016 (10 μ M). The relative TAARs mRNA expression in the kidney were significantly elevated in SHRs compared to WKY.

Conclusions: The results obtained from this study would suggest that TAAR1 are involved in tryptamine-, PEA-, and T1AM-induced response in the WKY and SHR rats perfused kidney. The responses were also inhibited by indomethacin, NS398, SC560, and HET0016 would suggest that the vasoconstriction involved the production of arachidonic acid metabolites via activation of cyclooxygenase and non-cyclooxygenase pathways. All TAARs 1-9 mRNA expression were higher in SHRs compared to WKY rats, except for TAAR 4 and TAAR 6.

[1] Borowsky B, Adham N, Jones KA, et al. Trace amines: identification of a family of mammalian G protein-coupled receptors. Proc Natl Acad Sci U S A. 2001;98(16):8966-8971. doi:10.1073/pnas.151105198

PP068. Potentially inappropriate anticholinergic drugs use - a Portuguese study with older outpatients

Rodrigues D^{1,2}, Herdeiro M³, Mateos-Campos R⁴, Figueiras A⁵, **Roque F^{1,2}**

¹Polytechnic Institute of Guarda, Guarda, Portugal, ²Health Sciences Center (CICS-UBI), Covilhã, Portugal, ³Institute of Biomedicine (iBIMED-UA), Aveiro, Portugal, ⁴University of Salamanca, Salamanca, Spain, ⁵University of Santiago de Compostela, Santiago de Compostela, Spain

Introduction: Drugs with strong anticholinergic properties are widely prescribed [1] and deemed potentially inappropriate for use in older adults [2]. So, minimizing the use of such drugs is recommended for this population. This study aims to characterize the use profile of anticholinergic drugs among Portuguese older adults and to identify factors associated with the potentially inappropriate use of these medications.

Methods: A retrospective study was conducted on a randomly selected sample of 1200 older adults (≥ 65 years old) receiving care at primary health care facilities under the Regional Health Administration (Administração Regional de Saúde – ARS) of Centro (ARSC) in Portugal from April 2021 to August 2022. The assessment of potentially inappropriate anticholinergic drugs was based on the 2023 Beers criteria [3]. Logistic regression analyses were employed to identify associations between independent variables (sex, age, diagnoses, medications, and medical conditions) and the potentially inappropriate use of anticholinergic drugs.

Results: At least one potentially inappropriate anticholinergic drug was found to be taken by 8.9% of the older adults, with amitriptyline being the most frequently dispensed (2.0%). Multivariate analysis indicated that anticholinergic use was associated with a higher average number of medications (OR 1.17, 95% CI 1.11-1.23), as well as diagnoses of depression (OR 2.89, 95% CI 1.79-4.68) and psychiatric disorders (OR 1.65, 95% CI 1.00-2.73).

Conclusion: Understanding utilization patterns could inform strategies aimed at reducing anticholinergic drug use among older adults. As such, primary health care should implement tailored interventions to optimize the use of anticholinergic drugs in this population.

Funding: Daniela A. Rodrigues is funded by an individual grant from the Foundation for Science and Technology (FCT) (ref 2022.10605.BD).

- [1] Ghossein N, Kang M, Lakhkar AD. Anticholinergic Medications. 2023.
- [2] Nishtala PS, Salahudeen MS, Hilmer SN. Anticholinergics: theoretical and clinical overview. Expert Opin Drug Saf. 2016.
- [3] American Geriatrics Society 2023 updated AGS Beers Criteria® for potentially inappropriate medication use in older adults. J Am Geriatr Soc. 2023.

PP069. Impact of COVID-19 restrictions on antipsychotic utilization in Turkey: Findings from Changes in Nationwide Drug Consumption During the Pandemic Study

Vizdiklar C¹, Aydin V², Akici A¹

¹*Department of Medical Pharmacology, School of Medicine, Marmara University, Istanbul, Turkey,*

²*Department of Medical Pharmacology, International School of Medicine, Istanbul Medipol University, Istanbul, Turkey*

Introduction: The COVID-19 pandemic and the associated restrictions have resulted in out-of-routine practices [1]. These extraordinary circumstances, in addition to the potential changes in the prevalence of mental health disorders, might have been reflected in chronic drug consumption levels. We aimed to evaluate the alterations in utilization of antipsychotics throughout the periods determined by COVID-19 restrictions.

Methods: We obtained nationwide outpatient drug sales data spanning from March 2018 to December 2022, along with the nationwide projection data of prescribing from IQVIA Turkey. Among six different pharmacotherapy main groups of interest of the comprehensive study, antipsychotic utilization was evaluated in this analysis. We examined the average monthly antipsychotic utilization and costs, as well as the quarterly prescribed antipsychotic levels in three periods: “before restriction” (BfR, 01.03.2018-31.03.2020), “during restriction” (DuR, 01.04.2020-31.03.2022), and “after restriction” (AfR, 01.04.2022-31.12.2022). We assessed the utilization with the “defined daily dose/1000 inhabitants” (DID) metric.

Results: Antipsychotic consumption increased from 8.4 ± 0.6 DID in BfR to 9.9 ± 1.6 DID in DuR ($p < 0.001$) and reached 10.2 ± 0.9 DID in AfR ($p < 0.001$ vs. BfR). The number of prescribed antipsychotics showed a decrease while transitioning from BfR (673.1 ± 55.3 thousand units) to DuR (403.9 ± 51.0 thousand units, $p < 0.001$), then a subsequent rise insufficient to reach the initial level (541.7 ± 89.0 thousand units, $p = 0.011$ vs. BfR, $p = 0.008$ vs. DuR). Consumption of atypical antipsychotics significantly increased following BfR (7.6 ± 0.5 DID), escalating to 9.0 ± 1.4 DID in DuR ($p < 0.001$), then 9.3 ± 0.9 DID in AfR ($p < 0.001$ vs. BfR). Typical antipsychotics demonstrated an increasing consumption trend from BfR (0.8 ± 0.2 DID) to DuR (0.9 ± 0.1 DID, $p = 0.03$). In contrast, their consumption in AfR (0.9 ± 0.1 DID) did not significantly differ from previous periods ($p > 0.05$ vs. both). Expenditure on antipsychotics increased from 16.7 ± 1.1 million Euros ($m\text{€}$) in BfR to 19.0 ± 2.7 $m\text{€}$ in DuR ($p < 0.001$), then slightly reduced in AfR (18.3 ± 1.9 $m\text{€}$, $p > 0.05$ vs. both).

Conclusions: Our study revealed a surge of antipsychotic utilization in Turkey with the onset of the pandemic. This contrasts with the observed decrease in prescribing, which might be linked to difficulties in accessing a prescriber. Additionally, the discordance between increased consumption and observed cost trends might potentially be associated with reduced use of expensive treatment regimens due to interrupted physician follow-ups.

- [1] Mendez-Brito A, El Bcheraoui C, Pozo-Martin F. Systematic review of empirical studies comparing the effectiveness of non-pharmaceutical interventions against COVID-19. J Infect. 2021;83(3):281-293.

PP070. Deletion of 3-mercaptopyruvate sulfurtransferase reduces atherosclerosis

Zampas P^{1,2}, Varela A³, Davos H C³, Papapetropoulos A^{1,2}

¹*Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece*, ²*Clinical, Experimental Surgery and Translational Research Center, Biomedical Research Foundation Academy of Athens, Athens, Greece*, ³*Cardiovascular Laboratory, Biomedical Research Foundation Academy of Athens, Athens, Greece*

Aim: Atherosclerosis is a complex vascular inflammatory disease that correlates strongly with metabolic disorders, such as dyslipidemia. Hydrogen sulfide (H₂S) is a signaling molecule that is produced mainly by three enzymes, cystathionine γ -lyase (CSE), cystathionine β -synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (MPST). H₂S is known that to be involved in atherosclerosis and lipid metabolism affecting multiple pathophysiological pathways. Here in, we investigated the impact of MPST in atherosclerosis progression. For this purpose, we generated a double APOE/MPST knockout mouse (Apoe-/-Mpst-/-).

Methods: Mice (n=6/group) were fed a western diet (WD; 42% kcal from fat and 1.25% cholesterol) for 14 weeks to induce obesity and dyslipidemia. Changes in body weight, glucose tolerance and blood pressure were determined, while mice underwent echocardiographic assessment of left ventricular (LV) function. Furthermore, serum total cholesterol, low density lipoproteins (LDL-C), high density lipoproteins (HDL-C) and triglycerides were measured. En face staining was used for quantification of atherosclerotic lesion area in isolated whole aortae. Subsequently, the expression of H₂S production enzymes and proteins related to lipid metabolism were measured by western blot in the liver, while H₂S levels were measured using lead acetate assay.

Results: H₂S production was reduced in the liver of double knockout mice (1.00 ± 0.015 vs 0.85 ± 0.042 ; p<0.05 vs Apoe-/-), while expression of CSE and CBS was not altered. No differences between the two genotypes were observed for body weight, glucose tolerance and blood pressure. Interestingly, we observed that Apoe-/-Mpst-/- mice were characterized by an improved lipid profile compared to Apoe-/-, with reduced levels of serum total cholesterol (1519 ± 138.0 vs 807.6 ± 65.78 ; mg/dl), LDL-C (1183 ± 100.8 vs 674.8 ± 50.47 ; mg/dl) and triglycerides (114.7 ± 17.97 vs 63.18 ± 1.95 ; mg/dl) (p<0.05 vs Apoe-/-). Consequently, atherosclerotic plaque development was decreased in Apoe-/-Mpst-/- mice compared to Apoe-/- (8.82 ± 0.99 vs 4.95 ± 0.92 ; % lesion area normalized per lumen area; p<0.05). LV function was also found to be improved in double knockout mice (Fractional shortening; 39.86 ± 0.40 vs 41.09 ± 0.32 ; p<0.05 vs Apoe-/-). From the proteins tested, apolipoprotein B (APOB) (1.00 ± 0.17 vs 3.03 ± 0.42 vs 1.77 ± 0.19) and apolipoprotein A1 (APOA1) (1.00 ± 0.08 vs 0.89 ± 0.15 vs 0.27 ± 0.07) (p<0.05, WT vs Apoe-/- vs Apoe-/-Mpst-/-) levels were found to be reduced in the liver of Apoe-/-Mpst-/- mice compared to Apoe-/-.

Conclusion: Our results indicate that MPST deletion leads to decreased atherosclerotic plaque formation in the Apoe-/- dyslipidemia model, likely through the regulation of hepatic lipid metabolism.

PP071. Risk of new onset diabetes mellitus with pitavastatin as compared to atorvastatin and rosuvastatin: A systematic review and meta-analysis

Singh H¹, Kaur S¹, Kaushal P², Singla M¹

¹*Government Medical College and Hospital, Chandigarh, India, Chandigarh, India*, ²*All India Institute of Medical Sciences, New Delhi, India, New Delhi, India*

Introduction: Statins have been found to be associated with the risk of new onset diabetes mellitus (NODM). In most of the published research studies, atorvastatin and rosuvastatin have been found to be associated with NODM and it has been found that pitavastatin carries a lower risk. The aim of the present systematic review was to evaluate the impact of pitavastatin in causing NODM as compared to atorvastatin and rosuvastatin.

Methods: We performed a systematic literature search (from inception to October 28, 2022) through PubMed, CENTRAL, EMBASE and ClinicalTrials.gov to select the relevant research studies addressing the comparison of pitavastatin, atorvastatin and rosuvastatin with respect to incidence of NODM and their impact on glycemic parameters. Two study authors independently performed the literature search and screening, risk of bias (ROB) and quality assessment, and data extraction. Joanna Briggs Institute checklist (for cross-sectional studies), Newcastle-Ottawa Scale (for cohort/ prospective observational studies), and Scottish intercollegiate Guidelines Network checklist (for RCT) were used to assess ROB and quality of included studies. Data was collected and noted with respect to type of statin used, dose, duration and incidence of NODM and other pertinent information. The analysis was conducted using RevMan version 5.4, using risk ratio (RR), along with 95% confidence intervals (CI) and corresponding P values. Heterogeneity was scrutinized using the I^2 statistic,

Results: Initial search yielded 517 potential records. After screening potential records, 13 research studies were included in this systematic review. The included studies were observational studies (retrospective, cohort and cross-sectional studies), real world studies, and randomized controlled trials (RCTs). Most of the studies were primarily evaluating the risk of NODM, however, few studies also evaluated the risk of NODM and impact of statins on glycemic parameters, as secondary objectives. Most of the included studies were of good quality (low ROB) and showed pitavastatin to be associated with a lower or no risk of developing NODM as compared to atorvastatin and rosuvastatin. For meta-analysis, 8 studies qualified for comparison of pitavastatin (n=7471) with atorvastatin (n=65796) and 7 for comparison of pitavastatin (n=7159) with rosuvastatin (n=19278). Pitavastatin showed a lower risk of NODM as compared to atorvastatin (RR=0.86 and 95% CI=0.79-0.93, p=0.0002) and rosuvastatin (RR=0.77 and 95% CI=0.71-0.84, p<0.00001).

Conclusion: Pitavastatin carries a lower risk of developing NODM as compared to other commonly used statins. It may serve as a safer option in patients requiring long term statin therapy.

PP072. MIR27A rs895819 TC genotype increases risk of fluoropyrimidine-induced severe toxicity in Greek cancer patients

Ragia G^{1,2}, Biziota E³, Koukaki T³, Amarantidis K³, Manolopoulos V^{1,2,4}

¹Laboratory of Pharmacology, Medical School, Democritus University of Thrace, Alexandroupolis, Greece, ²Individualised Medicine & Pharmacological Research Solutions Center (IMPReS), Alexandroupolis, Greece, ³Department of Medical Oncology, University General Hospital of Alexandroupolis, Medical School, Democritus University of Thrace, Alexandroupolis, Greece, ⁴Clinical Pharmacology Unit, Academic General Hospital of Alexandroupolis, Alexandroupolis, Greece

Background: DPYD rs3918290, rs55886062, rs67376798, and rs75017182 upfront genotyping has been endorsed in Europe [1], guiding fluoropyrimidine dosing and leading to significantly reduced incidence of fluoropyrimidine-induced severe toxicity. We have previously shown that DPYD defective variants are associated with any grade fluoropyrimidine-induced toxicity, and with grade 3-4 toxicity (OR: 6.493, p=0.014) in Greek cancer patients [2]. Albeit their frequency is rather low, estimated at 2.7%. MicroRNA 27a (miR-27a) has derived as a significant regulator of DPD activity. MIR27A rs895819T>C polymorphism modulates miR-27a expression [3] and has gained attention in fluoropyrimidine pharmacogenomic studies. The aim of the present study was to analyze the association MIR27A rs895819T>C polymorphism with fluoropyrimidine-induced toxicity in Greek cancer patients treated with 5-fluorouracil or capecitabine.

Methods: Study group consisted of 313 fluoropyrimidine-treated cancer patients previously genotyped for DPYD rs3918290, rs55886062, rs67376798 and rs75017182 [2]. MIR27A rs895819 polymorphism was genotyped on QuantStudio™ 12K Flex Real-Time PCR System by use of pre-designed TaqMan® assay (ThermoFisher Scientific).

Results: In the whole population, any grade toxicity prevalence was estimated at 66.5%, while 12% of patients experienced grade 3-4 toxicity. In grade 3-4 toxicity cases, MIR27A rs895819CC genotype was absent, 8 cases were TT homozygous and 17 cases were TC heterozygous. MIR27A rs895819TC genotype was associated with grade 3-4 toxicity independently of DPYD (p=0.005), and, together with DPYD variants, could predict 82% of grade 3-4 cases. In adjusted polygenic regression model, the odds of grade 3-4 toxicity were increased by both DPYD deficiency (OR: 8.923, p=0.006) and MIR27A rs895819 TC genotype (OR: 3.865, p=0.002).

Conclusions: DPYD genotyping can guide fluoropyrimidine dosing in Greece. Combined with DPYD, MIR27A genotyping identifies a significant proportion of fluoropyrimidine-induced grade 3-4 toxicity cases. Integration of additional genes that are associated with fluoropyrimidine response in dosing-guidelines, holds promise to increase the prognostic value of DPYD genotyping and improve safety of fluoropyrimidine-based chemotherapy [4].

- [1] EMA (2020). EMA recommendations on DPD testing prior to treatment with fluorouracil, capecitabine, tegafur and flucytosine.
- [2] Ragia G, Maslarinou A, Atzemian N, et al. Implementing pharmacogenetic testing in fluoropyrimidine-treated cancer patients: DPYD genotyping to guide chemotherapy dosing in Greece. Front Pharmacol. 2023;14:1248898.
- [3] Sun Q, Gu H, Zeng Y, et al. Hsa-mir-27a genetic variant contributes to gastric cancer susceptibility through affecting miR-27a and target gene expression. Cancer Sci. 2010;101(10):2241-2247.
- [4] Maslarinou A, Manolopoulos VG, Ragia G. Pharmacogenomic-guided dosing of fluoropyrimidines beyond DPYD: time for a polygenic algorithm?. Front Pharmacol. 2023;14:1184523.

PP073. MiRNome analysis in DOAC-treated male atrial fibrillation patients reveals novel mechanistic insights of drug action

Ragia G^{1,2}, Thomopoulos T³, Pallikarou M^{1,2}, Chalikias G⁴, Trikas A³, Tziakas D⁴, Manolopoulos V^{1,2,5}

¹Laboratory of Pharmacology, Medical School, Democritus University of Thrace, Alexandroupolis, Greece, ²Individualised Medicine & Pharmacological Research Solutions Center (IMPReS), Alexandroupolis, Greece, ³Department of Cardiology, "Elpis" General Hospital of Athens, Athens, Greece, ⁴Department of Cardiology, Academic General Hospital of Alexandroupolis, Alexandroupolis, Greece, ⁵Clinical Pharmacology Unit, Academic General Hospital of Alexandroupolis, Alexandroupolis, Greece

Background: Direct Oral Anticoagulants (DOACs) are recommended as first line treatment in atrial fibrillation (AF) patients. The potential role of microRNAs (miRs) as epigenetic biomarkers of DOAC response has been scarcely studied [1,2]. To fill this gap, we are currently conducting a prospective clinical trial aiming to follow in time changes of miR expression in naïve AF patients starting DOAC therapy.

Methods: This is an ongoing study. So far, we have enrolled 76 AF male and female patients treated with dabigatran, rivaroxaban or apixaban and 22 non-AF controls. Circulating plasma miRs have been isolated from all participants at baseline (t0), and for patients at 7 (t1) and 28 (t2) days of DOAC treatment. In this preliminary miRNome analysis, to avoid confounding by sex, twelve male patients were included, 4 treated with each of dabigatran, rivaroxaban, and apixaban. The expression of 754 human miRs was analyzed at t0, t1 and t2 by use of TaqMan™ OpenArray™ Human Advanced MicroRNA Panel on QuantStudio™ 12K Flex Real-Time PCR System (ThermoFisher Scientific).

Results: In our study, sixteen minor bleeding events occurred; 5 bleeding cases were among the analyzed samples. A total of 557 miRs were present in plasma at t0, t1 or t2. In pooled samples analysis, DOAC treatment led to up-regulation of miR-204-5p (12.209 fold-change, p<0.0001) and miR-20a-5p (2.314 fold-change, p=0.015) at t1. Analysis for each DOAC showed that rivaroxaban altered the expression of 10 miRs, dabigatran altered the expression of 8 miRs, while apixaban led to altered expression of over 90 miRs. When patients were stratified in bleeding cases (n=5) and controls (n=7), expression of 9 miRs differed at baseline between cases and controls; the strongest association was for miR-518d-5p (40.806 fold-change, p=0.008).

Conclusions: DOAC treatment induces changes on miRNome. MiR-204 and miR-20a are key components of the PI3K/AKT pathway holding a role in inflammation, fibrosis, cardiac remodeling/hypertrophy, angiogenesis, and conduction abnormalities, suggesting that DOACs potentially exert beneficial actions beyond anticoagulation. Each DOAC induces a distinct pattern of miRNome expression, thus, DOAC pleiotropic effects may involve different mechanisms. Studying the expression of miRs in bleeding cases can help in biomarker identification towards safer DOAC treatment.

- [1] Zhang H, Zhang Z, Liu Z, et al. Circulating miR-320a-3p and miR-483-5p level associated with pharmacokinetic-pharmacodynamic profiles of rivaroxaban. *Hum Genomics*. 2022;16(1):72
- [2] Rytkin E, Bure IV, Bochkov PO, et al. MicroRNAs as novel biomarkers for rivaroxaban therapeutic drug monitoring. *Drug Metab Pers Ther*. 2021;37(1):41-46

PP074. A randomized controlled trial to evaluate efficacy and safety of glibenclamide in moderate to severe traumatic brain injury: The STRATEGY study

Kakkar A¹, Tripathi M¹, Ahuja C¹, Kaur M¹, Batra S¹, Malhotra S¹

¹*Postgraduate Institute of Medical Education and Research, Chandigarh, India*

Introduction: According to a recent analysis of Global Burden of Disease (GBD) Study 2019, it was found that there were approximately 27.2 million new cases and 49 million prevalent cases of traumatic brain injury(TBI) in 2019. The years lived with disability (YLDs) for TBI amounted to 7.08 million. Regionally, Eastern Europe had the highest burden of TBI in the same year[1]. Among young adult population, TBI is a leading cause of mortality besides causing disability across all age groups. While cerebral edema and contusion expansion significantly contribute to the clinical and economic impacts of TBI, current treatments options are often inadequate, mainly reactionary, and lack robust evidence for their effectiveness. Few preclinical and small clinical studies have evaluated the role of sulfonylurea receptor 1 (SUR1)—TRPM4 channel modulation in TBI[2].

Methods: This study assesses the efficacy and safety of glibenclamide in participants with moderate/severe traumatic brain injury in terms of functional outcomes, contusion volumes and quality of life of these patients. The study is double-blind, randomized, placebo-controlled, parallel-group trial being conducted at Advanced Trauma Center of PGIMER Chandigarh, India with a sample size of 150. The trial is registered at Clinical Trial Registry of India (Ref: CTRI/2023/12/060716) and study protocol has received approval from Institutional Ethics Committee(IEC). The study participants after screening and meeting the inclusion/exclusion criteria will undergo randomization to receive either glibenclamide or matching placebo and followed up for 180 days.

Results: The study is open for enrollment. The patients are managed according to standard guidelines for management of TBI based on clinical assessment. The study intervention or placebo is administered in addition to usual standard of care(SOC) in all the patients. The patients are monitored for efficacy and safety including hypoglycemia as per pre-defined schedules or as clinically indicated. Primary outcome is improvement in Glasgow Outcome Scale – Extended(GOS-E) at day 90 and 180. Secondary outcome measures include improvement in disability rating scale, days spent in ICU, duration of hospitalization, quality of life using QOLIBRI scale, incidence of adverse events including serious adverse events.

Conclusion: This study plans to evaluate the utility and feasibility of successful clinical translation of glibenclamide in TBI patients. If found effective, it can be made available at affordable costs to the patients.

Acknowledgment: Financial support from the Indian Council of Medical Research(ICMR), India.

[1] Guan B et.al. BMJ Open. 2023;13(10):e075049-e075049.

[2] Lerouet D et.al. Fundamental & Clinical Pharmacology. 2021;35(3):524-538.

PP075. Exploring the real-world association between statin use and acute renal failure risk: A pharmacovigilance study using FDA Adverse Event Reporting System

Kaur K¹, Singh H²

¹All India Institute of Medical Sciences Rishikesh, Rishikesh, India, ²Government Medical College and Hospital Chandigarh, Chandigarh, India

Introduction: The widespread use of statins has raised questions regarding their potential impact on renal health. This study aimed to investigate the association between different statins and the risk of acute renal failure using real-world evidence extracted from the FDA Adverse Event Reporting System (FAERS) database.

Methods: In this investigation, we utilized real-world evidence from the FAERS database to scrutinize the correlation between different statins and the risk of acute renal failure. Employing disproportionality analysis, we assessed the relationship across various statins, including simvastatin, atorvastatin, rosuvastatin, pitavastatin, pravastatin, and fluvastatin. OpenVigil FDA software (contains data till 2023Q3) facilitated data extraction and analysis, utilizing the MedDRA Low-Level Term (LLT) classification for adverse events. To ascertain statistical significance, we applied the 2001 Evans criteria, necessitating a report count greater than 3, a Proportional Reporting Ratio (PRR) exceeding 2, and a Chi-squared value surpassing 4. Reporting Odds Ratio (ROR) with a 95% confidence interval (CI) was also calculated. Additionally, we observed the gender-wise distribution of the event of interest.

Results: We found a statistically significant association of all the statins with acute renal failure except pitavastatin. Simvastatin exhibited a notable correlation, recording 3434 acute renal failure events among 244,782 adverse event reports (Chi-squared value=13275.89, PRR=6.03, ROR = 6.10, 95% CI 5.89 to 6.32). Atorvastatin displayed a similar trend, with 1613 events amidst 219,924 reports (Chi-squared value=2095.89, PRR=3.01, ROR = 3.03, 95% CI 2.88 to 3.18). Rosuvastatin accounted for 1055 events against 176,055 total events (Chi-squared value=873.57, PRR=2.43, ROR = 2.44, 95% CI 2.30 to 2.60). Likewise, pravastatin revealed 126 events against 15,853 total reports (Chi-squared value=187.51, PRR=3.19, ROR = 3.21, 95% CI 2.69 to 3.82), and fluvastatin leading to 127 events among 6893 reports (Chi-squared value=696.97, PRR=7.40, ROR = 7.52, 95% CI 6.31 to 8.97). In contrast, pitavastatin exhibited no significant association with acute renal failure, reporting 32 events against 8300 total events (Chi-squared value=5.64, PRR=1.54, ROR = 1.54, 95% CI 1.09 to 2.19). Higher percentage of events were found among male patients as compared to female patients.

Conclusion: This study raises concerns about a notable association between acute renal failure and statin use, excluding pitavastatin. Emphasizing the necessity for personalized risk assessments when prescribing statins, these findings contribute to a more detailed understanding of their potential impact on renal health in clinical practice.

PP076. Metformin, a Principal Actress in the Global Oxidative Status in Patients with Calcific Aortic Stenosis and Diabetes Mellitus

Tejerina T¹, Corbacho-Alonso N², Rodriguez-Sanchez E³, Perales I⁴, Mouríño L⁵, Ruiz-Hurtado G⁶, Gonzalez-Barderas M⁷

¹School of Medicine, Universidad Complutense de Madrid, Spain, Madrid, Spain, ²Hospital Nacional de Paraplejicos, SESCAM, Toledo, Spain, Madrid, España, ³Cardiorenal Translational Laboratory, Instituto de Investigación Imas12, Hospital Universitario 12 de Octubre, Madrid, España,

⁴Department of Vascular Physiopathology, Hospital Nacional de Paraplejicos, Toledo, Madrid, España, ⁵Department of Vascular Physiopathology, Hospital Nacional de Paraplejicos, Toledo, Madrid, España, ⁶Cardiorenal Translational Laboratory, Instituto de Investigación Imas12, Hospital Universitario 12 de Octubre, Madrid, España, ⁷Department of Vascular Physiopathology, Hospital Nacional de Paraplejicos, Toledo, Madrid, España

Calcific aortic stenosis (CAS) and diabetes mellitus 2 (DM2) are related and often concomitant pathologies that are accompanied by common comorbidities, such as hypertension or dyslipidaemia. The oxidative stress (OS) is one of the mechanisms that triggers CAS as well as vascular complications in DM. Metformin can inhibit (OS), yet its effects have not been studied in the context of CA and DM. In this work, we assessed the global oxidative status in plasma samples from a population with CAS with and without DM2 (under treatment with Metformin) using multimarker scores of systemic oxidative damage (OxyScore) and antioxidant defence (AntioxyScore). The OxyScore was determined by measuring carbonyls, oxidized LDL (oxLDL), 8-hydroxy-20-deoxyguanosine (8-OHdG), and xanthine oxidase (XOD) activity. The AntioxyScore was determined by catalase (CAT) activity, superoxide dismutase (SOD) activity and total antioxidant capacity (TAC). Patients with CAS present an increase of oxidative stress compared with healthy subjects that probably overwhelms their antioxidant capacity. Interestingly, patients with DAS and DM present a decrease of oxidative stress that might be caused by the benefits produced thanks to the pharmacological treatment to diabetic patients with metformin. In Conclusions reducing oxidative stress or enhancing antioxidant capacity with personalized therapies in CAS patients could be a good strategy for managing the disease, focusing on a personalized medicine.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: The authors thank the Service of Microscopy and Image Analysis at the HNP.

PP077. Characterization of 5-HT receptor subtypes involved in the serotonergic sympatho-modulation of vascular tone in female rats

Terol Úbeda A^{1,2}, Fernández González J^{1,2}, García Domingo M^{1,2}, Martín M^{1,2}, Morán A^{1,2}, García Pedraza J^{1,2}

¹Laboratorio de Farmacología, Departamento de Fisiología y Farmacología, Facultad de Farmacia. Universidad de Salamanca, Salamanca, Spain, ²Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain

Cardiovascular functionality is influenced by peripheral serotonergic system. We have demonstrated that 5-hydroxytryptamine (5-HT) modulates sympathetic vascular neurotransmission, inhibiting it via 5-HT1A/1D receptors and potentiating it by 5-HT3 activation in male Wistar rats [1,2]. In female rats, the sympatho-inhibitory effect is via 5-HT1 receptors, while the enhancement is mediated by 5-HT2/3 activation. The aim of this study was to determinate, in female rats, the 5-HT1 and 5-HT2 receptor subtypes involved in the serotonergic vascular sympatho-regulation.

Female Wistar rats (n=40) were anaesthetized with pentobarbital (60 mg/kg; i.p.), pithed and prepared for the stimulation of the entire sympathetic outflow [3]. Mean blood pressure (MBP) and heart rate (HR) were continuously monitored. Electrical stimulation of the entire spinal cord (0.1; 0.5; 1 and 5 Hz; 15 ± 3 V) was performed to obtain frequency-dependent increases in MBP (control curve). Then, animals received a continuous i.v. infusion of saline (1mL/h) or 10 µg/kg/min of the selective: 5-HT1A (8-OH-DPAT), 5-HT1B (CP 93,129), 5-HT1D (L-694,247), 5-HT1F (LY344864), 5-HT2A/2B/2C (α -methyl-5-HT), 5-HT2B (BW723C86), 5-HT2C (MK212) receptor subtype agonists. Then, new curves were constructed to evaluate their role on electrical-induced vasopressor responses. Statistical significance (ANOVA) was accepted at p < 0.05.

Basal MBP and HR were 48 ± 1 mmHg and 313 ± 4 beats/min, respectively. The electrically-induced increases in MBP were 2.1 ± 0.1, 9.8 ± 0.3, 21.1 ± 0.7, 46.3 ± 1.1 mmHg for 0.1, 0.5, 1 and 5 Hz, respectively (control curve). In females, L-694,247 (5-HT1D agonist) exerted an inhibitory effect and α -methyl-5-HT (5-HT2A/2B/2C agonist) increased electrically induced vasopressor responses. Neither 5-HT1A/1B/1F nor 5-HT2B/2C agonists modified noradrenergic vasopressor responses.

In conclusion, in female rats, serotonergic sympatholytic action at vascular level is due to 5-HT1D receptor activation, whereas 5-HT2A activation is involved in the potentiating action of serotonergic system in vascular sympathetic neurotransmission.

[1] Morán A, Velasco C, Salvador T, Martín ML, San Román L. Inhibitory 5- hydroxytryptamine receptors involved in pressor effects obtained by stimulation of sympathetic outflow from spinal cord in pithed rats. Br J Pharmacol. 1994;113(4):1358-1362.

[2] Morán A, Fernández MM, Velasco C, Martín ML, San Román L. Characterization of prejunctional 5-HT1 receptors that mediate the inhibition of pressor effects elicited by sympathetic stimulation in the pithed rat. Br J Pharmacol. 1998; 123(6):1205-1213.

[3] García-Pedraza JA, López C, Fernández-González JF, Martín ML, Morán A, García-Domingo M. Vascular sympathetic neurotransmission and its serotonergic regulation are modified by chronic fluoxetine treatment. J Pharmacol Sci. 2021;147(1):48-57.

PP078. Exploration of new mechanisms for the protection of peroxisome proliferator-activated receptor alpha agonists against endothelial inflammation and atherogenesis

Pu Y^{1,2,3}, Dong P², Zuo Z³, Wang L², Huang Y²

¹*City University of Hong Kong Shenzhen Research Institute, Shenzhen, China*, ²*Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, China*, ³*School of Pharmacy, The Chinese University of Hong Kong, Hong Kong, China*

Introduction: Fibrates, peroxisome proliferator-activated receptor alpha (PPAR α) agonists, are commonly used to treat hypertriglyceridemia in clinical practice. The mechanisms by which these agonists protect against endothelial activation and atherogenesis are yet to be fully understood. This study aims to investigate the function of endothelial PPAR α in the agonists-produced inhibition of vascular inflammation and atherogenesis.

Methods: RNA sequencing was performed to analyze the correlation between genes and diseases or pathways in human endothelial cells (ECs) or mouse aortic ECs (n=3-13). AAV-Cas9-sgPpar α or AAV-Cdh5-Ppar α was injected into ApoE-/- mice through tail veins to induce endothelium-specific knockdown or overexpression of PPAR α , and PPAR α agonist pemetrexed was orally administered to mice for 2 weeks with a dosage of 0.1 mg/kg/day (n=7-8). The expression and phosphorylation of proteins were determined using western blot, qPCR, and immunostaining (n=3-6).

Results: Gene Set Enrichment Analysis (GSEA) of RNA sequencing data revealed negative correlations between PPAR signaling and abnormal endothelial function in circulating ECs of patients (n=6-13) and in atherosclerotic mouse aortas (n=3). Additionally, PPAR phosphorylation was reduced in the human coronary arterial endothelium with atherosclerotic plaques (n=6). Pro-inflammatory cytokines or oscillatory shear stress inhibited PPAR α expression and phosphorylation in cultured human ECs (n=3-6). These findings revealed decreased endothelial PPAR α activity in atherosclerotic arteries.

Pemetrexed treatment reduced the formation of atherosclerotic plaques induced by Western diet (n=7-8) or carotid partial ligation (n=3-4) in ApoE-/- mice. However, these beneficial effects were reversed by endothelium-specific PPAR α knockdown, suggesting that endothelial PPAR α mediates a large part of the vaso-protective effect of pemetrexed treatment. Moreover, endothelium-specific PPAR α overexpression inhibits the formation of atherosclerotic plaques in the aortas and the expression of pro-inflammatory cytokines in carotid arteries of ApoE-/- mice fed a Western diet (n=6-7).

The hallmark analysis in human ECs with PPAR α overexpression enriched hippo-merlin signaling. The heatmap shows PPAR α overexpression inhibited the expression of YAP target genes (n=3). These results were further confirmed in vivo and in vitro (n=5-7). PPAR α overexpression resulted in increased YAP phosphorylation at S127 in cultured human ECs and mouse carotid arteries (n=5-6). PPAR α overexpression or activation decreased YAP activation-induced expression of pro-inflammatory genes (n=6). These findings suggest that PPAR α promotes YAP phosphorylation at S127, which inhibits the transcriptional activity of YAP, ultimately reducing endothelial activation and atherogenesis.

Conclusions: This study reveals that endothelial PPAR α mediates the protective effect of pemetrexed on vascular inflammation and atherogenesis through inhibiting endothelial YAP activity (supported by HMRF-07181286, T12-101/23-N, SRFS-2021-4S04, and NSFC-82300491).

PP079. Novel Biomarkers in Anti-Tuberculosis Treatment-Induced Liver Injury (ATT-DILI): A Profound Analysis of Clinical Profiles and Severity Assessment

Kumar A¹, Shafiq N¹, Duseja A², Aggarwal A³, Sharma N⁴, Kakkar N⁵, Singh H⁴, Malhotra S¹

¹*Department of Pharmacology, Post Graduate Institute of Medical Education and Research, Chandigarh, India*, ²*Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India*, ³*Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India*, ⁴*Department of Internal Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India*, ⁵*Department of Histopathology, Postgraduate Institute of Medical Education and Research, Chandigarh, India*

Aim: Drug-induced liver injury (DILI) continues to be a significant clinical concern, posing persistent challenges in treatment. Existing blood biomarkers fall short in effectively identifying and anticipating DILI outcomes. Traditional markers demonstrate limited specificity and sensitivity. This study aimed to evaluate Cytochrome c, Aldehyde Dehydrogenase 1A1 (ALDH1A1), Argininosuccinate Synthase (ASS1), and Bile acids (DCA, GCA, TCA) as novel biomarkers in individuals undergoing Drug-Induced Liver Injury (DILI) caused by anti-tuberculosis treatment (ATT), with a specific focus on assessing the severity of the disease.

Methods: Patients were categorized into two cohorts, Control group and the DILI group. The Control group comprised adults who had been exposed to ATT but did not develop DILI. The evaluation of Cytochrome c, Aldehyde Dehydrogenase 1A1 (ALDH1A1), and Argininosuccinate Synthase (ASS1) levels using ELISA kits. The quantification of three individual bile acids (IBA), namely deoxycholic acid (DCA), glycocholic acid (GCA), and taurocholic acid (TCA), was performed using the LC-MS/MS method.

Results: 90 patients were enrolled (42 control and 48 DILI). Cholestatic injury were observed in 39.5%, hepatocellular in 37.5%, and mixed-type in 22.9% patients. Jaundice was present in 29.1% patients. The mean concentration of GCA and TCA were significantly higher in patients with drug-induced liver injury (DILI) compared to the control group (7698 ng/mL vs control [599.3 ng/mL], and 4886 ng/mL vs control [97 ng/mL], p < 0.05, respectively). Conversely, DCA levels were significantly lower in the control group (24.1 ng/mL vs control [58.3 ng/ml], p < 0.05). Bile acid levels exhibited significant differences based on the severity of DILI. The levels were GCA- 2951.5 ng/ml vs 14543.5 ng/ml; TCA- 1051.4 ng/ml vs 10708.9 ng/ml; DCA- 32.1 ng/ml vs 16.1 ng/ml as compared to mild vs severe DILI patients.

The mean concentration of Cytochrome c and ALDH1A1 were significantly higher in patients with drug-induced liver injury (DILI) compared to the control group (216.7 ng/L vs control [173.7 ng/L], and 116.3 ng/L vs control [50.9 ng/L], p < 0.05, respectively). While ASS1 showed no significant difference compared to the control group (166.4 ng/L vs control [142.8 ng/L]).

Conclusion: In summary, our findings suggest that Cytochrome c, ALDH1A1, DCA, GCA, and TCA exhibit promise as biomarkers for anti-tuberculosis treatment-induced liver injury (ATT-DILI). These markers demonstrate potential and could be employed for diagnostic purposes in instances of drug-induced liver injury (DILI), as well as for evaluating the severity of the associated disease.

PP080. 3-mercaptopyruvate sulfurtransferase (MPST) is required for the cardioprotective action of thiosulfate

Ravani S^{1,2}, Papapetropoulos A^{1,2}

¹Center of Clinical, Experimental Surgery & Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, ²Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Acute myocardial infarction (AMI) is responsible for millions of fatalities worldwide annually. Recent preclinical studies have highlighted the cardioprotective effect of sodium thiosulfate (STS) in myocardial ischemia/reperfusion injury [1]. Thiosulfate, a clinically used drug, is a breakdown product of the signaling molecule hydrogen sulfide (H₂S) but has also been shown to generate H₂S. 3-mercaptopyruvate sulfurtransferase (MPST), is a H₂S-producing enzyme with strong expression in the myocardium capable of converting thiosulfate to H₂S in vitro in the presence of reducing agents or hypoxic conditions [2,3]. In this study, we aimed to investigate whether STS-induced cardioprotection depends on MPST.

Methods: 8-week-old C57BL/6J WT and Mpst^{-/-} mice (n=5/group) anesthetized with ketamine/xylazine intraperitoneally were subjected to 30 min occlusion of the left anterior descending coronary artery followed by 120 min reperfusion. STS (45,6 mg/kg) was dissolved in water for injection and administered intravenously 10 min prior to reperfusion. In different groups of animals, the H₂S donors Na₂S: 1 μmol/kg, GYY4137: 25 μmol/kg and AP39: 0,25 μmol/kg were similarly administered. Infarct size was estimated by 2,3,5-triphenyltetrazolium staining, while the area at risk was calculated using Evans blue.

Results: WT mice exhibited an infarct size of 37,65±2,31% (infarcted area/area at risk). Administration of STS to WT mice significantly reduced the infarcted area (12,21±3,57%). STS's efficacy in reducing infarct size was comparable to other H₂S donors; infarct size after administration of the fast H₂S releasing donor Na₂S was 14,03±2,69%, while GYY-4137 a slowly releasing H₂S donor reduced infarct size to 16,16±1,47%. The mitochondrial donor AP39 attenuated infarct to 9,66±4,43%. No differences were observed in the area at risk/all among groups. Consistent with previous reports from our group, genetic ablation of Mpst attenuated myocardial injury (27,03±3,06%). STS administration to Mpst^{-/-} mice did not further reduce infarct size (26,18±1,78%) compared to vehicle-treated Mpst^{-/-} Control group (mean±SD, one-way ANOVA, significance p<0.05, Tukey's post hoc test)

Conclusion: We conclude that MPST is required for the cardioprotective effect of STS in myocardial ischemia/reperfusion injury, possibly through H₂S generation.

[1] Ravindran S, et al. Sodium thiosulfate post-conditioning protects rat hearts against ischemia reperfusion injury via reduction of apoptosis and oxidative stress. Chem Biol Interact. 2017;274:24-34.

[2] Mikami Y, et al. Thioredoxin and dihydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide. Biochem J. 2011;439(3):479-485.

[3] Olson KR, et al. Thiosulfate: A readily accessible source of hydrogen sulfide in oxygen sensing. Am J Physiol - Regul Integr Comp Physiol. 2013;305(6):592-603.

PP081. Adenosine Receptor Modulation in Asthma Combination Therapy Adenosine Blockers: Bronchial Reactivity

Mustafa L¹, Cvetkovski A¹, Havolli M¹, Islami H¹, Voca Abazi F¹, Alidema F¹, Hoti A¹, Spahiu R¹

¹*Iliria College, Prishtina, Kosovo*

Bronchial asthma is a chronic respiratory disorder characterized by airway inflammation, bronchial hyperresponsiveness, and reversible airflow obstruction. Despite advances in therapy, asthma remains a significant global health burden, with increasing prevalence worldwide. Current research suggests that adenosine receptors play a crucial role in the pathogenesis of bronchial asthma, with their activation contributing to inflammation and bronchoconstriction. This study aims to investigate the synergistic effects of adenosine receptor blockers and bronchodilators in the management of bronchial asthma. The study protocol involves the administration of methylxanthines (such as bambamifylline) and other bronchodilators, including beta2-adrenergic receptor agonists, anticholinergics, and antihistamines, both individually and in combination with adenosine receptor blockers. A total of 60 patients aged 15 to 65 years will be enrolled in the study and divided into three groups based on their bronchial reactivity: non-reactive, moderate reactive, and highly reactive. Pulmonary function parameters will be assessed using body plethysmography before and after treatment with the study medications. Specific airway resistance and conductance will be calculated to evaluate bronchial reactivity. The study will employ a statistical analysis to compare the effectiveness of different treatment regimens in improving bronchial reactivity. Any adverse effects will be closely monitored, and therapy will be discontinued immediately in case of serious adverse events. The anticipated results of this study hold significance for optimizing asthma management strategies. By elucidating the synergistic effects of adenosine receptor blockers and bronchodilators, the study aims to provide insights into more effective therapeutic approaches for bronchial asthma. These findings may ultimately inform clinical practice and contribute to better outcomes for patients with respiratory diseases. In conclusion, this study addresses an important gap in current asthma therapy by investigating the potential synergistic effects of adenosine receptor blockers and bronchodilators. The results may pave the way for personalized and more efficacious treatment strategies for bronchial asthma, ultimately improving the quality of life for affected individuals.

PP082. The Impact of GLP-1R Agonist Liraglutide on Oxidative Stress in Human Venous and Arterial Endothelial Cells under Hyperglycemic Conditions

Zeybek E¹, Gökçe M^{1,2}, Civelek D², Şen A¹, Doğan S¹, Alp Yıldırım J¹

¹*Istanbul University Faculty of Pharmacy, Department of Pharmacology, Istanbul, Turkey,*

²*Bezmialem Vakif University Faculty of Pharmacy, Department of Pharmacology, Istanbul, Turkey*

Aim: Endothelial dysfunction, altered cell signaling, increased oxidative stress, and activation of proinflammatory processes are the molecular mechanisms responsible for diabetic vascular complications. This study aimed to investigate the effect of GLP-1R agonist Liraglutide, an antidiabetic agent, on oxidative stress in human venous and arterial endothelial cell cultures comparatively, under hyperglycemic conditions.

Materials and Methods: Human umbilical vein endothelial cells (HUVEC) and human coronary artery endothelial cell (HCAEC) cultures were exposed to Liraglutide (10 nM or 100 nM) under normoglycemic (5.5 mM) or hyperglycemic (25 mM) conditions, for 48 hours. Oxidative stress was evaluated by total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI).

Results: Hyperglycemic environment and/or Liraglutide incubation did not cause any significant change in TAS levels in HUVEC cultures. On the other hand, concomitant exposure to hyperglycemic conditions and 10 nM Liraglutide, reduced the TOS levels compared to hyperglycemic condition alone. In HUVEC culture, hyperglycemic environment increased OSI levels and surprisingly, Liraglutide incubation concentration-dependently caused an additional increase in OSI levels. In regard to HCAEC, hyperglycemic environment decreased TAS levels, while 100 nM Liraglutide incubation reversed the increment in TAS levels. Hyperglycemic environment and/or Liraglutide incubation did not cause any change in TOS levels. In HCAEC, hyperglycemic condition increased OSI levels, while 10 nM Liraglutide reduced hyperglycemia induced increase in OSI levels, in contrary to HUVEC.

Conclusion: This study suggests that, Liraglutide may have a potential impact to reduce cellular oxidative stress induced by hyperglycemia, while this effect vary between human venous and arterial endothelial cells.

PP083. Effect of resveratrol on the pharmacokinetics of everolimus in rats: a drug-phytochemical interaction

Ozturk Seyhan N¹, Unal Adiguzel B¹, Ozturk Civelek D², Sancar S³, Akyel Y⁴, Okyar A¹

¹*Department of Pharmacology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey,*

²*Department of Pharmacology, Faculty of Pharmacy, Bezmialem Vakif University, Istanbul, Turkey,*

³*Department of Biology, Faculty of Science, Istanbul University, Istanbul, Turkey, ⁴Department of Medical Pharmacology, School of Medicine, Istanbul Medipol University, Istanbul, Turkey*

Introduction: Today, there is a rising trend in the use of resveratrol, both as a food supplement and a complementary/alternative treatment especially in the elderly with multiple chronic diseases. This increases the risk of drug-resveratrol interactions, particularly when used with drugs with narrow therapeutic index like everolimus. Resveratrol is an orally-used antioxidant with its anti-aging, anti-inflammatory, cardioprotective/neuroprotective, immunomodulatory and anti-tumor effects [1]. Everolimus is widely used orally in organ transplantation with its immunosuppressant effects, and in the treatment of various cancers with its anti-tumor effects [2]. Since everolimus is metabolized by CYP3A4 enzyme and transported by P-glycoprotein efflux transporter, co-administration with resveratrol, a phytochemical capable of modulating these proteins, may lead to clinically significant drug interactions [3]. Therefore, in this study we aimed to investigate the effect of resveratrol on everolimus pharmacokinetics in rats.

Methods: Everolimus (5 mg/kg), alone and with resveratrol at low (50 mg/kg) and high doses (100 mg/kg) was administered orally to male Wistar rats (n=8 per groups). Both drugs were dissolved in 30:70 propylene glycol:distilled water. Blood samples were collected at 0.5, 1, 2, 4, 8 and 24h following last dose. Everolimus levels in whole blood were determined by high-performance liquid chromatographic/tandem mass spectrometric assay (LC-MS/MS). Pharmacokinetic parameters were calculated, and statistical analysis was performed with one way-ANOVA.

Results: Resveratrol, increased the oral bioavailability of everolimus. Compared to the control, Cmax of everolimus elevated 35.05% (p<0.05), and 39.60% (p<0.01); plasma AUC0-24h increased 38.51% (p=0.057) and 53.86% (p<0.01) following low and high single doses of resveratrol co-administration, respectively. Tmax did not changed and was calculated as 1h in all experimental groups.

Conclusions: In conclusion, orally-administered resveratrol significantly increased the intestinal absorption and exposure of everolimus suggesting the inhibitory effect on P-glycoprotein-mediated drug efflux and CYP3A4-mediated metabolism in the intestine and/or liver. If these results would be confirmed in clinical experiments, the dosage of everolimus should be readjusted when it is used concomitantly with resveratrol.

*The present work was supported by the Research Fund of Istanbul University. Project No. TSA-2022-38734.

[1] Guthrie AR, Sherry Chow HH, Martinez JA. Effects of resveratrol on drug- and carcinogen-metabolizing enzymes, implications for cancer prevention. *Pharmacol Res Perspect*. 2017;5(1):e00294.

[2] de Fijter JW. Cancer and mTOR Inhibitors in Transplant Recipients. *Transplantation*. 2017;101(1):45-55.

[3] Booth J, Robeva A, Rosamilia M. Everolimus (RAD001) Investigator Brochure, 2010, Edition 10, Novartis.

PP084. Aripiprazole potentiates the anticancer activity of paclitaxel in NSCLC in vitro and in vivo models

Andriopoulou C¹, Kofinas A¹, Koutsogianni F², Epeslidou E¹, Leondaritis G¹, Dimas K², **Konstandi M¹**

¹Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece, ²Department of Pharmacology, Faculty of Medicine, University of Thessaly, Larissa, Greece

Introduction: Lung cancer is the second most frequent cancer worldwide, which accounts for 75-80% of lung cancer-related deaths¹. Several epidemiological studies reported significantly lower cancer incidence in male schizophrenic patients following antipsychotic treatment compared to general population, although these patients are usually heavy smokers and adopt dietary habits that are largely related to carcinogenicity^{2,3}.

Aim: The present study investigated the potential anticancer effect of aripiprazole (ARP) and its role in the anticancer effect of paclitaxel (PTX), a standard anticancer drug in the treatment of non-small cell lung cancer (NSCLC).

Methods: Different NSCLC cell lines were treated with ARP alone or in combination with PTX. Cell proliferation was assessed using the SRB test and the induction of apoptosis was evaluated by the Annexin V-PI protocol. The impact of the combined treatment was also assessed using an in vivo murine model, the NSCLC H460-xenograft model developed in NOD/SCID mice, and its effect on cancer-related signaling pathways was assessed using Western Blot analysis.

Results: The in vitro study indicated that ARP markedly enhanced the PTX-mediated reduction of H460 cell population ($P<0.001$, $n=12$). Furthermore, ARP strengthened the PTX-mediated repression of tumor size and weight in the H460 xenografts ($P<0.05$, $n= 50$). Notably, sub-therapeutic doses of ARP reduced the IC₅₀ of PTX. The enhanced anticancer effect of the combined treatment appears to be mediated by activation of the PI3K/AKT/GSK3/pS6 and MAPK-linked pathways.

Conclusion: Present data suggest that ARP potentially displays anticancer properties by inducing apoptotic mechanisms via activation of the PI3K/AKT/GSK3/pS6 and MAPK-linked pathways. Furthermore, ARP and PTX, when administered in combination even in sub-therapeutic doses, show a strong anticancer effect. This finding, if confirmed in the framework of clinical studies, could ensure an improved anticancer outcome and side effect profile in patients with NSCLC.

PP085. Pterostilbene directly promotes vasodilation in the abdominal inferior vein cava

Zogu M¹, Daci A¹, Elshani A¹

¹*University of Prishtina "Hasan Prishtina", Prishtina, Kosovo*

Background: Stilbene derivatives have demonstrated the ability to elicit vasodilation, alongside possessing antioxidant, anti-inflammatory, anti-cancer, anti-ageing, as well as a range of additional beneficial properties. Pterostilbene (PTS), a dimethyl ether analog of resveratrol, has been shown to provide these positive effects. There has been a study conducted in the thoracic aorta, showing the endothelium-dependent relaxation promoted by PTS. However, there is a lack of information covering the direct effect of this phytonutrient on the venous tone, or more precisely on the tone of the Inferior Vena Cava (IVC).

Aims: Our objective was to explore the direct effect of PTS on the abdominal Inferior Vena Cava (IVC) isolated from rats.

Methods: Abdominal IVC was isolated in small segments from Wistar rats and was prepared for the tissue organ bath apparatus. The direct vasodilatory effect of PTE (10-7 – 3x10-5M) was tested in venous tissues precontracted with endothelin-1 (ET-1 1nM). The test was conducted in the absence or presence of inhibitors as follows: non-selective K⁺ channel inhibitor - tetraethylammonium chloride (TEA 5mM), non-selective NO-synthetase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME 2x10-4 M), selective soluble guanylyl cyclase inhibitor 1h-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ 10-5M) and cyclooxygenase inhibitor indomethacin (10-5M).

Results: PTE has demonstrated that it can completely relax the abdominal IVC to its baseline tone (Emax=100%). Its relaxation activity was diminished in the presence of NO and non-selective potassium channel inhibitor (PTE: pEC50=5.113±0.06, versus pEC50= 5.708±0.07, P<0.001).

Conclusion: PTE has stimulated vasodilation in the abdominal IVC which was precontracted with ET-1. This effect may partially involve the NO-cGMP pathway and K⁺ channels.

PP086. Oleoylethanolamide attenuates the cardio-renal-metabolic alteration secondary to obesity

Comella F¹, Pirozzi C¹, Aragón Herrera A², Lama A¹, Annunziata C¹, Melini S¹, Opallo N¹, Meli R¹, **Mattace Raso G¹**

¹University of Naples, Federico II, Naples, Italy, ²Cellular and Molecular Cardiology Unit and Department of Cardiology, Institute of Biomedical Research of Santiago de Compostela (IDIS-SERGAS), Santiago de Compostela, Spain

The cardio-renal-metabolic (CRM) syndrome is a serious healthcare issue globally, with high morbidity and mortality. Metabolic alterations, especially obesity, exert pathophysiological effects on the heart and kidneys, including hyperglycemia, insulin resistance, lipotoxicity, chronic inflammation and fibrosis [1]. Peroxisome proliferator-activated receptor (PPAR)- α is a member of ligand-activated transcription factors and is mainly involved in regulating inflammation, fibrogenesis, and lipid oxidation [2]. Oleoylethanolamide (OEA) is an endogenous mediator belonging to the N-acylethanolamines family. OEA, a PPAR- α high-affinity agonist, controls feeding behaviour, body weight, and energy homeostasis [3]. This study aimed to investigate OEA's potential in counteracting cardio-renal metabolic alterations secondary to obesity and metabolic syndrome.

to this purpose, C57BL/6J male mice were fed with a high-fat diet (HFD) for 12 weeks to acquire the obese phenotype and secondary cardio-renal-metabolic alteration. Then, mice were treated with OEA (2.5mg/kg/day) for eight weeks along with HFD feeding.

In obese mice, OEA treatment reduced the body weight measured throughout the experimental period compared to untreated HFD mice. Before sacrifice, we performed the OGTT, and the glycaemia levels in HFD mice peaked at approximately 300 mg/dL, whereas OEA-treated mice showed an improvement in glucose disposal. Long-term HFD feeding determined an alteration of kidney functionality, while OEA treatment restored serum creatinine, BUN and markers of tubular damage such as NGAL and kidney injury molecule 1. It's well known that obesity induces the secretion of many cytokines and chemokines and the production of profibrotic mediators that contribute to fibrosis both in the heart and kidney. OEA showed a marked anti-inflammatory and antifibrotic effect as showed by histological analysis, Hematoxylin&Eosin and Masson's Trichrome staining of cardiac and renal tissues, as further confirmed by the reduced transcription of pro-inflammatory and pro-fibrotic markers. Furthermore, OEA normalizes cardiac metabolic factors, modulating tissue lipid profile by increasing the expression of fatty acid translocase CD36 and regulating glucose homeostasis by the activation of the AMPK/AKT/AS160 pathway. Contemporary OEA improves renal lipid metabolism, regulating the expression of fatty acid-binding protein 4, which plays a key role in regulating lipid trafficking.

These results indicate that OEA may be a promising molecule for treating cardio-renal-metabolic alterations, limiting the molecular mechanisms associated with inflammation, fibrosis, glucose and lipid metabolism secondary to obesity and other metabolic disorders.

- [1] Akiyama H. et al., Front Endocrinol. 2023, doi: 10.3389/fendo.2023.1111984
- [2] Lin Y. et al., Front Endocrinol 2022, doi: 10.3389/fendo.2022.1074911
- [3] Bowen KJ. et al., Prog Lipid Res. 2017 doi: 10.1016/j.plipres.2017.04.001

PP087. Role of elastase-2 in mice abdominal aortic aneurysms: a novel finding in disease pathogenesis

Mestriner F¹, Fora Dugaich V¹, Brüch Dantas P¹, Z. Kovacs H¹, Serra Ribeiro M¹, Becari C²

¹Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil, ² Department of Biological Science, School of Dentistry of Bauru, Bauru, Brazil

Introduction: The abdominal aortic aneurysm (AAA) is pathological dilation in abdominal aorta that can lead to death, and no pharmacological treatment other than repair surgery. The continuous angiotensin II (AngII) infusion induces AAA in mice¹. Elastase-2 (ELA-2), a chymotrypsin-serine protease elastase family member 2A, is an Ang II enzyme in arteries and contributes to resistance arteries and vascular remodeling in animal models². Therefore, ELA-2 is a candidate factor contributing to AAA formation and/or development. We sought to investigate the expression of ELA-2 in the AAA mice.

Methods: Male wild type (C57bl/6, WT) and ELA-2 knockout (CELA-2aTm1Bdr; ELA-2KO) mice treated with saline (WT+Sal n=10, ELA-2KO+Sal, n= 9) or AngII (1.500ng/kg/min, WT+AngII n=8, ELA-2KO+AngII n=8) for twenty-eight days by subcutaneously mini-pumps infusion. Vascular ultrasonography (US, Vevo 2100) analyzed, before and after treatment, aortic expansion index, aortic systolic and diastolic diameter parameters. Aorta RNAm ELA-2 expression was done by real-time PCR. The histological parameters were performed. Data are shown mean±SEM. Statistical analyses using t-test or one-way-ANOVA or Kruskall-Wallis.

Results: WT+Ang II mice developed AAA, and ELA-2KO mice did not have AAA by infusion of Ang II. Aorta ELA-2 expression up-regulated in WT-AngII vs. WT+Sal ($p=0.0001$). Aortic systolic diameter increased in the WT+AngII vs WT+Sal (0.8vs.1.5mm, $p<0.0001$). Aortic diastolic diameter increases in the WT+AngII vs WT+Sal (1.2 vs.0.6mm, $p<0.0001$). Aortic expansion index decreases in WT+ Ang II vs WT+ Sal (11.4 vs. 17.1%, $p=0.0008$). WT+Ang II aorta has higher collagen deposition and fibrosis and lower elastin than WT+Saline ($p=0.001$). ELA-2KOAngII vs. ELA-2SAL showed no difference in elastin, collagen, and fibrosis.

Conclusion: ELA-2 expression is up-regulated in AAA mice's aorta and may contribute to developing aneurysms. ELA-2KO might be less susceptible to developing AAA induced by Ang II infusion. ELA-2 plays a relevant role in AAA pathophysiology and could be a target for therapeutic treatment to prevent AAA development.

Funding: FAPESP (# 2017/21539-6; 2018/23718-8, 2019/11485-1), CAPES, FAEPA.

- [1] Becari C et al. Elastase-2, an angiotensin II-generating enzyme, contributes to increased angiotensinII in resistance arteries of mice with myocardial infarction. Br J Pharmacol. 2017;174(10):1104-1115. Doi:10.1111/bph.13755
- [2] Lu H, et al. Subcutaneous AngiotensinII Infusion using Osmotic Pumps Induces Aortic Aneurysms in Mice. J Vis Exp. 2015;(103). Doi:10.3791/53191

PP088. Effect of time span between exposing enamel slabs to calcium lactate and sodium fluoride on enamel uptake of alkali soluble fluorides

Kullashi Spahija F¹, Sutej I¹, Basic K¹, Spahija K², Peros K¹

¹*University of Zagreb School of Dental Medicine, Zagreb, Croatia*, ²*Dental Polyclinic Center, Peja, Kosovo*

Introduction: Pretreatment with calcium lactate when used prior to sodium fluoride solution enhances the enamel uptake of alkali-soluble fluoride. This study aimed to establish the effect of time span between exposing enamel slabs to calcium lactate and to sodium fluoride, on enamel uptake of alkali soluble fluorides.

Materials: All experimental procedures were conducted in accordance with the Declaration of Helsinki's recommendations guiding physicians in biomedical research. This blind and randomized in vitro study was approved by The Ethics Committee of the School of Dental Medicine University of Zagreb under the protocol number(05-PA-30-17-4/2023).Total of 10 non-carious human wisdom teeth, extracted for orthodontic reasons were used. Each tooth was cut into 4 slabs and randomly allocated into one of four treatments groups as follow(4x10): group "a" was treated with calcium lactate solution followed by sodium fluoride solution with minimal delay (just drying between the two treatments),group "b" was treated with calcium lactate solution followed by sodium fluoride solution with delay of one hour(leaving time for calcium to eventually adhere or penetrate the enamel),group "c" was treated with sodium fluoride only, as positive control. Last group "d" was negative control group-the group without treatment. Extraction of alkali-soluble fluoride from enamel slabs was made using 1M KOH solution for 24h and under agitation of the shaker at the room temperature, by method of Caslavská[1].The extracts were analyzed using fluoride ion-specific electrode(Orion Research EA 940) by ISO 19448:2018 standard method. Wilcox matched pairs test and Friedman ANOVA were used to analyze the effect of substrate and treatments.

Results: There were no statistically significant differences observed between any pair of treatment groups as indicated by the p-values being greater than 0.05 in all comparisons(0.635 ± 0.443 vs 0.661 ± 0.838 vs 0.382 ± 0.18 vs $0.7052 \pm 0.241 \mu\text{g F/cm}^2$,for groups Ca lactate with NaF, Ca lactate with delayed NaF, NaF only, no treatment; respectively).The only statistically significant difference was observed between enamel slabs treated with sodium fluoride only(group c) and those in the negative control group(group d) with a p-value of 0.028, that was expected effect of sodium fluoride.

Conclusions: The time span between exposing enamel slabs to calcium lactate and sodium fluoride did not significantly affect enamel uptake of alkali soluble fluorides. Based on these findings, it is suggested that the sodium fluoride products may be applied immediately after calcium lactate.

- [1] Caslavská V, Moreno EC, Brudevold F. Determination of the calcium fluoride formed from in vitro exposure of human enamel to fluoride solutions. Archives of Oral Biology,1975; doi:10.1016/0003-9969(75)90023-0.

PP089. The comparative study of the rapid (IKr) and slow (IKs) delayed rectifier potassium currents in undiseased human, dog, rabbit and guinea pig cardiac ventricular preparations

Jost N¹, Agoston M¹, Kohajda Z², Virág L¹, Bitay M¹, Papp J¹, Varro A^{1,2}

¹*University of Szeged, Albert Szent-Gyorgyi Medical School, Szeged, Hungary*, ²*HUN-REN-SZTE Research Group for Cardiovascular Pharmacology, Hungarian Research Network, Szeged, Hungary*

To understand the inter-species variation in the drug effect on repolarization, the properties of the rapid (IKr) and the slow (IKs) components of the delayed rectifier potassium currents were compared in myocytes from undiseased human donor (HM), dog (DM), rabbit (RM) and guinea pig (GM) ventricles by applying the patch-clamp techniques at 37 °C. The amplitude of the E-4031 sensitive IKr tail current measured at -40 mV after a 1 s long test pulse to 20 mV, was similar in HM and DM (0.35 ± 0.07 and 0.38 ± 0.02 pA/pF, respectively, n=12-15) but larger in RM and GM (0.66 ± 0.05 pA/pF and 1.0 ± 0.08 pA/pF, respectively, n=10). The L-735,821 sensitive IKs tail current was larger in GM (amplitude at -40 mV, after a 5 s long test pulse to 50 mV was 3.3 ± 0.6 pA/pF, n=10) than in RM (1.22 ± 0.7 pA/pF, n=7) and DM (0.9 ± 0.05 pA/pF, n=24). In HM IKs tail was even smaller than in DM (0.2 ± 0.05 pA/pF, n=14). IKr activated rapidly and monoexponentially in each studied species. The activation time constants measured at 30 mV were: 36 ± 3 ms in HM, 53 ± 6 ms in DM, 35 ± 3 ms in RM and 30 ± 2 ms in GM, respectively (n=6-26). The deactivation of IKr in HM, DM and RM measured at -40 mV, after a pulse to 30 mV was slow and biexponential ($\tau_1 = 0.6 \pm 0.05$ s and $\tau_2 = 6.7 \pm 0.9$ s in HM; $\tau_1 = 0.4 \pm 0.02$ s and $\tau_2 = 3.3 \pm 0.3$ s in DM; $\tau_1 = 0.6 \pm 0.03$ s and $\tau_2 = 6.5 \pm 0.3$ s in RM, respectively, n=8-26), while in GM the IKr current was best fitted triexponentially ($\tau_1 = 0.14 \pm 0.01$ s, $\tau_2 = 0.8 \pm 0.01$ s and $\tau_3 = 6.6 \pm 0.06$ s, n=10). IKs measured at 30 mV, activated slowly and had apparently a monoexponential time course in HM, DM and RM ($\tau = 0.9 \pm 0.2$ s in HM, $\tau = 1 \pm 0.1$ s in DM, and $\tau = 0.8 \pm 0.05$ in RM, respectively, n=6-21). In contrast, in GM the activation was biexponential ($\tau_1 = 0.5 \pm 0.02$ s and $\tau_2 = 3.2 \pm 0.01$ s, n=10). In HM, DM and RM IKs deactivation measured at -40 mV, was fast and monoexponential ($\tau = 0.15 \pm 0.02$, $\tau = 0.14 \pm 0.01$ s and $\tau = 0.16 \pm 0.05$, respectively, n=6-22), while in GM, in addition to the fast component ($\tau_1 = 0.16 \pm 0.01$ s, $A_1 = 860 \pm 98$ pA) an another slower component was also revealed ($\tau_2 = 0.6 \pm 0.1$ s, $A_2 = 670 \pm 79$ pA, n=10). These results suggest that IK in HM resembles that measured in DM and RM, and considerably differs from that observed in GM. These findings suggest that the dog and rabbit are more appropriate species than the guinea pig for preclinical evaluation of new potential drugs expected to affect cardiac repolarization.

PP090. The Effects of Curcumin and Nicorandil on ECG-Telemetered Findings of Nilotinib Treated Rats

Harmancı N¹, Kaltus Z^{1,2}, Eroglu E^{1,3}, Cengelli Unel C¹, Yigitaslan S¹, **Sırmagül B¹**

¹Eskişehir Osmangazi University Department of Medical Pharmacology, Eskisehir, Turkey, ²Eskişehir City Hospital Ethics Committee, Eskisehir, Turkiye, ³Turkish Medicines and Medical Devices Agency Clinical Research Department, Ankara, Turkiye

Introduction: Long QT syndrome(LQTS) is a syndrome characterized by QT prolongation, arrhythmia, and sudden death [1]. Nilotinib(Nilo) is a TKI used in the treatment of imatinib-resistant CML that can cause LQTS through KATP inhibition. Curcumin(Curc), which is frequently used together due to its anti-tumor effect, also inhibits KATP. Nicorandil is a dual-acting antianginal that also has a KATP activator effect[2]. The study aimed to investigate the effects of curcumin and nicorandil on QTc prolongation caused by nilotinib.

Methods: The radiotelemetry transmitter was placed into the abdominal cavity of 74 adult male Sprague-Dawley rats.(Ethics approval no: 2018/681-1) Nilo was administered(p.o) at doses of 10,30, and 50mg/kg(n=3). ECG data of Nilo(10mg/kg, n=3), Curc(100mg/kg,n=3) and Nico(10mg/kg,n=3) were recorded. Rats were divided into 7 groups: Control, Nilo, Curc, Niko, Nilo+Curc, Nilo+Nico, Nilo+Curc+Nico. ECG parameters were recorded at 1000 Hz in 15-minute periods. Heart tissue samples were collected. One-way analysis of variance following Tukey's test were used for statistical analysis. p<0.05 was considered as significant.

Results: Nilotinib significantly prolonged QTc interval at increasing doses($p=0.03$, $p<0.001$, $p<0.001$). The lowest significant dose was determined as 10mg/kg. Nilo(10mg/kg) caused significant change in 3rd hour and Curc(100mg/kg) at the 60th minute($p<0.001$). QTc interval increased significantly in all groups except the Nico group compared to the control group($p<0.001$). There was a significant increase in the Nilo+Curc group and a significant decrease in the Nilo+Nico group($p<0.001$) compared to the Nilo group. There was a significant increase in the Nilo+Curc group compared to the Curc group, and a significant decrease in the Nilo+Curc+Nico group compared to the Nilo+Curc group($p<0.001$). TNF- α ($p<0.05$) and TAS($p<0.001$) increased significantly in the Nilo group, while they decreased in combination groups compared to the control. There was no histological alteration.

Conclusion: Nilotinib significantly prolonged the QTc interval in a dose-dependent manner, and the addition of curcumin increased this effect. Nicorandil appeared to have a reversal effect on long QT. These results suggest that nicorandil may be a potential agent in the treatment of LQTS.

Supported by BAP commission (2018-2319).

- [1] Shah, S.R., K. Park, and R. Alweis, Long QT Syndrome: A Comprehensive Review of the Literature and Current Evidence. *Curr Probl Cardiol*, 2019. 44(3): p. 92-106.
- [2] Takahashi, N., et al., Clinical suppression of bradycardia dependent premature ventricular contractions by the potassium channel opener nicorandil. *Heart*, 1998. 79(1): p. 64-8.

PP091. The influence of CYP2C19 polymorphism on the safety and efficacy of voriconazole in pediatric patients with hematologic malignancy receiving voriconazole treatment due to an invasive fungal infection

Gumustekin M¹, Ertem O¹, Karadas N², **Kantarci M¹**, Yilmaz S³, Karapinar D², Oren H³, Pirmohamed M⁴

¹*Department of Medical Pharmacology, Faculty of Medicine, Dokuz Eylul University, Izmir, Türkiye,*

²*Department of Pediatric Hematology, Faculty of Medicine, Ege University, Izmir, Türkiye,*

³*Department of Pediatric Hematology, Faculty of Medicine, Dokuz Eylul University, Izmir, Türkiye,*

⁴*Department of Clinical Pharmacology, Faculty of Medicine, Liverpool University, Liverpool, United Kingdom*

Introduction: Voriconazole is used to the first line therapy for the treatment of invasive fungal infections in children. Voriconazole is primarily metabolized by cytochrome P450 (CYP) 2C19, and more than 30 variants in its gene have been identified. Since it has a narrow therapeutic window, the high inter/intra-individual variability and nonlinear pharmacokinetics, therapeutic drug monitoring (TDM), if possible, pharmacogenetic-guided dose selection are recommended in pediatric patients to optimize clinical outcomes.

Objective: We aimed to evaluate the impacts of voriconazole drug monitoring and CYP2C19 polymorphisms on the efficacy and safety of voriconazole in pediatric patients.

Methods: This is two centre, observational cohort study. Proper approval and permissions have been obtained from Clinical Research Ethics Committee of Dokuz Eylul University and the Ministry of Health. Written informed consent was obtained from the children (if possible) and their parents. Patients (2-18 years old) were recruited from the Pediatric Hematology Service of Dokuz Eylul and Ege University Hospital. Data collection and follow-up were performed from medical records. Two blood samples were taken from the patients for TDM (just before the 9th dose of voriconazole) and pharmacogenetics analysis.

Results: 57 patients were included in the study. Serum voriconazole levels were found to be subtherapeutic in 24 patients, within the therapeutic range in 17 patients, and at the supratherapeutic level in 8 patients (therapeutic range 1-5.5 mcg/mL). Adverse events occurred in 13 patients. Hallucination (n=2), skin findings and green vision (n=1) and elevated liver function tests were observed. Treatment was discontinued in 3 patients (due to hallucinations and LFT elevation). Analysis of variations in the 2*, 3* and 17* alleles in the gene encoding the CYP2C19 enzyme that metabolizes voriconazole is ongoing.

Conclusion: Serum trough voriconazole levels appears to be within the therapeutic range in only 31% of patients. We will be able to more clearly demonstrate the effects of pharmacogenetic variations and TDM on the effectiveness and safety of voriconazole in our pediatric patients after data analysis and pharmacogenetic analyzes are completed.

[1] Hope W, Johnstone G, Cicconi S, et al. Software for Dosage Individualization of Voriconazole: a Prospective Clinical Study. *Antimicrob Agents Chemother*. 2019;63(4):e02353-18.

[2] Sharad N, Singh G, Xess I, et al. Therapeutic Drug Monitoring of Voriconazole in Children with Hematologic Malignancy and Invasive Fungal Infections: An RCT from a Tertiary Care Centre in India. *Cardiovasc Hematol Disord Drug Targets*. 2023;23(4):285-292.

PP092. How to tackle antimicrobial resistance: A novel approach to inspire University students

Lopez-Cadenas C¹, Carrera-Gonzalez C², Diez-Laiz R¹, Fernandez-Dominguez J³, Fernandez-Martinez N¹, Garcia-Sierra J⁴, Gutierrez-Martin C⁵, Lopez-Rodriguez C⁶, de la Puente Garcia R¹, Rubio-Langre S⁷, San Andres-Larrea M⁷, **Sahagun-Prieto A¹**

¹Biomedical Sciences, Institute of Biomedicine (IBIOMED) University of Leon, Leon, Spain,

²Pharmacology, Pharmacy & Pharmaceutical Technology, University of Santiago de Compostela, Lugo, Spain, ³Labor and Social Security Law, University of Leon, Leon, Spain, ⁴Mechanical, Informatics, Aerospace & Computers Technology Engineering, University of Leon, Leon, Spain,

⁵Animal Health, University of Leon, Leon, Spain, ⁶Castilla & Leon Health Department, Leon, Spain, ⁷Pharmacology & Toxicology, Complutense University, Spain

Introduction: Antimicrobial resistance (AMR) is a complex and critical global problem affecting human, environmental, and animal health¹. It is important to frame it within an integrated and multifarious approach such as the One Health perspective, to better fight against it. In this context, we present the preliminary results of a Jean Monnet Action focused on AMR and supported by the European Commission, aimed to university students of Health Sciences at the University of Leon (Spain), as a way to increase their awareness on this problem.

Methods: The European Action was structured as an annual course with a monthly lecture (in-person or online). In the first half of the course (five months), the students have attended 5 lectures related to AMR, which were explained and discussed from different points of view. They also developed several interactive activities during each lecture. After the first 5 months, a satisfaction survey was carried out to assess their opinion on the strategies developed. The level of satisfaction was statistically assessed (Chi squared test, $p \leq 0.05$).

Results: Forty students (80% female) were recruited and enrolled in the first annual course. They were mainly studying the Nursing (37.5%) or Veterinary Medicine degrees (37.5%). A mean of 25.3 ± 5.4 students followed the lectures and carried out the designed activities. Half of those enrolled completed the satisfaction survey (70% female). Many of the latter belonged to the Veterinary (45%) or Nursing Degrees (20%). Students were satisfied (50%) or highly satisfied (35%) with the topics of the lectures, as well as with the design of lectures and activities implemented (50% highly satisfied; 35% satisfied). The wide majority felt that lectures and the case studies were not difficult to follow (85% and 65%, respectively). A few students found difficult (20%) some concepts, but most of them had no problem during the course already given (75%). Finally, they were mostly satisfied or highly satisfied (90%). No significant differences were revealed in the answers according to students' gender or modality followed (in-person/online).

Conclusions: The course developed is an excellent opportunity to work with students of Health Sciences on the problem of AMR. The students showed a high motivation to follow and participate in the course, and a high degree of satisfaction with the activities implemented was obtained.

[1] World Health Organization (WHO). Global action plan on antimicrobial resistance. Geneva: WHO Paper; 2015.

PP093. Learning Pharmacology by ear: An approach to engage students

Diez-Laiz R, Lopez-Cadenas C¹, de la Puente-Garcia R¹, Romero-Gomez B¹, Vazquez-Acero M¹, Rodriguez-Lago J¹, Martinez-Lopez M¹, **Sahagun-Prieto A¹**

¹Biomedical Sciences, Institute of Biomedicine (IBIOMED), University of Leon, Leon, Spain

Introduction: Multimedia tools have helped to change formal education plans. Among them, the use of podcasts as educational tools has increased over the last 20 years¹. The study describes the outcomes of integrating short podcasts as supplementary resources into teaching of the subject Drug-Food Interactions to undergraduate students (Degree in Food Science and Technology).

Methods: Eight podcasts explaining various topics related to drug-food interactions were provided to students over the semester previously to theoretical classes. The number of downloads for each podcast, partial and final marks, and the students' level of satisfaction were evaluated at the end of the course to assess the success of the innovative strategy carried out. Mean values of partial and final marks were compared with those obtained the previous academic year (without podcast activity) ($p \leq 0.05$).

Results: Almost all the students decided to voluntarily take part in the activity (16 students; 94.1 % of those enrolled in the subject; 44% male). The number of downloads ranged from 8 to 14 depending on the podcast considered, whereas partial and final academic scores did from 6.7 to 9.9 points (out of 10 points) and 7.5 to 9.7, respectively. Marks were higher than those achieved the previous academic year, but only those of the first partial exam were significantly higher (Mann-Whitney U test, $p = 0.22$). Seven students answered the satisfaction survey (43.7% of those participating in the innovation strategy). Most of them (71.4%) indicated that the number of podcasts provided was adequate, and considered their difficulty level as mild. More than half (57.1%) said that the podcast length was adequate or very adequate, and the vast majority (85.7%) were satisfied with the activity carried out.

Conclusions: The findings demonstrated evidence that the integration of podcasts into the subject had a positive impact on students' motivation and academic performance.

[1] Drew C. Educational podcasts: A genre analysis. E-Learn Digit Med. 2017;14:201–11

PP094. Trends in Dispensed Opioid Analgesic Prescriptions in Croatia 2013 to 2022, is there a reason for concern?

Vranic L¹, Bašić K¹, Peroš K, Šutej I¹

¹*School of Dental Medicine University in Zagreb, Zagreb, Croatia*

Introduction: Prescription opioid abuse, associated with many opioids and especially with oxycodone, is a well-established public health crisis in developed countries like UK, US and Australia. Opioids have a limited role in general dental practice since non-steroidal anti-inflammatory drugs (NSAIDs) are superior to opioids in dental pain management. The aim of this study was to assess prescribing rates of dental opioids in 10 year period in Croatia.

Materials and Methods: Data on opioid prescribing practices for this study were provided by the Croatian Health Insurance Institute. The analysis included the number of prescriptions, costs, and the number of packages prescribed.

Results: Over the 10 years, dentists in Croatia prescribed total of 8130 opioid prescriptions, representing on average 11% of all analgesic prescriptions, and 0,2% of all dental prescriptions. The number of opioid prescriptions per 1,000 dental patients was on average 1,39. Tramadol/paracetamol and tramadol were the most prescribed opioids, with average annual number of prescriptions 622,7 (64% of opioids prescriptions) and 114,5 (11% of opioids prescriptions) respectively. Trends in opioid prescription varied, especially during pandemic years (2020-2022), when tramadol rates decreased while fixed combination medications tramadol/paracetamol and tramadol/dexaprophen increased.

Conclusion: Dentists in Croatia are restrictive and conservative in prescribing opioids, and there is no need for intervention, but because of increasing trends, observing the prescribing pattern in the future is highly recommended.

PP095. Practices in supplements use among athletes: Correlation with sociodemographic characteristics

Petrovic A¹, Srb N¹, Hefer M¹, Klaric F², Kralik K³, Smolic R¹, Vcev A¹, Smolic M¹

¹*Faculty of Dental Medicine and Health Osijek, J. J. Strossmayer University of Osijek, Croatia, Osijek, Croatia*, ²*Faculty of Kinesiology Osijek, J. J. Strossmayer University of Osijek, Croatia*, ³*Faculty of Medicine Osijek, J. J. Strossmayer University of Osijek, Croatia*

Introduction: Athletes, professionals or amateurs, constitute a demographic that frequently relies on dietary supplements, often without medical oversight. Recent research has highlighted a concerning rise in the prevalence of mislabeled supplements that contain anabolic androgenic steroids (AASs). This can result in serious AASs-induced disorders, underscoring the importance of promoting comprehensive education and vigilant oversight in the procurement of these products [1]. In our study, we investigated supplement usage practices, as well as education and acquiring of the supplements among athletes, as well as its correlation with sociodemographic characteristics. Ultimately, our aim was to provide insight into the potential need for better education among athletes regarding supplement intake.

Method: The study was designed as a cross-sectional study and included 107 athletes from professional and amateur sports clubs in Croatia. Data was collected using an anonymous questionnaire, which included questions regarding their sociodemographic characteristics and practices in use, education and purchasing of the supplements. Statistical analysis was performed accordingly.

Results: 42% participants reported using supplements. Most commonly used (40%) are protein supplements. Only 12% of the participants takes supplements based on a doctor's recommendation, while a significant 30% of the participants take supplements without any advice. The correlation between the participants' age, years of training and weekly training frequency with the use of dietary supplements showed that older participants tend to use them more for enhancing their athletic performance ($\text{Rho} = 0.324$). Longer period of training positively correlates with dietary supplements usage for performance enhancement ($\text{Rho} = 0.329$). As the weekly number of workouts increases, participants are more likely to use dietary supplements for injury prevention ($\text{Rho} = 0.347$) or injury recovery ($\text{Rho} = 0.429$). Most common reason for not taking supplements (42%) is that participants don't find them necessary, however this was not significantly associated with any of other statements nor sociodemographic characteristics.

Conclusion: This study showed a potential need for enhancing professional medical advice and education of athletes, considering that a high number of participants opts out for supplements without any recommendation. Also, in athletes with more rigorous regimens, an increased use for injury prevention and recovery has been reported, showing the need for pharmacovigilance especially at the higher level of professionalism.

- [1] Daher J, El Khoury D, Dwyer JJM. Education Interventions to Improve Knowledge, Beliefs, Intentions and Practices with Respect to Dietary Supplements and Doping Substances: A Narrative Review. *Nutrients*. 2021; 13(11):3935. <https://doi.org/10.3390/nu13113935>

PP096. Epac1 activation prevents LPS-induced endothelial barrier disruption

Seoane N¹, Picos A¹, Campos-Toimil M¹, Viña D¹

¹*Physiology and Pharmacology of Chronic Diseases (FIFAEC), Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago De Compostela, Santiago De Compostela, Spain*

Introduction: The pathophysiology of multiple chronic neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, or multiple sclerosis, has been often associated with the loss of the blood-brain barrier (BBB) integrity [1]. Cyclic adenosine monophosphate (cAMP) is a second messenger involved in the regulation of many biological processes and exerts its effects through two different effectors, protein kinase A (PKA) and Rap guanine nucleotide exchange factor 3 (Epac1). cAMP participates in the maintenance of the endothelial barrier permeability, so the activation of the cAMP signaling pathway represents a promising strategy to improve endothelial function in health and disease [2]. The aim of this study was to evaluate the effect of forskolin and rolipram (cAMP-elevating agents), 6-Bnz-cAMP (PKA agonist) or 8-pCPT-2'-O-Me-cAMP (Epac activator) against lipopolysaccharide (LPS)-induced inflammation in murine vascular endothelial cells.

Methods: Murine cerebral microvasculature endothelial cell line bEnd.3 considered a useful BBB model, was used. For all the experiments, bEnd.3 cells were exposed to LPS (10 µg/mL), forskolin (10 µM), rolipram (50 µM), 6-Bnz-cAMP (300 µM) and/or 8-pCPT-2'-O-Me-cAMP (200 µM) for 24 hours. The barrier integrity was assessed by transendothelial electrical resistance (TEER) measurement. Western Blot analysis was performed to measure the protein levels of claudin-5 and the phosphorylation of STAT3. IL-6, VCAM-1 and ICAM-1 mRNA expression was quantified using RT-qPCR. Claudin-5 localization was verified by immunostaining of bEnd.3 monolayers fixed in 4% PFA.

Results: Forskolin and rolipram co-treatment prevented LPS-induced 10-fold increase in VCAM-1 and ICAM-1 mRNA expression. However, it failed to counteract the increases in IL-6 mRNA expression and STAT3 phosphorylation. LPS induced a loss of barrier integrity, significantly decreasing TEER by 35% and claudin-5 protein levels by 50%. Under these conditions, forskolin and rolipram restored TEER and claudin-5 levels. The selective activation of Epac1 by 8-pCPT-2'-O-Me-cAMP increased TEER after LPS exposure by 50%, while PKA activation by 6-Bnz-cAMP fails to improve barrier integrity.

Conclusions: The activation of the cAMP signaling pathway partially reverts LPS-induced inflammation and protects bEnd.3 barrier integrity. Epac1 selective activation by 8-pCPT-2'-O-Me-cAMP prevents inflammation-induced barrier disruption. However, more studies are needed to elucidate the specific downstream effectors that are responsible for this effect.

- [1] Sweeney MD, Sagare AP, Zlokovic BV. Blood-brain barrier breakdown in Alzheimer's disease and other neurodegenerative disorders. *Nat Rev Neurol.* 2018, 14 (3): 133-150.
- [2] Viña D, Seoane N, Vasquez EC, Campos-Toimil M. cAMP Compartmentalization in Cerebrovascular Endothelial Cells: New Therapeutic Opportunities in Alzheimer's Disease. *Cells.* 2021, 10 (8): 1951.

PP097. Rapamycin weekly treatment delays replicative senescence of HUVEC in culture while maintaining endothelial identity and function

Picos A¹, Seoane N¹, Álvarez E², Viña D¹, Campos M¹

¹*Physiology and Pharmacology of Chronic Diseases (FIFAEC), Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela, Santiago De Compostela, Spain, ²Health Research Institute of Santiago de Compostela (IDIS), USC University Hospital Complex (CHUS), SERGAS, Santiago de Compostela, Spain, Santiago de Compostela, Spain*

Introduction: Aging is the main risk factor for chronic diseases in developed countries, in which cardiovascular diseases and cancer are the leading causes of death [1]. Cellular senescence is one of the so-called hallmarks of aging [2] and it's been proposed as an underlying mechanism of the process of aging itself. Rapamycin treatment have shown to extend lifespan of numerous preclinical models [3], and its off-label use for longevity purposes is increasingly widespread. In this study, we wanted to evaluate the effect of rapamycin treatment in replicative senescence of human endothelial cells, as a possible mechanism for cardiovascular diseases prevention and lifespan extension in humans.

Methods: Human umbilical vein endothelial cells (HUVEC) were obtained from healthy donors and maintained in culture until end of replication. Treatment with rapamycin (10 nM) was performed weekly for 24 hours. Two separated experiments with 5 different donors (n=5 for each group) were performed to further evaluate the reproducibility of the results.

Population doubling level were calculated for each passage until end of lifespan. Senescence associated β -galactosidase staining, RNA and protein samples, immunostaining of CD144 and α -smooth muscle actin (α -SMA), growing curves, cell cycle analysis, wound healing and angiogenesis assays were performed at passages 4, 10, 15, 20 and 25.

Western blot and RT-qPCR were performed to evaluate cyclin dependant kinases inhibitors p16 and p21.

Expression of endothelial markers CD31 and vWF were analysed by RT-PCR.

Results: Rapamycin treated HUVEC showed a heterogeneous increase in culture lifespan between 4 and 20 population divisions. Cell senescence markers, such as β -galactosidase activity at pH 6, displayed incremental improvement with treatment through passages. Both control and treatment group keep an endothelial phenotype evidenced by the expression of endothelial markers CD144, CD31 and vWF, as well as absence of mesenchymal markers such as α -SMA. HUVEC ability to form new capillaries and migrate to close wounds remained intact in both groups during their lifespan in culture.

Conclusions: Under described conditions rapamycin delays replicative senescence of HUVEC, maintaining cell identity and endothelial capacities such as wound closure and formation of new vessels.

- [1] Niccoli T, Partridge L. Ageing as a Risk Factor for Disease. *Curr Biol*. 2012;22(17):R741-R752. doi:10.1016/j.cub.2012.07.024
- [2] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. *Cell*. 2023;186(2):243-278. doi:10.1016/j.cell.2022.11.001
- [3] Liu GY, Sabatini DM. mTOR at the nexus of nutrition, growth, ageing and disease. *Nat Rev Mol Cell Biol*. 2020;21(4):183-203. Doi:10.1038/s41580-019-0199-y

PP098. Study of the effect of bradykinin B2 receptor activation, in the management of hypercholesterolemia and cardiovascular risk

Mparnia V¹, Vachlioti E², Giannatou K¹, Kakafoni G¹, **Zvintzou E¹**, Rassias G², Kypreos K¹

¹*Pharmacology Laboratory, Department of Medicine, School of Health Sciences, University of Patras, Greece, Patra, Greece*, ²*Synthetic Organic Chemistry Laboratory, Department of Chemistry, School of Natural Sciences, University of Patras, Greece, Patra, Greece*

According to the World Health Organization (WHO) cardiovascular disease remains, for the last 20 years, the leading cause of death worldwide. An estimated 17.9 million people died from cardiovascular disease in 2019, accounting for 32% of all deaths worldwide. Of these deaths, 85% were due to heart attack and stroke. Although, hypolipidemic therapies, such as statins, ezetmibe and PCSK9 inhibitors play a crucial role in the management of hyperlipidemia and development and progression of cardiovascular disease, a significant residual risk still exists in treated patients, which leads to the continuous investigation of novel pharmacological targets. High risk patients often suffer from more than one disorder, and the coexistence of risk factors such as high lipid levels, hypertension, and insulin resistance leads to a difficult to manage, disease profile. Given the direct association of hypertension with hypercholesterolemia [1], but also the significant effect of elevated cholesterol levels on the development of atherosclerosis, in the present study we investigated the effect of bradykinin B2 receptor activation, through the administration of a non-peptide partial agonist, in the management of hypercholesterolemia of low density lipoprotein (LDL) receptor (LDLR)-deficient experimental animals.

For this purpose, high-fat diet was administered for twenty weeks to two groups of male LDLR deficient mice (*ldlr*-/-) one of which received in the last six weeks, the B2 receptor agonist through food. After twenty weeks, the experimental animals were sacrificed, and blood and tissues were isolated for biochemical and molecular analyses.

Our results showed that the bradykinin B2 receptor agonist led to a significant reduction in plasma cholesterol levels, which was mainly due to a reduction in LDL cholesterol. In addition, the reduction in LDL cholesterol levels was accompanied by a significant reduction in the levels of apolipoprotein B, an apolipoprotein that has been directly associated with cardiovascular risk [2]. Furthermore, the bradykinin B2 receptor agonist caused a significant reduction in hepatic cholesterol levels but also had a significant effect on the glycemic profile of experimental animals to which it was administered.

Based on these observations, we propose that bradykinin B2 receptor activation could be a potential pharmacological target for the management of hypercholesterolemia, glucose-related disorders and for the reduction of cardiovascular risk in patients with multiple metabolic disorders.

PP099. RNA editing alterations in sporadic ALS highlight novel associations with disease-related targets, promoting new intervention approaches

Kanata E¹, Karagianni K², Kyriakidis K¹, Pettas S^{1,2}, Xanthopoulos K¹, Dafou D², Sklaviadis T¹

¹*Laboratory of Pharmacology, Department of Pharmacy, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*, ² *Department of Genetics, Development, and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Introduction: Amyotrophic Lateral Sclerosis (ALS) displays progressive deterioration of upper and lower motor neurons, leading to respiratory failure and death. It manifests as familial (fALS, 10-15 %) or sporadic (sALS, ≥90-95%) and no cure is currently available. Effective therapeutic approaches require a better understanding of disease-related mechanisms and, given the high disease heterogeneity, patient stratification. RNA editing is an RNA modification mechanism with adverse functional effects, including expression regulation. Its study in ALS may contribute to deciphering disease-related mechanisms further aiding novel therapeutic approaches. We studied RNA editing in a subset of sALS patients with emphasis on RNA editing-expression correlations in synaptic function related transcripts.

Methods: We focused on sALS cases presenting a common transcriptomic profile characteristic of oxidative stress (ALS-Ox). Utilizing an 'in-house' bioinformatics pipeline, we established gene expression and global RNA editing profiles. Differentially edited transcripts (editing frequency between sALS and controls, p<0.05) were ranked by disease related functionality, guided by pathway analysis and literature experimental data. Gene expression-editing levels correlation (Pearson correlation) on selected targets was performed to unravel effects of RNA editing on gene expression. Bioinformatic analysis results (two datasets) were experimentally verified on human brain tissue from sALS and control cases, using targeted re-sequencing on cDNA and matching gDNA samples and quantitative Real-Time PCR.

Results: We identified significant reduction of RNA editing in sALS-Ox cases compared to controls (p < 0.001, Wilcoxon's test, Bonferroni adjusted) in two datasets (GSE124439, n=17 sALS, n=6 non-neurological controls, GSE153960, n=48 sALS, n=48 non-neurological controls). A cluster of differentially edited sites in CACNA1C was verified in both datasets. CACNA1C expression was significantly reduced in sALS cases (GSE124439: p<0.01, GSE153960: p<0.0001) and positively correlated with RNA editing levels (GSE124439: Pearson r: 0.477, p=0.053, GSE153960: Pearson r: 0.651, p<0.0001). Differential editing and the positive correlation between CACNA1C expression and RNA editing levels in sALS was experimentally verified using human brain tissue material by MiSeq targeted-resequencing and qRT-PCR (n=3 sALS, n= 3 Control).

Conclusions: We highlighted RNA editing alterations affecting CACNA1C expression in a subset of sALS cases. CACNA1C encodes the α1 subunit of an L-type calcium channel, previously implicated in ALS. Focusing on defined disease subsets for identification of pathogenetic mechanisms that would aid disease subtype-specific interventions is increasingly suggested. Studies to delineate RNA editing mediated mechanisms on CACNA1C expression/function and test the effects of RNA editing modulation in appropriate in vitro systems, potentially suggesting novel intervention approaches, are required.

PP100. A Comparative Study of RgIA4, VC1.1, Baclofen on Motion-induced Emesis and Thermoregulation in *Suncus murinus* (House Musk Shrew)

Li Z¹, SO R², Rudd J¹

¹*CUHK, Hong Kong, Hong Kong,* ²*HKUST, Hong Kong, Hong Kong*

Motion sickness (MS) occurs in ~10% of the population and represents a cluster of symptoms including nausea, vomiting and hypothermia. Current drugs for MS are not very efficacious and are associated with their own side effects including unwanted sedation. There are $\alpha 9\alpha 10$ nicotinic acetylcholine receptors ($\alpha 9\alpha 10$ nAChR) in vestibular hair cells that contribute to MS-induced changes in thermoregulation. RgIA4 is a selective antagonist of $\alpha 9\alpha 10$ nAChRs. VC1.1 not only blocks $\alpha 9\alpha 10$ nAChRs, but also activates GABAB receptors. Baclofen selectively activates GABAB receptors. In the present study, we compared the potential of RgIA4, VC1.1, and baclofen to reduce MS and associated changes in temperature in *Suncus murinus*, an insectivore commonly used in anti-emetic research. Diphenhydramine, an antihistamine, was used as a positive anti-emetic control.

Male *Suncus murinus* were administered RgIA4 (10-100 μ g/kg, s.c.), VC1.1 (0.1-1000 μ g/kg, s.c.), baclofen (1-10 mg/kg, s.c), diphenhydramine (3-30 mg/kg, s.c) or their respective vehicles, as a 30 min pretreatment before being exposed to provocative motion (1 Hz, 4-cm horizontal displacement for 30 min). Infrared imaging was used to assess surface temperatures.

Motion induced approximately 15 episodes of retching and/or vomiting. RgIA4 and VC1.1 did not reduce motion-induced emesis (n=4-9), but baclofen at 1, 3, and 10 mg/kg, significantly reduced the number of episodes induced by by $82.5 \pm 5.9\%$, $95.4 \pm 3.0\%$ and 100.0% , respectively (n=7, P<0.05; ID50 = 5.10 mg/kg). Diphenhydramine at 30 mg/kg was effective to reduce the number of episodes by $77.5 \pm 8.0\%$ (n=6, P<0.05). Baseline thermal imaging of control animals revealed a approximate body and eye temperature of 33.2 °C, 35.0 °C, respectively; these temperatures were reduced by motion to 32.4 °C, 33.2 °C (n=6, P<0.05). The baseline tail temperature was 27.9°C and this increased to approximately 31.5 °C by motion (n=6, P<0.05). RgIA4 and VC1.1 failed to affect significantly motion-induced changes in temperature, but baclofen and diphenhydramine appeared to reduce the effect (n=6-8, P<0.05).

The action of baclofen relative to RgIA4 and VC1.1 suggests that GABAB, but not $\alpha 9\alpha 10$ nAChRs, are involved in mechanisms of MS.

This study was supported by the Research Grants Council of the HKSAR, China (14108021).

PP101. New trick for an old dog: Pyronaridine against hard-to-treat human echinococcosis

Wang W¹, Duan L¹, Li J², Zhang W²

¹National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, China, ²State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia, the First Affiliated Hospital of Xinjiang Medical University, Urumqi, China

Introduction: Human cystic echinococcosis (CE) is a parasitic disease caused by the larval stage of the tapeworm *Echinococcus granulosus*. CE is globally distributed, affecting mainly low-income and rural populations. Human CE is considered an orphan and neglected disease by the WHO. Clinically, CE causes damage or dysfunction of target organs, predominantly the liver and lungs. Since early stages of *E. granulosus* infections are usually asymptomatic, most patients seeking medical help are in the late stages and left with limited treatment options. The only drug recommended by WHO, albendazole, exhibits limited efficacy against echinococcosis. Alternative anti-echinococcal drug is urgently required.

Methods: An in vitro phenotypic screening of an approved drug library against *E. granulosus* protoscoleces was conducted to discover novel anti-echinococcal agents. Therapeutic efficacy studies were performed in experimentally *E. granulosus* infected female Balb/c mice ($n = 10$ or 12). Pyronaridine was administered either intraperitoneally (14, 28 and 57 mg/kg/day, q.d. for 3 days) or orally (57 mg/kg/day, q.d. for 30 days). Cyst weight and mortality were determined. Blood samples were collected for safety assessment. A microinjection procedure was developed to mimic the clinical percutaneous drainage treatment.

Results: Drug repurposing strategy identified pyronaridine, an approved antimalarial drug, for the treatment of CE. Following a three-dose intraperitoneal regimen (57 mg/kg/day), pyronaridine caused 100% cyst mortality in the infected animals. Oral administration of pyronaridine at 57 mg/kg/day significantly reduced the parasite weight (42.4%) compared with that of unmedicated mice ($p < 0.05$), and revealed more potent parasiticidal ability (cyst mortality: 90.7%) than that of albendazole ($p < 0.001$). Hematological parameters and liver and renal function indices of pyronaridine-treated animals were all within normal limits. Using a microinjection procedure, pyronaridine (200 μ M) significantly inhibited the parasite growth in mice ($p < 0.05$, compared with saline group), which demonstrated its potential application in clinical practice. Pyronaridine predominantly distributed in the liver and lungs, which are the most affected organs of echinococcosis. Functional analysis and molecular docking studies demonstrated that pyronaridine inhibited recombinant *E. granulosus* topoisomerase I ($IC_{50} = 209.7 \mu M$) and triggered classical apoptotic hallmarks in protoscoleces, including DNA fragmentation and caspase activation.

Conclusions: Given its approved clinical safety and established drug profiles, the repurposing of pyronaridine offers a rapidly translational option for the hard-to-treat human echinococcosis.

PP102. Activity-dependent proteolysis of cyclooxygenase-2 yields cleavage products that modulate cellular functions in a prostaglandin-independent manner

Hartal-Benishay L¹, Abd Elkader A¹, Ehsinieh O¹, Tal S¹, Schwartz A¹, Kleifeld O², Mikl M¹, **Barki-Harrington L¹**

¹*Department of Human Biology, University of Haifa, Haifa, Israel*, ²*Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*

Introduction: Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme for the production of all prostaglandins (PGs), bioactive lipids that are central mediators of inflammation. Except for a few tissues where it is constitutively expressed, COX-2 expression is usually low and undergoes rapid upregulation in response to a wide range of inflammatory and pathological signals. The PGs generated by the actions of COX-2 bind to several different receptors, thus initiating multiple signaling pathways that comprise a major part of the inflammatory response. Here we propose that COX-2 also fulfills additional cellular functions that are independent of its enzymatic activity, which are mediated through its controlled cleavage and interaction of the resulting fragments with specific cellular proteins.

Methods: COX-2 cleavage following exposure to arachidonic acid (AA) was studied in several cancer-derived cell lines with endogenous expression of the protein, and detected by immunoblotting. Specific cleavage sites were identified using mass spectrometry and the putative cleavage products were subcloned and expressed in cells using a doxycycline-inducible gene expression system (Tet-On). The effects of fragment expression on proliferation and metabolism were assessed.

Results: Probing for the presence of COX-2 in multiple cancer-derived cell lines (A549, U87, HeLa, MCF10A, and others) following a brief exposure to AA showed the presence of a ~40 kD COX-2 immunoreactive band, in addition to the full-length protein (~72 kD). The appearance of this band was prevented by pre-treatment of the cells with ibuprofen or with certain fatty acids, suggesting that its formation is dependent upon enzymatic activity. Mass spectrometry analysis of cells expressing COX-2 following AA stimulation revealed the presence of two adjacent putative cleavage sites, roughly in the middle of the protein. Point mutations in these sites prevented AA-mediated cleavage of COX-2. Finally, the inducible expression of each of the COX-2 fragments had a differential effect on cell growth, mitochondrial function, and glucose metabolism.

Conclusions: Our findings propose the existence of a second wave of COX-2-mediated response, which begins after the enzyme is catalytically active. The resulting COX-2 fragments, which lack enzymatic activity modulate cellular functions. Characterization of this PG-independent pathway and discovery of additional functions of COX-2 will further our understanding of the complex role of COX-2 in pathologies such as chronic inflammation and proliferative diseases, and can lead to the identification of novel targets for intervention against components of this pathway.

PP103. Melanin Regulates Ultraviolet A-Induced Signaling in Human Epidermal Melanocytes

Hafez S^{1,2,3}, Oancea E³

¹College of Nursing, King Saud Bin Abdulaziz University for Health Sciences, Jeddah, Saudi Arabia, Jeddah, Saudi Arabia, ²King Abdullah International Medical Research Center, Jeddah, Saudi Arabia, jeddah, Saudi Arabia, ³Brown University, Department of Neuroscience, Providence, RI, USA

Introduction: Human epidermal melanocytes (HEMs) synthesize melanin as a protective mechanism against solar ultraviolet radiation (UVR), which consists of 95% long-wavelength UVA and 5% short-wavelength UVB [1]. Natural melanin exists as a mix of photoprotective black-brown eumelanin (EM) and photoreactive yellow-red pheomelanin (PM), the amount and ratio of which determine an individual's basal skin pigmentation and susceptibility to skin cancer [2]. We recently showed that physiological doses of UVA evoke a signaling pathway in HEMs involving crosstalk between reactive oxygen species (ROS) and cytosolic and mitochondrial Ca²⁺ that regulates early melanin synthesis [3]. HEMs are particularly susceptible to excessive ROS levels because, while EM is a ROS scavenger, PM, and melanin synthesis itself are pro-oxidant. The ROS balance in HEMs depends on external factors (such as UVA) and internal factors (such as Melanin synthesis, EM, and PM). Indeed, the role of melanin on ROS is context dependent making the contribution of melanin in the UVA-induced melanoma development complex. Thus, here we wanted to further investigate the cellular effects of all these factors combined on ROS in HEMs to better elucidate the role of melanin in UVA-induced melanoma development (Buonocore et al., 2010; Srinivas et al., 2018).

Methods: We mainly used fluorescence live cell imaging and biochemical tools. For live cell imaging, we have a unique setup with filtered output comparable to solar UV radiation (~90% UVA, ~10% UVB) that allowed for stimulation with physiological doses of UVA (320--400 nm) to cultured cells while monitoring changes in intracellular Ca²⁺ or ROS responses by fluorescence imaging [3].

Results: Here, we show that when exposed to physiological doses of UVA, melanocytes with equal EM-to-PM ratios and low total melanin content exhibit an amplified ROS response and increased incidence of DNA double strand breaks compared to melanocytes with high total melanin content. We also demonstrate that ROS generated by melanin synthesis do not contribute to the observed increase in ROS in response to UVA exposure.

Conclusion: Our results elucidate the potential mechanisms of the connection between melanoma and melanin in individuals with red-hair and fair skin.

- [1] Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. Photochem Photobiol. 2008;84(3):539-49.
- [2] Barsh GS. What controls variation in human skin color? PLoS Biol. 2003;1(1):E27.
- [3] Dumbuya H, Hafez SY, Oancea E. Cross talk between calcium and ROS regulate the UVA-induced melanin response in human melanocytes. FASEB J. 2020;34(9):11605-23.

PP104. Discovery of key residues of GLUT9 for uric acid transport using Molecular Dynamics and its clinical evaluation from renal hypouricemia

Cho S¹

¹*Department of Ajou University School of Medicine, Suwon, Republic of Korea*

Uric acid is the end product of purine metabolism. Uric acid transporters in the renal proximal tubule plays a key role in uric acid transport. GLUT9 has been reported as a key transporter for uric acid reuptake in renal proximal tubule. GLUT9 mutation is known as causal gene for renal hypouricemia due to defective uric acid uptake. However, the effect of mutation is not fully investigated and hard to predict the change of binding affinity. We comprehensively described the effect of GLUT9 mutation for uric acid transport using molecular dynamics and investigated the specific site for uric acid binding differences. R171C and R380W showed the significant disruption of the structure not affecting transport dynamics whereas L75R, G216R, N333S, and P412R showed the reduced affinity of the extracellular vestibular area towards urate. Interestingly, T125 M showed a significant increase in intracellular binding energy, associated with distorted geometries. Our investigation will provide the important clue for the chemical screening and foster GLUT9 inhibitor development.

PP105. 3-mercaptopyruvate sulfur transferase (MPST) is a key regulator of macrophage inflammatory responses: possible implications on Metabolic-associated fatty liver disease (MAFLD)

Savvoulidou O¹, Arora A¹, Tripodi G¹, Katsouda A^{2,3}, Papapetropoulos A^{2,3}, Ketelhuth D¹, Peleli M¹

¹*Department of Renal and Cardiovascular Research, Institute of Molecular Medicine (IMM), University of Southern Denmark (SDU), Odense, Denmark,* ²*Biomedical Research Foundation Academy of Athens, Athens, Greece,* ³*Laboratory of Pharmacology, National and Kapodistrian University of Athens, Athens, Greece*

Introduction: Activated macrophages are a key cell type supporting chronic inflammation in metabolic diseases such as obesity and MAFLD. MPST is a main enzyme involved in H2S synthesis, an endogenous molecule able to regulate inflammation and cell metabolism. However, the role of MPST in regulating the function of macrophages in the context of MAFLD remains unknown.

Methods: We examined MPST gene expression and inflammation using publicly available liver transcriptome data from MAFLD and cirrhotic patients and controls (GSE164760). Bone marrow-derived macrophages (BMDM), from C57BL/6 wild type (WT) and MPSTKO (N=4-6/group) mice, were incubated with or without Palmitate (PA), 0.5 mM, to mimic a steatotic environment; subsequently, cells were challenged with LPS (10ng/ml) to promote cell activation. After 24 hours, the levels of pro-inflammatory cytokines, such as TNF, IL-1 β , and IL-6, as well as the anti-inflammatory cytokine IL-10 were measured in the supernatants by ELISA. Correlation studies used two-tailed Pearson's R analysis, while comparisons between >3 groups used ordinary One-Way ANOVA with Fischer's LSD test (p <0.05 denoted statistical significance).

Main Results:

- Transcript analysis showed MAFLD/cirrhosis patients have significantly lower MPST expression than healthy controls, and inversely correlated with macrophage marker CD68, M1-like markers TNF, CCR1, CD86, and CCI2, and positively correlated with M2-like marker ARG1.
- PA induced a mild increase in all tested cytokines which was markedly enhanced in the presence of LPS.
- MPSTKO BMDMs treated with PA+LPS secreted significantly higher levels of IL-1 β (991.9 ± 198.3 and 1422 ± 255.49 pg/mL for WT and MPSTKO, respectively).
- MPSTKO BMDMs treated with LPS secreted significantly lower levels of IL-10 (176.6 ± 22.0 and 105.8 ± 23.99 pg/mL for WT and MPSTKO, respectively) and a similar trend was seen for PA+LPS treatment (107.8 ± 16.84 and 59.59 ± 18.73 pg/mL for WT and MPSTKO, respectively; P = 0.052).

Conclusions: We found that hepatic MPST mRNA is downregulated and inversely correlated with pro-inflammatory macrophage profile in MAFLD/cirrhosis patients. In line with the previous, MPST KO-derived macrophages showed a pro-inflammatory response with higher IL-1 β and lower IL-10 levels than WT. Our findings suggest that MPST might play a key regulatory role in metabolic disorders involving macrophage-mediated inflammation.

*Note: Peleli M, and Ketelhuth F.J D, share last authorship

PP106. Introduction of novel silver nanoparticles materials on dental acrylic samples as alternative medication delivery to oral mucosa

Rezić I², Somogy M², Kraljević Šimunković S¹, Bando I¹, **Šutej I¹**

¹*School of Dental Medicine University in Zagreb, Zagreb, Croatia*, ²*University of Zagreb Faculty of Textile Technology, Zagreb, Croatia*

Introduction: How to deliver medication on the oral mucosa has been an old struggle in oral medicine. The imperative for discoveries becomes evident along with the new technology. Therefore, the aim of this research was to produce novel nanoparticle coatings on dental acrylic samples combined with natural and antimicrobial substances. The initial focus was on achieving transparency and resistance to saliva while ensuring medical activity would not be compromised.

Materials and Methods: In this study, the silver nanoparticles were utilized due to their antibacterial and antifungal properties, while natural substances (propolis, miconazole, and fluconazole) were chosen for their antimicrobial and non-cytotoxic characteristics. Propolis, miconazole, and fluconazole were individually prepared in various formulations, incorporating a natural plasticizer (glycerol) and collagen. Afterwards the deposition of sol-gel-derived hybrid thin films with nanoparticles onto acrylate was conducted through dip coating. The morphology of the materials was analyzed before and after modification using a Dino-lite microscope, pH testing, an artificial saliva solution method, infrared spectroscopy (FTIR), and scanning electron microscopy (SEM).

Results: The results were functional and medically active dental acrylic samples modified with antimicrobial coatings containing natural compounds and sol-gel-derived hybrid films with nanoparticles.

Conclusion: Novel materials with antimicrobial silver nanoparticles, known for their significance in medicine and dentistry, were efficient alternatives to dental fillers.

PP107. T cell phenotype changes in Immunosenescence: A Comparative Analysis Across Young, Healthy Elderly and Frail subjects

di Flora A¹, Rasini E¹, Albizzati M¹, Servello M¹, Cosentino M¹, Marino F¹

¹*Università Degli Studi Dell'insubria, Varese, Italy*

Introduction: Frailty is an age-associated multi-organ disorder, exerting detrimental effects on health even in the absence of specific diseases. Frailty is sustained by intricate interplay of biological changes¹ and different frailty biomarkers, including those related to inflammation, immunosenescence, and cellular aging have been postulated². To gain better insight about the role of peripheral immunity in frailty, we investigated circulating CD4+ and CD8+ T cells in frail and not-frail subjects and their correlation with frailty scores.

Methods: We studied three groups (G) of subjects: G1 (young; ≤55 years); G2 (non-frail-elderly) and G3 (frail-elderly), both >65 years. Frailty in all groups was assessed using the SHARE-FI score.³ Immunophenotypic analysis of circulating CD4+ and CD8+ T cells was performed by flow cytometry.

Results: In comparison to G1, G2 and G3 had lower absolute and percentage of CD4+CD45RA+CCR7+ and CD8+CD45RA+CCR7+ cells (naïve), and higher CD3+CD4+CD45RA-CCR7- and CD3+CD8+CD45RA-CCR7- cells (effector memory). Both CD4+CD28+CD57- and CD8+CD28+CD57- cells (not-activated/early-activated) were lower in G2 and G3 in comparison to G1, and in G3 in comparison to G2. Both CD4+CD28+CD57- and CD8+CD28+CD57- are inversely correlated with the SHARE-FI score in all groups. Both CD4+CD28-CD57+ and CD8+CD28-CD57+ cells (senescent/late-activated) were higher in G2 and G3 in comparison to G1, and in G3 in comparison to G2. CD4+CD28-CD57+ and CD8+CD28-CD57+ are directly correlated with the SHARE-FI score in all groups.

Conclusions: The present findings show that elderly and frail subjects differ from young subjects as regards both CD4+ and CD8+ T naïve/memory, not-activated/early-activated cells and senescent/late-activated. The last two also correlate with frailty scores. Senescent cells are a pivotal factor in understanding age- and frailty-related immunological alterations. Immunosenescence is associated with complex changes in adaptive immunity, characterized by substantial variations that are linked to both age and frailty. This study sheds light on the intricate relationship between age, frailty, and T cells.

- [1] Cerreta F. et al., (2016). Baseline frailty evaluation in drug development. *J Frailty Aging*, 5, 139-140.
- [2] Cardoso A. et al., (2018). Towards frailty biomarkers: Candidates from genes and pathways regulated in aging and age-related diseases. *Ageing research reviews*, 47, 214-277.
- [3] Fried, L. P., et al., (2001). Newman a B, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*, 56(3), M146-56.

PP108. Acute toxicological profiles of the selected modified natural clays in rats

Jaćević V^{1,2,3}, Obradović M⁴, Milovanović Z⁵, Amidžić L^{6,7}, Nežić L⁸, Knežević M⁹, Krajišnik D¹⁰, Daković A⁴

¹Department for Experimental Toxicology and Pharmacology, National Poison Control Centre, Military Medical Academy, Belgrade, Serbia, ²Medical Faculty of the Military Medical Academy, University of Defence, Belgrade, Serbia, ³Department of Chemistry, Faculty of Science, University of Hradec Králové, Hradec Králové, Czech Republic, ⁴Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia, ⁵Special Police Unit, Ministry of Interior, Belgrade, Serbia, ⁶Center for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina, ⁷Department for Human Genetics, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina, ⁸Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina, ⁹Military Academy Belgrade, University of Defence, Belgrade, Serbia, ¹⁰Department of Pharmaceutical Technology and Cosmetology, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia

Introduction: Since natural clays have their toxicological profiles and their modification can change this property, acute toxicological evaluation on the two modified natural clays by chitosan, signed as modified bentonite (MBNT) and modified halloysite (MHAL) was performed in this study.

Method: An acute toxicity study, for MBNT and MHAL was performed on adult male and female Wistar rats according to the procedures published earlier [1,2]. The median lethal dose (LD50) of each clay was established by using three groups of male and female rats ($n = 5$). Increasing doses of clays were applied by p.o. the route, in a separate group of rats, both genders. After treatment, all animals were observed daily within 14 days for any toxic symptoms. Then, gastric samples from each animal were prepared for standard histopathological analysis, as previously described [3]. All experimental procedures were approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade (No. 21/2020) and the Veterinary Directorate, Ministry of Agriculture and Environmental Protection, Serbia (No. 323-07-11720/2020-05).

Results: The LD50 value of MBENT was higher than 3000 mg/kg in male and female rats, while MHAL showed somehow lower acute toxicity in male and female rats ($LD50 > 3500$). Besides, in animals treated with these highest doses of MBNT and MHAL, focal desquamation of superficial epithelial cells and diffuse oedema and hyperemia of the tunica submucosa were seen in all gastric samples. These histological alterations, expressed as gastric damage score (GDS) were higher in animals treated with 1.0LD50 MBENTs (GDS was 2.00 ± 0.94) than in 1.0LD50 MHAL-treated animals (GDS was 1.64 ± 0.98).

Conclusions: Presented results can help to predict likely adverse acute effects for selected modified natural clays.

Acknowledgement: This research was supported by the Science Fund of the Republic of Serbia (project AniNutBiomedCLAYS, No. 7748088).

- [1] Jaćević V, Obradović M, Milovanović Z, Amidžić Lj, Nežić L, Knežević M, Krajišnik D, Daković A (2023) Evaluation of acute toxicological profile of the selected natural clays in rats. British Journal of Pharmacology 180(S1):839–840.
- [2] Jaćević V, Nepovimova E, Kuča K (2019) Interspecies and intergender differences in acute toxicity of K-oximes drug candidates. Chemico-Biological Interactions 308:312-316.
- [3] Jaćević V, Nepovimova E, Kuča K (2019) Acute toxic injuries of rat's visceral tissues induced by different oximes. Scientific Report 9:16425.

PP109. Effect of cytoprotector amifostine on survival rate, general condition and body weight of rats subjected to high doses of doxorubicin

Dragojević-Simić V^{1,2}, Iačević V^{2,3,4}

¹*Centre for Clinical Pharmacology, Military Medical Academy, Belgrade, Serbia*, ²*Medical Faculty of the Military Medical Academy, University of Defense in Belgrade, Belgrade, Serbia*, ³*Department for Experimental Toxicology and Pharmacology, National Poison Control Centre, Military Medical Academy, Belgrade, Serbia*, ⁴*Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove, Czech Republic*

Introduction: The therapeutic application of antineoplastic agent doxorubicin (DOX), the one with the widest indication in oncology, is unfortunately limited due to its myelosuppression and cardiotoxicity. It is well known that cytoprotector amifostine (AMI), the gold standard in radioprotection, also offers protection against the toxic effects of numerous radiomimetic anticancer agents. The study aimed to evaluate the general protective effect of AMI in rats subjected to very high doses of DOX, to choose the adequate doses of DOX for following experiments on potential cardioprotective effects of AMI.

Methods: Adult male Wistar rats were used in experiments. Investigation of AMI single dose efficacy (300 mg/kg ip 20 minutes before DOX) in the prevention of acute DOX toxicity (given in a dose of 6, 10, 15, 20 and 25 mg/kg ip) was performed during 10 days, as well as 8 weeks. The general protective efficacy of AMI was estimated based on animal survival rate, changes in body mass and mass of some organs (thymus, spleen, heart, kidneys, adrenal glands, liver and testes) obtained after they were sacrificed.

Results: AMI in a dose of 300 mg/kg administered 20 minutes before DOX provided excellent protection for rats. In rats protected with AMI, seven days after the application of DOX in a dose of 10 mg/kg 94.1% of animals survived, what was significantly more than in the group which was treated with DOX only. The application of AMI was also successful after the application of higher doses of DOX, although the Dose Reduction Factor (DRF) with a value of 1.29 was not significant in comparison to the control. Good results were also obtained when the protective efficacy of AMI was evaluated 8 weeks after application of DOX in a dose of 6, 10, 15 and 20 mg/kg. It was shown that the percentage of survived rats in groups protected with AMI was higher or significantly higher in comparison to those which were administered with DOX only. This protective effect was substantiated by the general condition of animals and body weight during followed periods since the body weight of protected rats was significantly higher than in non-protected ones.

Conclusion: Taking into account the percentage of survival of rats protected by AMI after the administration of individual high doses of DOX and monitoring of their general condition, body weight and weight of selected organs, DOX doses of 6 and 10 mg/kg were selected for further studies.

PP110. Further evaluation of oxidative balance in rats' brains following subacute exposure to oxime K027

Jaćević V^{1,2,3}, Grujić-Milanović J⁴, Milovanović Z⁵, Amidžić L^{6,7}, Vojinović N⁷, Nežić L⁸, Marković B⁹, Dobričić V⁹, Milosavljević P¹⁰, Knežević M¹¹, Nepovimova E³, Kuča K³

¹Department for Experimental Toxicology and Pharmacology, National Poison Control Centre, Military Medical Academy, Belgrade, Serbia, ²Medical Faculty of the Military Medical Academy, University of Defence, Belgrade, Serbia, ³Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove, Czech Republic, ⁴University of Belgrade, Institute for Medical Research, National Institute of the Republic of Serbia, Department for Cardiovascular Research, Belgrade, Serbia, ⁵Special Police Unit, Ministry of Interior, Belgrade, Serbia, ⁶Center for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina, ⁷Department for Human Genetics, Faculty of Medicine, University of Banja Luka, Bosnia and Herzegovina, ⁸Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Bosnia and Herzegovina, ⁹University of Belgrade – Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Belgrade, Serbia, ¹⁰Veterinary Services Center, Military Health Department, Belgrade, Serbia, ¹¹Military Academy Belgrade, University of Defence, Belgrade, Serbia

Introduction: Our previously published data have demonstrated that oxime K027, as one of the most promising K-oximes, showed the lowest subacute brain injuries [1-3]. Accordingly, we continued investigation into the relationship between different markers of oxidative stress in the brain of Wistar rats induced by repeated application of low doses of standard acetylcholinesterase reactivators (asoxime and obidoxime), and oxime K027.

Methods: Each oxime (0.1 of LD50/kg i.m.) was given 2 times/week for 4 weeks. The rats' brain oxidative status was done on day 35 of the study. The concentration of Thiol groups and paraoxonase (PON1) were measured in the brain homogenates. The statistical analysis was performed by one-way analysis of variance (ANOVA). Thus, p < 0.05 was considered statistically significant. The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade (No. 01-386/6) and the Veterinary Directorate, Ministry of Agriculture and Environmental Protection, Serbia (No. 323-07-07549/2020-05/23).

Results: In the brain tissue of the K027-treated animals, Thiol groups increased significantly, by 20% to 38%, compared with the obidoxime and asoxime- treated groups, respectively. Application of oxime K027 significantly enhanced levels of PON1 activity (p < 0.001) compared to the other two oxime groups, respectively. As expected, this parameter was significantly better in the K027-treated group, thus it had almost the same values as the control group.

Conclusions: These results confirm the potential therapeutic effect of oxime K027 which is expressed through improving the oxidative status and attenuating signs of inflammation in rats' brains.

Acknowledgement: This study is supported by the following projects: MFVMA01/23-25, PrF UHK 2216/2023-2024, 451-03-47/2023-01/200015, and 451-03-47/2023-01/200161. We would like to thank Jelena Dumanovic for her help in the experiment's conduction.

[1] Dumanović J, Grujić-Milanović J, Milovanović Z, Amidžić Lj, Vojinović N, Nežić L, Milosavljević P, Nepovimova E, Kuča K, Jaćević V (2022) Oxidative status assessment of rats' brain injury following subacute exposure to K-oximes. Military Medical Science Letters 91(Suppl. 1):89-90.

[2] Jaćević V, Dumanović J, Grujić-Milanović J, Milovanović Z, Amidžić Lj, Vojinović N, Nežić L, Milosavljević P, Nepovimova E, Kuča K (2023) Oxidative balance in rats' brains following subacute exposure to different oxime. British Journal of Pharmacology 180(S1):438–439.

[3] Jaćević V, Dumanović J, Grujić-Milanović J, Milovanović Z, Amidžić Lj, Vojinović N, Nežić L, Marković B, Dobričić V, Milosavljević P, Nepovimova E, Kuča K (2023) Oxidative stress status assessment of rats' brains injury following subacute exposure to K-oximes. Chemico-Biological Interactions 383:110658.

PP111. Study of the toxicity of ARBM101 (Methanobactin SB2) compared to other iron chelators

Wachinger V¹, Akdogan B², Eberhagen C², Reinold Q¹, Zischka H^{1,2}

¹Institute of Toxicology and Environmental Hygiene, School of Medicine, Technical University Munich, Munich, Germany, ²Institute of Molecular Toxicology and Pharmacology, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany

Introduction: Hemochromatosis is an iron homeostasis disorder characterized by increased intestinal iron uptake and accumulation. Chelation therapy, e.g., deferoxamine (DFO), deferasirox (DFX), or deferiprone (DFP), currently are insufficient therapeutical options because of low iron excretion capacity and adverse side effects [1].

Methanobactins, peptides serving as copper acquisition systems in certain methanotrophs, alongside copper binding abilities, show iron-binding abilities [2,3]. This work, therefore, aims to investigate the toxicity of ARBM101 (Methanobactin SB2) compared to common chelators and its ability to reduce iron.

Methods: Cellular toxicity was assessed in Huh7 cells by CellTiter-Glo assay compared to the untreated control group (N=3, n=3-12) in two settings: Viability of Huh7 cells after treatment with ARBM101, DFP, DFX, and DFO at increasing concentrations without or after preloading with iron for 24 hours (h).

Additionally, the iron reduction ability of ARBM101 compared to other chelators was assessed at 23,4µM in horse spleen ferritin by spectrometry at 562nm using a ferrozine assay.

Statistical analysis was done by student's t-test compared to the untreated control.

Results and Conclusion: Cells treated with different concentrations of ARBM101 (N=3, n=3) show a mean viability of about 120% (0.1mM: 122.3%, P=0.0456). Cells treated with DFP or DFO (N=3, n=3) displayed 90-100% viability, while those treated with DFX (N=3, n=3) exhibited decreasing viability with increasing concentration.

The mean viability of iron preloaded cells treated with ARBM101 (N=3, n=3) ranges from 80-90% at all concentrations tested (0.1mM: 78.58%, P=0.0056; 0.5mM: 85.32%, P=0.0153). Cells treated with DFP or DFO (N=3, n=3) displayed around 70% or less viability, while those treated with DFX (N=3, n=3) showed decreasing viability with increasing concentration.

To summarize, ARBM101 displays less toxicity than other chelators with or without iron-preloading.

Spectrometric analysis (N=2) showed higher absorbance values after ARBM101 treatment than previously named chelators (significant difference of final values ARBM101 compared to DFX: P=0,0394).

This indicates that ARBM101 reduces Fe(III) to Fe(II) on a larger scale than other chelators, suggesting that ARMB101 is better able to dissolve ferritin-bound iron.

[1] Zoller H, Schaefer B, Vanclooster A, et al. EASL Clinical Practice Guidelines on Haemochromatosis. J of Hepatol.2022;77(2):479-502.

[2] Dershitz P, Bandow NL, Yang J, et al. Oxygen Generation via Water Splitting by a Novel Biogenic Metal Ion-Binding Compound. Appl Environ Microbiol. 2021;87(14):e00286-21. doi: 10.1128/AEM.00286-21.

[3] Einer C, Munk DE, Park E, et al. ARBM101 (Methanobactin ARBM101) Drains Excess Liver Copper via Biliary Excretion in Wilson's Disease Rats. Gastroenterology. 2023;165(1):187-200.

PP112. Cytotoxic activity of novel GnRH analogs conjugated with mitoxantrone in ovarian cancers

Markatos C¹, Karageorgos V¹, Biniari G², Chepurny O³, Tsakalakis N¹, Venihaki M⁴, Holz G³, Tselios T², Liapakis G¹

¹Dept. of Pharmacology, School of Medicine, University of Crete, Heraklion, Greece, ²Dept. of Chemistry, University of Patras, Patras, Greece, ³Dept. of Medicine and Pharmacology, SUNY Upstate Medical University, Syracuse, USA, ⁴Dept. of Clinical Chemistry, School of Medicine, University of Crete, Heraklion, Greece

Introduction: The hypothalamic gonadotropin-releasing hormone (GnRH) exerts its actions through its interaction with the GnRH receptor (GnRH-R). GnRH-R is also expressed in hormone-dependent ovarian cancer cells (OCC), thus constituting an important target for their treatment. In this study, we examined the antitumor effects of con3 and con7 on OCC. Con3 and con7 are GnRH analogs conjugated through a disulfide bond with the anticancer mitoxantrone. It is expected that the cytotoxic effects of con3 and con7 are due to the release of the cytotoxic mitoxantrone inside the cell upon their binding to the GnRH-R of cells. This could be achieved through the thioredoxin system of cancer cells, which reduces the disulfide bond between mitoxantrone and GnRH analog.

Methods: We determined the pharmacological properties (ability to stimulate calcium release) of con3 and con7 and their ability to release mitoxantrone into the ovarian cancer SKOV3 cells, using a Fura-2 Calcium assay and confocal microscopy, respectively. Mitoxantrone is excited at 610 or 660 nm and emits at 685 nm. Cell death and cytotoxicity induced by con3 and con7 were determined by flow cytometry assay (using Annexin V and PI) and MTT assay, respectively.

Results: Calcium release assays have shown that con3 and con7 are agonists displaying potencies of 0.8 and 1.8 nM, respectively. Confocal microscopy experiments revealed the accumulation of mitoxantrone in the cytoplasm and nucleus of SKOV3 cancer cells after 6-hour incubation with con3 or con7, which is inhibited by pretreatment of cells with cisplatin. MTT experiments revealed that con3 and con7 reduced the proliferation of SKOV3 cells in a dose- and time-dependent manner. Specifically, the cytotoxic potency of con3 and con7 by treating cells for 2, 3, and 4 days, was 0.80-0.93 μ M, and 0.68-0.94 μ M, respectively. Flow cytometry experiments showed that the cytotoxicity of con3 and con7 is mainly associated with apoptosis rather than necrosis. In marked contrast, the unconjugated GnRH analogs, named Con-P1 and Con-P2, are not cytotoxic, even though they display similar potencies to stimulate calcium release with con3 and con7.

Conclusions: The GnRH-mitoxantrone conjugates con3 and con7 are potent agonists for the GnRH-R and exert cytotoxic effects by releasing the cytotoxic mitoxantrone into the ovarian cancer cells, by exploiting the thioredoxin system of these cells. The cytotoxic effects are associated mainly with apoptosis of cells. Thus, con3 and con7 conjugates could be the basis for the development of novel targeted anticancer drugs against GnRH-dependent tumors.

PP113. Pharmacological characterization of a novel CRF1R non-peptide analog

Markatos C¹, Karageorgos V¹, Panagiotopoulos V², Liapakis N¹, Tsakalakis N¹, Komontachakis G¹, Islam R³, Fahmy H³, Matsoukas M², Liapakis G¹

¹Dept. of Pharmacology, School of Medicine, University of Crete, Heraklion, Greece, ²Dept. of Biomedical Engineering, School of Engineering, University of West Attica, Athens, Greece, ³Dept. of Pharmaceutical Science, College of Pharmacy & Allied Health Professions, South Dakota State University, Brookings, USA

Introduction: Corticotropin-releasing factor (CRF) plays a major role in maintaining homeostasis through its interaction with receptor type 1 (CRF1R), by regulating the function of many systems of our body, including the central nervous system. Dysfunction of CRF/CRF1R neuronal circuits is associated with anxiety and depression. Small non-peptide CRF1R selective antagonists, including antalarmin, displayed antidepressant and anxiolytic properties in preclinical studies. However, none of these analogs are in clinical use today. To develop novel CRF1R antagonists, we designed and synthesized a series of pyrimidine analogs. Among these compounds, analog F21 (or compound 43) was the most promising. The aim of the present study is the pharmacological characterization of F21.

Methods: Pharmacological characterization of F21 was performed using HEK 293 cells stably expressing CRF1R, in the following experiments: 1) binding studies, using the CRF analog, [125I]-Tyr0-sauvagine, to determine the binding affinity of F21, 2) cAMP accumulation studies to determine the ability of F21 to antagonize sauvagine, and 3) molecular simulations to determine the mode of interaction of F21 with CRF1R.

Results: The F21 binds to CRF1R with an affinity of 19 nM, similar to that of antalarmin. F21 antagonizes in a dose-dependent manner the ability of sauvagine (at the single concentration of 10 nM) to stimulate the accumulation of cAMP with a half-maximal inhibitory concentration of 7.5 nM. In addition, F21, at the single concentration of 1000 nM, reduced 6-fold the potency of sauvagine to stimulate cAMP accumulation (6 nM and 35 nM in the absence and presence of F21, respectively). Molecular simulation studies determined the mode of interaction of F21 with the CRF1R. Among the interactions of F21 with CRF1R the hydrogen bonds with N283(5.54b), T316(6.37b), and L320(6.43b) of receptor are important.

Conclusions: The novel CRF non-peptide analog F21 binds with high affinity to the CRF1R and it is a potent CRF1R antagonist. Among the interactions of F21 with CRF1R the hydrogen bonds with N283(5.54b), T316(6.37b), and L320(6.43b) of receptor are important. The higher binding affinity of analog F21 compared to the other pyrimidine analogs (previously tested) is most likely attributed to the different set of interactions between these ligands and CRF1R. For example, the hydroxyl group of analog F21 is possible to form an extra hydrogen bond with the main chain carbonyl oxygens of Leu320(6.41b) and Thr316(6.37b), partly explaining its higher affinity from other compounds, such as compound 6.

PP114. Novel GnRH conjugate analogs exert cytotoxic effects on endometrial cancer cells

Markatos C¹, Karageorgos V¹, Biniari G², Liapakis N¹, Komontachakis G¹, Sifakis K³, Venihaki M⁴, Tselios T², Liapakis G¹

¹Dept. of Pharmacology, School of Medicine, University of Crete, Heraklion, Greece, ²Dept. of Chemistry, University of Patras, Patra, Greece, ³Dept. of Experimental Oncology, School of Medicine, University of Crete, Heraklion, Greece, ⁴Dept. of Clinical Chemistry, School of Medicine, University of Crete, Heraklion, Greece

Introduction: Gonadotropin-releasing hormone (GnRH) is a key regulator of the reproductive axis by releasing gonadotropins from the anterior pituitary. GnRH-R is also expressed in endometrial cancer cells, which is one of the most common gynecological malignancies. In the present study, we determined the cytotoxic effects of con3 and con7 on endometrial cancer cells. Con3 and con7 are GnRH analogs conjugated through a disulfide bond with the anticancer agent mitoxantrone. It is anticipated that the cytotoxic effects of con3 and con7 are due to the release of the cytotoxic mitoxantrone inside the cell upon their binding to the GnRH-R of cells. This could be achieved through the thioredoxin system of cancer cells, which reduces the disulfide bond between mitoxantrone and GnRH analog.

Methods: We determined the ability of con3 and con7 to release mitoxantrone into the endometrial cancer Ishikawa cells, using confocal microscopy and the property of mitoxantrone to be excited at 610 or 660 nm and emit at 685 nm. Cell death and cytotoxicity induced by con3 and con7 were determined by flow cytometry assay (using Annexin V and PI) and MTT assay, respectively.

Results: The confocal microscopy experiments revealed the accumulation of mitoxantrone in the cytoplasm and nucleus of Ishikawa cancer cells after their incubation with con3 or con7. Pretreatment of cells with cisplatin resulted in a dramatic reduction of mitoxantrone inside the cells. Analysis of flow cytometry results showed that Ishikawa cell death after their treatment with con3 or con7 is associated mainly with apoptosis rather than necrosis. Moreover, in MTT experiments con3 and con7 reduced Ishikawa cell viability in a dose- and time-dependent manner. Specifically, the cytotoxic potency of con3 after exposure of Ishikawa cells for 2, 3, and 4 days was 1.7 μ M, 0.67 μ M, and 0.84 μ M, respectively, and for con7 was 0.76 μ M, 0.88 μ M and 0.77 μ M respectively.

Conclusions: Accumulation of the anticancer mitoxantrone from con3 and con7 into endometrial cancer cells is most likely associated with the decrease of cell proliferation and cell death mainly through apoptosis by these conjugates. The mechanism of mitoxantrone accumulation into cells is its release from the GnRH conjugates as a result of the reduction of the disulfide bond between GnRH analogs and mitoxantrone by the thioredoxin system of cancer cells. Con3 and con7 conjugates could, therefore put the basis for the development of novel targeted anticancer drugs against GnRH-dependent tumors.

PP115. Development of an in-situ gelling hydrogel for the controlled release of drugs with antidepressant-like potential by subcutaneous administration

Yáñez Gómez F^{1,2,3}, García-Fuster J^{1,2,3}

¹*University of Balearic Islands, Palma de Mallorca, España*, ²*IUNICS, Palma de Mallorca, España*,

³*IdISBA, Palma de Mallorca, España*

Introduction: Using controlled drug-release systems could improve in vivo pharmacological experimental procedures by providing a better therapeutic administration without distorting aspects (handling and/or animal stress), while improving the interpretation of the experimental results. In this study, and in the context of our line of research aiming at characterizing novel antidepressants for adolescent depression [1], we aimed at developing an in-situ gelling hydrogel for the controlled release of sub-anesthetic doses of ketamine in adolescent rats.

Materials and Methods: Pluronic F68, F127, alpha-cyclodextrin (α -CD; Sigma-Aldrich, Spain), and sodium hyaluronic acid (HA; Guinama, Spain) were the main components of the polymeric matrix. Ketamine (Richter Pharma, Austria) was selected as the active molecule (350 mg) [1]. Separate solutions of pluronic F68 and F127 at a concentration (w/v) of 32 and 42% were prepared at 4 °C under magnetic stirring overnight, as well as solutions of HA at 1, 2 or 3% (w/v). On the other hand, 19% (w/v) α -CD solution was prepared at 50 °C with stirring for 24 hours until completely dissolved. In these hydrogels, ketamine was included to study its incorporation and further controlled release.

Results and Discussion: Systems formed mainly by 42% pluronic F-127, 2 or 3% HA and 19% α -CD at 20 and 37 °C in vitro showed the best in-situ gelation properties. All of the gels were able to incorporate the initial amount of ketamine that was previously dissolved in a certain volume of α -CD, up to a final hydrogel volume of 800 μ l (suitable volume for subsequent in vivo study). These polymeric systems showed an instantaneous gelation capacity at room temperature and maintained their structure for 7 days at 37 °C.

Conclusions: We have found an in-situ hydrogel that could be used as a drug release platform at the body temperature in adolescent rats. Moreover, sub-anesthetic doses of ketamine incorporated perfectly in this structure. Current experimental studies are in the process of characterizing ketamine's release from the polymer in vitro and in vivo. Acknowledgments: Fundación Jané Mateu; PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033)

[1] Ledesma-Corvi S, et al. Transl Psychiatry. 2022.

PP116. Mechanisms of Lipopolysaccharide (LPS)-Induced Emesis and Physiological Changes Indicative of Nausea in *Suncus murinus* (House Musk Shrew)

Liu L¹, Tse K², Rudd J¹

¹*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, Hong Kong, ²Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong, Hong Kong*

Introduction: The development of anti-emetic compounds is limited by the lack of emesis in common laboratory animals. A previous investigation in piglets demonstrated that administration of lipopolysaccharide (LPS) induced retching and vomiting, but the activation of Toll-like receptor 4 (TLR4) was not confirmed. Here, we aim to develop a model of LPS-induced emesis in *Suncus murinus* to explore the anti-emetic potential of the TLR4 antagonist, resatorvid.

Methods: Male *Suncus murinus* ($n = 6-10$) were surgically implanted with radiotelemetry devices under general anaesthesia. Two biopotential wires of the radiotelemetry implant were inserted into the serosal wall of the antrum to record gastric myoelectric activity (GMA). Following a one-week recovery, animals were injected intraperitoneally with lipopolysaccharide (LPS) (2, 5 and 10 mg/kg) or vehicle (0.9% NaCl, 2 ml/kg). The animals were placed into whole-body plethysmography (WBP) chambers to permit 24-h recording of emetic behaviour and respiratory function. In other experiments, resatorvid (3 mg/kg) or vehicle (5%DMSO+5%Tween-80 in saline) was administered intraperitoneally as a 3-h pretreatment before LPS (5 mg/kg) administration.

Results: Intraperitoneal administration of LPS at 2, 5 and 10 mg/kg induced 15.08 ± 5.28 , 43.62 ± 10.02 , 41.08 ± 17.60 episodes of retching and/or vomiting, respectively ($P < 0.05$); the majority of emetic responses occurred between 40-150 min post LPS administration. LPS at 5 and 10 mg/kg also increased defecation at 2 and 4 h and reduced food intake at 4 and 24 h ($P < 0.05$). LPS increased body temperature (data from radiotelemetry devices) by 1.449 ± 0.144 , 1.377 ± 0.103 , 1.59 ± 0.099 °C between 90 and 450 min, respectively ($P < 0.05$). LPS reduced the dominant frequency of GMA from 15.08 ± 0.136 to 12.93 ± 0.377 , 12.45 ± 0.287 , 12.9 ± 0.314 cycle per minute (cpm) ($P < 0.05$), but did not significantly affect respiratory function. Pretreatment with resatorvid (3 mg/kg, i.p.) reduced the numbers of retching and/or vomiting induced by LPS from 38.8 ± 10.67 to 2.5 ± 2.5 ($P < 0.05$). Resatorvid antagonized the effects of LPS on hyperthermia (from 35.95 ± 0.199 to 34.64 ± 0.174 °C), defecation and reduced food intake.

Conclusion: This study revealed that LPS induced retching and vomiting in *Suncus murinus*, accompanied by behavioural and physiological modifications, which were antagonized by TLR4 antagonist, resatorvid.

All animal care and experimental procedures were conducted under license from the Government of the Hong Kong SAR and the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong.

PP117. Serendipity meets synergism: differential enantiomers activity of a RAD51-BRCA2 disruptor for an integrated, more robust synthetic lethality strategy in pancreatic cancer

Masi M¹, Poppi L², Previtali V¹, Myers S¹, Nelson S³, Varignani G¹, De Franco F⁴, Falchi F², Veronesi M^{5,6}, Ortega J¹, Bagnolini G², Farabegoli F², Di Stefano G⁷, Oliviero G⁸, Pellicciari R⁴, Walsh N³, Roberti M², Girotto S⁵, Cavalli A^{1,2}

¹Computational and Chemical Biology, Italian Institute of Technology, Genova, Italy, ²Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy, ³National Institute for Cellular Biotechnology, School of Biotechnology, Dublin City University, Dublin, Ireland, ⁴TES Pharma S.r.l., Perugia, Italy, ⁵Structural Biophysics, Italian Institute of Technology, Genova, Italy, ⁶D3-PharmaChemistry, Italian Institute of Technology, Genova, Italy, ⁷Department of Surgical and Medical Sciences, University of Bologna, Bologna, Italy, ⁸Systems Biology Ireland, School of Medicine, University College Dublin, Dublin, Ireland

Introduction: The innovative framework of chemically-induced synthetic lethality (SL) gained increasing attention for its promising application in the selective eradication of cancer cells [1]. Disrupting BRCA2-RAD51 interaction, key players in DNA repair by homologous recombination (HR), represents an interesting option within the SL paradigm [2]. We previously showed that combining Olaparib with a BRCA2-RAD51 disruptor (RS-35d) induced SL in pancreatic ductal adenocarcinoma (PDAC) cells. However, RS-35d displayed intrinsic cytotoxic effects even when administered alone [1]. To deepen our knowledge of this approach, we analyzed RS-35d-induced proteomic changes in PDAC cells and characterized the single contribution of its two enantiomers.

Methods: Proteomic profiling was performed through mass spectrometry and western blot. RAD51 binding and RAD51-BRCA2 binding inhibition were assessed through Molecular Docking, ELISA, and Microscale Thermophoresis. Cell-based experiments were performed on BxPC-3, Capan-1, H-6037 (non-cancerous), BxPC-3 3D spheroids, and PDM41 organoids. HR activity was analyzed through qPCR and fluorescence microscopy. RAD51 and γH2AX foci were analyzed via immunofluorescence. Cell viability was evaluated via MTT assay. Cell Death was assessed using CytoTox Green (Promega) and DAPI/PI (2D) or Calcein-AM/PI (3D). Proliferation, migration, and survival were analyzed through colony formation, scratch wound-healing, and Annexin V/PI staining. Kinase inhibition was performed by the external service Eurofins Scientific.

Results: Our data indicate clear differential RAD51-binding for the two enantiomers, where S-35d acts as a better RAD51-BRCA disruptor than R-35d. Consistently, S-35d significantly impairs HR and RAD51 nuclear localization, synergizing with Olaparib and resulting in more serious DNA damage, reduced cell viability/increased cell death, decreases proliferation and migration, and increased apoptotic rate. Only RS-35d racemate shows antineoplastic activity without Olaparib co-exposure due to the ability of its enantiomers to intrinsically synergize through the simultaneous inhibition of ATM, ATR, and DNA-PK in addition to RAD51. Notably, these proteins have emerged as innovative targets for SL in oncology, widening the plethora of combinations to kill cancer cells.

Conclusions: RS-35d intrinsic cytotoxicity, initially ascribed to potential off-targets, was unveiled as a serendipitous, built-in SL profile. This opens the way for novel anticancer SL strategies towards more robust personalized medicine approaches for unmet medical needs like BRCA-competent and PARP-resistant PDAC.

- [1] Bagnolini G et al. Synthetic Lethality in Pancreatic Cancer: Discovery of a New RAD51-BRCA2 Small Molecule Disruptor That Inhibits Homologous Recombination and Synergizes with Olaparib. *J Med Chem.* 2020;63(5):2588-2619.
- [2] Previtali V et al. Proteomic profile of BxPC-3 cells after treatment with BRC4. *J Proteomics.* 2023;288:104983.

PP118. Effects of mir-30c-5p modulation on the nucleolar stress response of dorsal root ganglion neurons after sciatic nerve injury

Francés R¹, Lafarga M^{2,5}, Marques J^{3,4}, Saguillo A^{3,5}, Hurlé M^{3,5}, **Tramullas M**^{3,5}

¹*Energy & Memory, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, Paris, France,*

²*Departamento de Anatomía y Biología Celular, Facultad de Medicina, Universidad de Cantabria, Santander, Spain,* ³*Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Santander, Spain,* ⁴*Servicio de Rehabilitación, Hospital Universitario Marqués de Valdecilla (HUMV), Santander, Spain,* ⁵*Instituto de Investigación Sanitaria Valdecilla (IDIVAL), Santander, Spain*

Neuropathic pain (NP) is a prevalent and debilitating chronic syndrome highly refractory to current analgesics. The development and maintenance of NP includes long-term pathological plasticity in the nervous system. The nucleolar stress response is an important sensor of neuronal dysfunction in several neurodegenerative disorders. However, the impact of nucleolar dysfunction on NP development after nerve injury remains elusive. MicroRNAs (miRNAs) are small noncoding RNAs that modulate post-transcriptional gene expression. Previous results of our group support a major role for miR-30c-5p in neuropathic pain development [1]. Our study aims to assess the nucleolar stress in neurons of the DRG in response to sciatic nerve injury (SNI) and evaluate the consequences of miR-30c-5p-gain and loss-of-function on the nucleolar stress triggered by sciatic nerve injury and their relationship with NP establishment. NP was induced to rats by sciatic spared nerve injury (N=5-6 rats per experimental group). Mechanical allodynia was assessed with von Frey monofilaments. On day 5 and 10 post-SNI, lumbar dorsal root ganglions (DRG) were obtained and processed for immunofluorescence (N=3 rats per experimental group). All procedures meet the following requirements of the EU Directive 2010/EU/63. Our results indicate that SNI induces important structural alterations of the nucleolus in the DRG primary neurons, in association with NP development ($p<0.001$). The harmful effect of SNI was potentiated by the treatment with miR-30c-5p mimic, with pro-allodynic consequences ($p<0.001$). In contrast, SNI-induced DRG damage was prevented by the treatment with miR-30c-5p inhibitor with anti-allodynic consequences ($p<0.001$). In conclusion, the nucleolus is one of the cellular organelles affected by SNI, which is especially vulnerable to the modulation of miR-30c-5p expression.

Supported by PID2019-104398RB-I00/MCIN/AEI/ 10.13039/501100011033.

[1] Tramullas, M. Francés R, de la Fuente R, et al. MicroRNA-30c-5p modulates neuropathic pain in rodents. *Sci Transl Med.* 2018;10(453). doi:10.1126/SCITRANSLMED.AAO6299

PP119. A novel positive modulator of alpha7 nAChR enhances cholinergic neurotransmission and promotes intracellular calcium responses in hippocampal interneurons

Pál I¹, Thán M¹, Kolok S¹, Marosi M¹, Ledneczki I¹, Visegrády A¹, Lendvai B¹, Némethy Z¹, Fodor L¹

¹*Gedeon Richter PLC, Budapest, Hungary*

Introduction: Homomeric alpha7 nicotinic acetylcholine receptors (nAChRs) are expressed in the central nervous system in cognition-relevant areas including the prefrontal cortex and the hippocampus. Numerous studies indicate the potential of nAChR ligands to improve cognitive functions. Despite the extensive efforts in the nAChR field, effective treatments remain an unmet medical need. The observed suboptimal efficacy of various alpha7 nAChR-selective agonists and partial agonists in clinical trials may be partially attributed to the desensitization-driven nAChR loss of function. Therefore, we have developed novel alpha7 nAChR selective positive modulator compounds.

Methods: Modulators were selected by their effect on intracellular Ca²⁺ elevation of a selective alpha7 nAChR agonist using plate-reader based fluorometry and on the kinetics of current evoked by choline using patch clamp in recombinant cells expressing human alpha7 nAChR. Intracellular Ca²⁺ concentration changes were measured simultaneously by multiphoton imaging in interneurons from rat hippocampal slices.

Results: Our compound (RGH-560) elicited a significant increase in both the potency and the efficacy of choline to evoke responses showing decelerating effect on current decay of choline induced current, up to 100 nM without agonist effect. From 1 μM it evoked an inward current. RGH-560 significantly enhanced choline-evoked intracellular Ca²⁺ responses in the dendrites of hippocampal interneurons. Moreover, it promoted the action potential firing of the interneurons evoked by choline. Spontaneous neuronal activity was also elevated showing network level effect of the compound. All these effects could be blocked by the alpha7 nAChR selective antagonist MLA. The compound was shown to be effective in vivo. RGH-560 produced a significant reversal of a scopolamine-induced cognitive deficit in the mouse place recognition test at 10 mg/kg p.o. and proved to be effective in delay-induced natural forgetting (novel object recognition) test in rats from 3 mg/kg p.o.

Conclusions: Our in vitro data uncover unique and promising properties of this novel positive modulator of alpha7 nAChR. Furthermore, RGH-560 displays in vivo efficacy in animal models of cognition, validating the targeted molecular mechanism of action. Further development of these compounds may provide an efficient strategy for treatment of cognitive disorders.

PP120. Pretreatment with (-)-epicatechin attenuates the development of NAFLD in vitro

Hefer M¹, Petrovic A², Omanovic Kolaric T³, Kuna Roguljic L⁴, Kizivat T⁵, Srb N⁶, Sikora R⁷, Smolic M⁸

¹*Faculty of Dental Medicine and Health, Osijek, Croatia*, ²*Faculty of Dental Medicine and Health, Osijek, Croatia*, ³*Faculty of Dental Medicine and Health, Osijek, Croatia*, ⁴*Faculty of Dental Medicine and Health, Osijek, Croatia*, ⁵*Faculty of Medicine Osijek, Osijek, Croatia*, ⁶*Faculty of Dental Medicine and Health, Osijek, Croatia*, ⁷*Faculty of Dental Medicine and Health, Osijek, Croatia*, ⁸*Faculty of Dental Medicine and Health, Osijek, Croatia*

Introduction: The accumulation of excess fat in the liver is the hallmark of non-alcoholic fatty liver disease (NAFLD). The pathogenesis of this metabolic disorder mainly involves alterations in lipid metabolism and transport. MTTP (Microsomal Triglyceride Transfer Protein) is a protein that plays an important role in lipid transport and contributes to the development of steatosis. (-)-Epicatechin (EPI), a polyphenol mostly found in cocoa beans and green tea, has demonstrated potential health benefits, including its antioxidant properties and involvement in lipid metabolism. The aim of this study was to explore the preventive effects of EPI on the development of steatosis in HepG2 cells induced by oleic acid (OA).

Methods: HepG2 cells were pretreated with 10-100 µM EPI for 4 hours, followed by exposure to 1.5 mM OA for 24 hours to induce NAFLD. Metabolic viability was assessed using the MTS assay, comparing the following experimental groups: DMEM (Dulbecco's Modified Eagle's Medium) only, OA only, and groups pretreated with varying concentrations of EPI followed by OA treatment (EPI 100-10/OA). Enzyme-linked immunosorbent assay (ELISA) was used for determining the intracellular MTTP levels to assessss the role of EPI in lipid transport.

Results: The results of the MTS assay revealed that HepG2 cells pretreated with EPI exhibited higher metabolic viability with respect to the concentration of EPI used (71.5 - 82.5%) compared to the OA-only group where the metabolic viability was 68.5% (** p < 0.001), suggesting a potential protective effect of EPI against the development of NAFLD. To elucidate the molecular mechanisms involving EPI and the development of NAFLD, the levels of MTTP were determined. Notably, the OA-only group exhibited the highest levels of MTTP, while the groups pretreated with EPI demonstrated a concentration-dependent decrease in MTTP levels (** p < 0.001).

Conclusions: The results of this study showed that EPI pretreatment attenuates the development of steatosis in HepG2 cells induced by OA. Increase in metabolic viability of the pretreated groups, along with the concentration-dependent decrease in MTTP levels, suggests a potential involvement of EPI in lipid metabolism and the prevention of NAFLD development. Therefore, further research is required to elucidate the role of EPI in mediating the lipid metabolism of NAFLD, and other liver diseases.

- [1] Rafiei H, Omidian K, Bandy B. Dietary Polyphenols Protect Against Oleic Acid-Induced Steatosis in an in Vitro Model of NAFLD by Modulating Lipid Metabolism and Improving Mitochondrial Function. *Nutrients*. 2019;11(3):541. Published 2019 Mar 3. doi:10.3390/nu11030541

PP121. Discovery of orally bioavailable phenothiazine aminoalcohols to fight against alveolar echinococcosis through targeting topoisomerase I

Duan L¹, Wang W¹, Li J², Zhang W²

¹National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, China, ²State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia, the First Affiliated Hospital of Xinjiang Medical University, Urumqi, China

Introduction: Human alveolar echinococcosis (AE) is a life-threatening parasitic disease caused by infection with the metacestode of the dog/fox tapeworm *Echinococcus multilocularis*. In Europe, AE is the top-ranked foodborne parasitic disease. Due to the tumor-like aggressive proliferative growth of metacestodes in the liver and the metastatic potential into other organs, the 10-year mortality of AE is > 90% without proper treatment. *E. multilocularis* topoisomerase I (EmTopo I) is an essential enzyme, which resolves topological barriers during most of the critical cellular processes involving DNA, including replication, transcription, recombination and repair. Inhibiting EmTopo I by small molecular drugs, thereby inhibiting the DNA replication machinery, can lead to parasite death.

Methods: An in vitro screening using a small molecule library to identify EmTopo I inhibitors was performed. The antiparasitic potential of the screening hits were assessed against *E. multilocularis* metacestodes. Therapeutic efficacy was evaluated in experimentally *E. multilocularis* infected female Balb/c mice ($n = 8$). Compound DLP2030 was orally administered at the doses of 50 and 80 mg/kg/day once daily for 30 days. The metacestode size and counts of each animal were determined as therapeutic effect evaluation indices. In vitro ADME profile was also determined.

Results: A novel phenothiazine aminoalcohol derivative, DLP2030, was identified as an EmTopo I inhibitor. DLP2030 exhibited potent parasitidal ability against metacestodes with an IC₅₀ value of 17.5 μ M, 10-fold more potent than that of albendazole, the only available drug approved for AE treatment. To mimic the natural infection of *E. multilocularis*, a hepatic AE mouse model was established and employed. The 30-day oral regimen of DLP2030 at the doses of 50 and 80 mg/kg/day led to significant reductions of 72.5% and 85.4% in metacestode counts and decreases of 75.8% and 76.8% in average metacestode size compared to those of vehicle controls (all p-values < 0.05); the treatment outcomes were more effective than those of albendazole-treated animals. In addition, DLP2030 has an attractive in vitro ADME profile, showing good stability in human plasma ($T_{1/2} = 4.1$ h), low clearance in human liver microsomes (Clint < 6.1 mL/min/kg), good permeability in MDCK-MDR1 cells ($P_{app} = 2.6 \times 10^{-6}$ cm/s), and no off-target inhibition of CYPs (IC₅₀s > 10 μ M).

Conclusions: Our findings demonstrated that targeting EmTopo I could be a promising strategy of finding a new cure for the life-threatening but neglected disease AE. Our study highlights the potential of DLP2030 for further development as a promising anti-AE candidate.

PP122. Enzymatic synthesis and biological evaluation of ganglioside GM3 derivatives as potential cancer immunotherapeutics

Lu D¹, Wang J, Jiang F

¹School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China

Introduction: Cancer is a leading cause of death worldwide. One strategy to expand the pool of available anticancer therapies is immunotherapy. Gangliosides, which belong to the glycosphingolipid family, are most abundant in eukaryotic biofilms, and have been implicated in many physiological processes, such as neurodevelopment and tumor development. The simplest ganglioside GM3 (NeuAc α 3Gal β 4Glc β 1Cer) is closely associated with human tumors, and has been validated as a promising target for cancer immunotherapy. However, GM3 is poorly immunogenic, which limits its application. The discovery of novel GM3 derivatives with higher immunogenicity has significant meaning for cancer immunotherapy.

Methods: A one-pot enzymatic method was developed to prepare a series of N-phenylacetyl GM3 derivatives. The resulting potential antitumor agents were screened by a wound-healing assay and Transwell assay in vitro, and were evaluated in tumor bearing animal models. Subsequently, the molecular mechanism was studied by RNA sequencing (RNA-seq) analysis and was verified by real-time quantitative polymerase chain reaction (qRT-PCR) and western blot (WB).

Results: We successfully prepared 14 ganglioside GM3 derivatives in high yields (22–41%) using one-pot enzymatic method. Wound-healing and Transwell assays revealed that all the synthesized GM3 derivatives had inhibitory activity on migration and invasion in vitro. N-12 was considered to have better activity among all the derivatives. In vivo studies confirmed the excellent anticancer capability of N-12 toward B16-Luc tumors in mouse models. N-12 showed better tumor suppressive activity in C57BL/6 mice than in BALB/C nude mice. Subsequent molecular biological analysis of cancer tissues and serum revealed that N-12 induced tumor inhibition via tumor necrosis and inflammatory response, especially in C57BL/6 mice. These data suggested that the activity of N-12 may be closely related to the immune response. An RNA-seq analysis was then performed and indicated that the antitumor mechanism of N-12 involved focal adhesion and ECM-receptor interaction signaling pathways.

Conclusions: N-12, as a novel GM3 derivative, can be further developed as an effective therapeutic for the treatment of cancer. GM3 and its derivatives may exert antitumor activity by regulating related genes in the focal adhesion and ECM-receptor interaction signaling pathways. The underlying mechanisms can help us get a step closer toward understanding the dynamic and perhaps the multiregulation of GM3 derivatives and the ganglioside family.

PP123. Role of JNJ7777120 (Histamine H4 receptor antagonist) in prevention and/or treatment of 5-FU-induced gastrointestinal mucositis in rats

Harmanci N¹, **Yigitaslan S**¹, Yildiz P¹, Yildirim E¹, Kaltus Z¹, Sahinturk V¹

¹*Eskisehir Osmangazi University, Eskisehir, Turkey*

Introduction: The pathogenesis of 5-FU induced gastrointestinal mucositis is quite complex and includes oxidative stress, NF-kB and some inflammatory events [1]. Histamine is a biogenic amine with broad activity in both physiological and pathological conditions and has at least 4 types of receptors. While the expression of H4R in the gastrointestinal tract is much lower than in other regions under physiological conditions, it increases with an inflammatory stimulus and modulates the function of mast cells, T cells, dendritic cells and eosinophils [2]. In this study, the effect of JNJ7777120, an H4 receptor antagonist, on 5-FU-induced mucositis in rats was investigated.

Methods: A total of 30 adult male Sprague Dawley rats were divided into 3 groups (ethical approval no. 2020-677/1). While the control group was given only saline treatment, the 5-FU group was given 60mg/kg i.p. 5-FU every other day and trauma was created in the oral cavity on other days. In the third group, in addition to the 5-FU+trauma application, JNJ7777120 was administered s.c. at a dose of 10 mg/kg/day from day 0, covering the five days after the day when oral mucositis was detected. At the end of the experiment, NF-kB, TNF-alpha and IL-6 levels and oxidative stress status were studied by ELISA in blood samples, and histological examination was performed in ileum samples.

Results: In the histological examination, findings such as shortening of the villi, flattening and shedding of the villus epithelium, inflammatory cell infiltration, and decrease in the crypts were observed in the 5-FU group. In the JNJ7777120-treated group, a histological appearance almost close to the control group was detected. Serum NF-kB, TNF-alpha and IL-6 levels and oxidative stress markers increased slightly with 5-FU application and decreased with JNJ7777120 treatment, but these differences did not reach statistical significance.

Conclusion: As a result, in the intestinal mucositis model induced by 5-FU in rats, the H4R antagonist JNJ7777120 provided significant histological improvement, but not biochemically. This study was financially supported by Scientific Projects Unit of Eskisehir Osmangazi University (201911045)

[1] Curra M, Soares Junior LAV, Martins MD, Santos P. Chemotherapy protocols and incidence of oral mucositis. An integrative review. Einstein (Sao Paulo). 2018;16(1):eRW4007.

[2] Coruzzi G, Adami M, Pozzoli C. Role of histamine H4 receptors in the gastrointestinal tract. Front Biosci (Schol Ed). 2012;4:226-39.

PP124. Antiseizure activity of new 2,5-dioxopyrrolidin-1-yl)propanamide derivatives with a probable novelty mechanism of pharmacological action

Rapacz A¹, Mogilski S¹, Abram M², Kamiński K²

¹*Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University, Medical College, Kraków, Poland*, ²*Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University, Medical College, Kraków, Poland*

Introduction: Although 70% of patients with new onset epilepsy have complete seizure control with current antiepileptic drugs, still, about one third of epilepsy patients suffer from seizures. Therefore, the aim of the study was to investigate the antiseizure activity of new 2,5-dioxopyrrolidin-1-yl)propanamide derivatives (namely AS-41, AS-44, AS-45 and AS-95).

Methods: Anticonvulsant activity was examined in the maximal electroshock test (MES) – a model of generalized tonic-clonic seizures and psychomotor (6 Hz, 32 mA) seizure test – a model of focal seizures, whereas neurotoxicity was assessed using rota-rod test (10 rpm), as previously reported [1]. Male CD-1 mice (20-26g) were used. The experimental groups consisted of 4 (initial pharmacology screening at a dose of 100 mg/kg) or 6 (anticonvulsant and neurotoxic studies) mice. Compounds were suspended in a 1% aqueous solution of Tween 80, and administered i.p. at a volume of 0.1 ml/10 g b.w., and after 0.5 h experiments were performed. Experimental procedures were carried out in accordance with EU Directive 2010/63/EU and approved by the I Local Ethics Committee for Experiments on Animals of the Jagiellonian University in Krakow, Poland.

Results: In the screening dose of 100 mg/kg antiseizure activity in the MES and 6 Hz tests demonstrated all tested compounds. In the next step, compounds were tested at lower doses to calculate ED₅₀ values in both tests. Among new compounds the highest activity in the MES test demonstrated AS-95 (ED₅₀=40.55 mg/kg), followed by AS-45 (ED₅₀=46.19 mg/kg), AS-41 (ED₅₀=50.87 mg/kg), and AS-44 (ED₅₀=69.72 mg/kg). Whereas, in the 6 Hz test the most potent was AS-45 (ED₅₀=52.66 mg/kg), followed by AS-41 (ED₅₀=57.69 mg/kg), AS-95 (ED₅₀=62.95 mg/kg), and AS-44 (ED₅₀=67.93 mg/kg). No neurotoxicity was observed for these compounds in rotarod test at two tested doses 100 mg/kg (screening) and 300 mg/kg (TD₅₀ > 300 mg/kg).

Conclusions: All tested compounds showed antiseizure activity in both tests. Compounds with that profile may be effective in the pharmacotherapy of wide range of human epilepsies. Importantly, the protective indices obtained for all compounds were higher than those obtained for reference drugs (ethosuximide and valproic acid). Moreover, our recent study has shown that compounds with a pyrrolidine-2,5-dione moiety revealed a unique and novel mechanism of action, as it is a selective PAM of glutamate transport by EAAT2 [2].

The study was financially Supported by Jagiellonian University Medical College grant (N42/DBS/000328).

- [1] Kamiński et al. Epilepsia. 2020 Oct;61(10):2119-2128.
- [2] Abram et al. JMedChem. 2022 Sep8;65(17):11703-11725.

PP125. Predict drug-induced vomiting and nausea using gastrointestinal pacemaker activity produced by the interstitial cell of Cajal as biomarkers: Glucagon-like peptide-1 agonists and Dipeptidyl Peptidase IV inhibitors as exemplar drugs

Liu Y^{1,2}, Tung M¹, Lau C¹, Chau C², Deng Y^{1,2}, Hui X¹, Rudd J^{1,2}

¹*The Chinese University of Hong Kong, Hong Kong*, ²*Gut Rhythm R&D (Hong Kong) Limited, Hong Kong, Hong Kong*

Introduction: Unexpected vomiting and nausea caused many patients to drop out from clinical trials. This study aims to explore whether pacemaker activity of interstitial cells of Cajal (ICC) can act as predictive biomarkers for vomiting and nausea. Vomiting and nausea are common following treatment with glucagon-like peptide-1 (GLP-1) agonists. Dipeptidyl peptidase IV (DPP4) inhibitors, which indirectly elevate GLP-1 levels, were hypothesized to produce lower gastrointestinal side effects.

Method: Gastrointestinal tissues were isolated from a vomit-capable animal model, *Suncus murinus*, and tested on a microelectrode array platform to record ICC pacemaker activities [1]. GLP-1 agonists: GLP-1(7-37) (1–100nM), semaglutide (10nM–1μM), liraglutide (10nM–1μM) and DPP4 inhibitors: linagliptin (1–100nM), saxagliptin (1–100nM) were added after a 5-min baseline recording. Raw data were filtered, and Fast Fourier transformed to derive dominant frequency and the period of waveform. Raw data was introduced into trained deep-learning models previously established using our novel Gastro-Intestinal Pacemaker Activity Drug Database (GIPADD) to predict gastrointestinal side effects [2-3] Training data included 100 drugs, 7,014 datasets. Liraglutide and linagliptin were used in model-training, while GLP-1(7-37), semaglutide and saxagliptin were used for external validation.

Results: The duodenal dominant frequency was reduced by GLP-1 agonists: GLP-1(7-37) (10nM) [-12.6%, n=6, p=0.014], semaglutide (100nM) [-4.0%, n=6, p=0.038] and liraglutide (1μM) [-6.2%, n=6, p=0.039]; the duodenal pacemaker waveform period was increased by GLP-1(7-37) (10nM) [+25.5%, n=6, p=0.025], semaglutide (1μM) [+7.3%, n=6, p=0.014] and liraglutide (1μM) [+12.4%, n=6, p=0.039]. The above effects were not found in DPP4 inhibitors, linagliptin [n=6-9] and saxagliptin [n=6]. The deep-learning model accuracy for vomiting [0.91] and nausea [0.91]. Predictive risk values were: GLP-1(7-37) [0.59, 0.91], semaglutide [0.42, 0.75], liraglutide [0.37, 0.96], linagliptin [0.19, 0.39], saxagliptin [0.43, 0.91] for vomiting and nausea respectively.

Conclusions: Inhibitory effects of GLP-1 agonists on duodenal pacemaker activity could represent the basis of a biomarker for drug-induced vomiting and nausea. The methodology together with the trained deep-learning models could be a novel pre-clinical fast-screening assay to predict risk of unexpected vomiting and nausea.

[1] Liu JYH, Du P, Chan WY, Rudd JA. Use of a microelectrode array to record extracellular pacemaker potentials from the gastrointestinal tracts of the ICR mouse and house musk shrew (*Suncus murinus*). *Cell Calcium*. 2019;80.

[2] Chau CH, Liu JYH, Rudd JA. Predict Drug-induced Nausea with Deep Learning using a new Gastro-Intestinal Pacemaker Activity Drug Database (GIPADD). British Pharmacological Society. Updated November 30, 2023. Accessed December 12, 2023. <https://my.bps.ac.uk/events/details/?id=66d40489-2b40-ee11-bdf3-6045bdd2c896>

[3] Gastro-Intestinal Pacemaker Activity Drug Database (GIPADD). Gut Rhythm. Updated December 12, 2023. Accessed December 12, 2023. https://www.gutrhythm.com/public_database

PP126. Assessment of the antibacterial and anti-efflux efficacy of novel synthetic chalcone derivatives

Čižmáriková M¹, **Franko O**¹, Garberová M², Szemerédi N³, Spengler G³, Takáč P⁴

¹Department of Pharmacology, Faculty of Medicine, P. J. Šafárik University, Košice, Slovakia,

²Department of Biochemistry, Institute of Chemistry, Faculty of Science, P. J. Šafárik University, Košice, Slovakia, ³Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary, ⁴Institute of Human and Clinical Pharmacology, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

Introduction: Chalcones, the precursors of flavonoids and isoflavonoids in plants, have been demonstrated to exhibit a wide range of biological activities, including antibacterial and anti-efflux properties [1,2]. In the present study, we conducted in vitro experiments to explore the antibacterial and efflux modulating effects of several novel acridine-chalcone derivatives. These compounds were previously recognized for demonstrating some antitumor effects [3].

Methods: The antibacterial and anti-efflux potential of (2E)-3-(Acridin-4-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (4b), (2E)-3-(Acridin-4-yl)-1-(2,6-dimethoxyphenyl)prop-2-en-1-one (4c), and (2E)-3-(Acridin-4-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (4e) was evaluated on strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* K-12 AG100. The minimum inhibitory concentration (MIC) of the compounds of concentrations from 100 µM to 0.195 µM was evaluated according to the standard guidelines. The impact of chalcones (50 and 100 µM) on efflux activity was determined using the Real-time ethidium bromide (EB) accumulation assay and the relative fluorescence index (RFI) of the last time point (minute 60) was calculated. Reserpine (25 µM) was applied as a positive control on ATCC 25923 and cyanide 3-chlorophenylhydrazone (50 µM) as a positive control on AG100. Student's t-test was employed to determine statistical significance. Difference was considered significant when p-values were lower than 0.05.

Results: The MIC values for all investigated chalcones on both bacterial strains were determined to be greater than 100 µM. The RFI values (from EB accumulation assay) exceeding or approaching 1 were exclusively documented for the compound 4b at both tested concentrations when applied to the *S. aureus* ATCC 25923 strain. Nevertheless, its anti-efflux activity observed as EB accumulation was notably lower than that observed for the positive control ($p < 0.05$). On the other hand, no compound displayed distinct anti-efflux activity on the *E. coli* strain.

Conclusions: No acridine-chalcone derivative exhibited inhibitory effects on the growth of the studied bacterial strains. However, chalcone 4b showed weak effectiveness in suppressing efflux mechanisms within the *S. aureus* ATCC 25923 subtype. Nevertheless, further studies are needed to elucidate these observations.

This study was supported by VEGA grant agency (No. 1/0446/22).

- [1] Henry EJ et al. Ferrocenyl chalcone derivatives as possible antimicrobial agents. *J. Antibiot.* 2020;73(5):299-308. doi: 10.1038/s41429-020-0280-y
- [2] Le MT et al. Chalcone Derivatives as Potential Inhibitors of P-Glycoprotein and NorA: An In Silico and In Vitro Study. *Biomed. Res. Int.* 2022; 2022:9982453. doi: 10.1155/2022/9982453
- [3] Vilková M et al. Discovery of novel acridine-chalcone hybrids with potent DNA binding and antiproliferative activity against MDA-MB-231 and MCF-7 cells. *Med. Chem. Res.* 2022; 31(8):1323-1338. doi: 10.1007/s00044-022-02911-0

PP127. The effects of lichen secondary metabolites on resistant colon cancer cells proliferation and ABCB1 efflux activity

Franko O¹, Čizmáriková M¹, Kello M¹, Goga M², Takáč P³, Mojžiš J¹

¹*Department of Pharmacology, Faculty of Medicine, P. J. Šafárik University, Košice, Slovakia,*

²*Department of Botany, Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Košice, Slovakia, ³Institute of Human and Clinical Pharmacology, University of Veterinary Medicine and Pharmacy, Košice, Slovakia*

Introduction: Lichens, symbiotic organisms, produce a diverse array of secondary metabolites with anticancer potential. The mechanisms by which they exert these effects mainly involve the inhibition of cell proliferation, angiogenesis, and apoptosis [1]. However, there is limited research on their impact on multidrug resistance induced by ABCB1 efflux transporter. The aim of our study was to evaluate the anti-proliferative effects of several secondary lichen metabolites derived from naturally occurring lichen species found in Slovakia on resistant colon cancer cells that over-express ABCB1 transporter and to determine the ABCB1 efflux modulation of the most active lichen metabolite.

Methods: The anti-proliferative potential of five lichen metabolites (atranorin, and physodic acid from *Pseudevernia furfuracea*; usnic acid (UA) from *Usnea hirta*; gyrophoric acid from *Umbilicaria hirsute* and evernic acid from *Evernia prunastri*) at several concentrations (200, 100, 50, 25, and 12.5 µM) was evaluated on parental (Colo 205) and ABCB1 over-expressing colon carcinoma cell line (Colo 320) using the colorimetric MTT assay (72 hours exposure). The elevated drug efflux in Colo 320 cells was validated using the EFLUXX-ID® Green Multidrug Resistance Assay Kit. The most active lichen metabolite was evaluated for its ability to modulate the activity of ABCB1 using the Multidrug Efflux Transporter P-Glycoprotein (ABCB1/MDR1/P-gp) Ligand Screening Kit. Student's t-test was employed to determine statistical significance. Difference was considered significant when p-values were lower than 0.05.

Results: Our results confirmed anti-proliferative potential of UA in both parental ($IC_{50}= 17.33 \pm 6.66 \mu M$) and ABCB1 over-expressing cancer cell lines ($IC_{50}= 22.33 \pm 2.52 \mu M$). UA demonstrated less pronounced anti-proliferative effect on ABCB1 over-expressing cancer cells; however, this difference did not achieve statistical significance. The IC_{50} values for all other lichen metabolites were greater than 100 µM. Significantly increased accumulation of fluorogenic ABCB1 substrate was observed in the presence of UA at concentrations of 100, 50, and 25 µM compared to control. The accumulation of fluorogenic ABCB1 substrate at 100 µM of UA was not statistically different from 100 µM of verapamil.

Conclusions: Our results suggest that UA could be a promising compound against colon cancer cells, including those with elevated expression of the ABCB1 transporter. Additionally, UA showed inhibitory action on the ABCB1 transporter. Nevertheless, further studies are needed.

This study was supported by VEGA grant agency (No. 1/0446/22).

- [1] Tripathi AH et al. A Review of Anti-Cancer and Related Properties of Lichen-Extracts and Metabolites. *Anticancer Agents Med Chem.* 2021;22(1):115-142. Doi: 10.2174/1871520621666210322094647

PP128. Platelet hyperactivity as a trigger of leukocyte trafficking to the lungs and subsequent respiratory exacerbations, in early-stage COPD

Marques P^{1,2,3}, Bocigas I⁴, Domingo E^{1,2}, Francisco V¹, Tarraso J^{1,4}, Morcillo E^{1,2}, Piqueras L^{1,2,5}, Signes-Costa J^{1,4}, Gonzalez C^{1,4}, Sanz M^{1,2,5}

¹*INCLIVA Biomedical Research Institute, Valencia, Spain*, ²*Faculty of Medicine, University of Valencia, Valencia, Spain*, ³*CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Madrid, Spain*, ⁴*University Clinic Hospital of Valencia, Valencia, Spain*, ⁵*CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Madrid, Spain*

Introduction: Chronic obstructive pulmonary disease (COPD), usually provoked by long-term tobacco smoking, has been associated with systemic inflammation. However, little is known about the systemic inflammatory status of patients with early-stage COPD (classified as GOLD 1) and long-term smokers with normal lung function (SLF). A description of the early changes in the associated inflammatory state of COPD-developing subjects might suggest new therapeutic targets to prevent its development or treat related exacerbations. Therefore, we characterized the inflammatory status of GOLD 1 patients and SLF.

Methods: Blood samples from 27 GOLD 1 patients, 27 SLF and 14 non-smokers were extracted and complete blood counts were analyzed. Leukocyte adhesion to TNF α -stimulated (20 ng/mL, 24h) pulmonary microvascular endothelium was determined by parallel-plate flow chamber. Platelet (CD62p and PAC-1 expression) and leukocyte subsets' activation (myeloid lineage: CD11b expression; lymphoid lineage: CD69 expression) and the platelet-leukocyte aggregates' counts (CD41+ leukocytes) were assessed by flow cytometry. Plasma levels of relevant cytokines were measured by ELISA. The study complied with the principles outlined in the Declaration of Helsinki and was approved by the institutional ethics committee of the University Clinic Hospital of Valencia (Valencia, Spain). All participants signed an informed consent.

Results: Enhanced platelet-leukocyte adhesiveness to TNF α -stimulated endothelium was found in GOLD 1 patients vs. SLF or non-smokers. Moreover, greater platelet reactivity (platelet count and activation, and fibrinogen levels) and higher frequency of platelet-leukocyte aggregates were detected in GOLD 1 patients than in the other two groups. Some of these findings correlated with the severity of lung dysfunction, while platelet hyperactivity correlated positively with leukocyte-platelet-endothelium interactions. Also, GOLD 1 subjects presented higher circulating levels of IL-17C and C-reactive protein than the other groups, while SLF had higher leukocyte counts and activation, and increased plasma levels of TNF α and IL-6 than non-smokers.

Conclusions: Our data suggest that platelet hyperactivity plays a pivotal role in leukocyte trafficking to the lungs in early-stage COPD, which in turn is responsible for respiratory exacerbations. Therefore, these findings might suggest respiratory benefits from anti-platelet therapy in COPD. Additionally, the altered inflammatory parameters detected in SLF may represent early biomarkers of COPD development. Accordingly, peripheral immune monitoring based on these parameters may be useful in preventing disease progression in SLF and early COPD.

This work was supported by the Spanish Ministry of Science and Innovation [PID2020-120336RB-I00]; the Carlos III Health Institute (ISCIII) and the European Regional Development Fund (FEDER) [PI21-00220]; the Generalitat Valenciana [APOTIP/2020/011, CIPROM/2022/45].

PP129. The effect of proinflammatory cytokines on soluble and membrane-bound dipeptidyl peptidase 4 levels of colonic epithelial cells

Gomes S^{1,2,3}, Melo F^{1,3}, Santiago M⁴, Assunção Silva R⁴, Magro F^{1,4,5,6,7,8}

¹Department of Biomedicine, Unit of Pharmacology and Therapeutics; Faculty of Medicine, University of Porto (FMUP), Porto, Portugal, ²Department of Public Health and Forensic Sciences and Medical Education, Unit of Medical Education; Faculty of Medicine, University of Porto (FMUP), Porto, Portugal, ³Center for Drug Discovery and Innovative Medicines (MedInUP); University of Porto, Porto, Portugal, ⁴Portuguese Study Group of Inflammatory Bowel Disease (GEDII), Porto, Portugal, ⁵Center for Health Technology and Services Research (CINTESIS), Porto, Portugal, ⁶RISE - Health Research Network, Porto, Portugal, ⁷Department of Gastroenterology, São João University Hospital Center, Porto, Portugal, ⁸Clinical Pharmacology Unit, São João University Hospital Center, Porto, Portugal

Introduction: Inflammatory bowel disease (IBD) is a chronic autoimmune condition including Crohn's disease (CD) and ulcerative colitis (UC). The etiopathogenesis is progressively becoming clearer with genetic predisposition, environmental factors, unbalanced gut microbiome, and dysregulated immune system as interconnected elements contributing to its development.

Dipeptidyl peptidase 4 (DPP4), a glycoprotein mainly expressed on the cell surface of epithelial cells, has drawn attention due to its potential as disease biomarker in IBD. Beyond its recognized role in regulating glucose metabolism, emerging evidence suggests the involvement of DPP4 in immune modulation, inflammation, cancer, and tissue remodelling[1]. IBD patients evidence decreased serum DPP4 levels in comparison with healthy volunteers or remissive patients[2], while fecal DPP4 is more reduced in UC patients than CD patients [3].

This work aims to investigate the effect of intestinal inflammation modulated by different proinflammatory cytokines on soluble and membrane-bound DPP4 levels (sDPP4 and mDPP4, respectively) in T84 colonic epithelial cells, unraveling the associated immunopharmacology mechanisms.

Methods: T84 colonic epithelial cells were cultured and exposed to Interleukin-17A (IL-17A), Interleukin-23 (IL-23), and Tumor Necrosis Factor-α (TNF-α). sDPP4 and mDPP4 were quantified by ELISA. Cell viability was assessed using Crystal Violet assay.

Results: Our results demonstrate that T84 cells increased sDPP4 when stimulated with IL-23 (1 and 100ng/mL) and TNF-α (0.1, 1, and 10µg/mL) for 12h. Higher sDPP4 was reported in 24h of exposure time for IL-23 (1 and 100ng/mL) and TNF-α (1 and 10µg/mL). mDPP4 was increased when cells were exposed to IL-23 (10 and 100ng/mL) for 12h, and 10µg/mL of TNF-α also improved mDPP4 at 30min, 12h, and 24h of exposure time. Decreased sDPP4 was observed in cells exposed to 1 and 100ng/mL of IL-17A for 12h. mDPP4 was also reduced in cells exposed to 1ng/mL of IL-17A for 12h. Cells were viable for all experimental conditions.

Discussion: Our findings suggest that variations in compound dosage and exposure time may reflect an effect in the DPP4 proteolytic function. Further in vitro studies are required to elucidate the immunopharmacological mechanism of DPP4.

- [1] Huang J, Liu X, Wei Y, et al. Emerging Role of Dipeptidyl Peptidase-4 in Autoimmune Disease. *Front Immunol*. 2022;doi:10.3389/fimmu.2022.830863
- [2] Pinto-Lopes P, Afonso J, Pinto-Lopes R, et al. Serum Dipeptidyl Peptidase 4: A Predictor of Disease Activity and Prognosis in Inflammatory Bowel Disease. *Inflamm Bowel Dis*. 2020;doi:10.1093/ibd/izz319
- [3] Pinto-Lopes P, Melo F, Afonso J, et al. Fecal Dipeptidyl Peptidase-4: An Emergent Biomarker in Inflammatory Bowel Disease. *Clin Transl Gastroenterol*. 2021;doi:10.14309/ctg.0000000000000320

PP130. Pharmacokinetics and lymphatic transport of two important cannabinoids – cannabidiol and cannabigerol

Paulusová V¹, Ryšánek P¹, Jelínek P², Kozlík P³, Sklenárová M¹, Arora M¹, Nováková A¹, Merdita S¹, Šteigerová M¹, Křížek T³, Slanař O¹, Šoós M², Šíma M¹

¹Institute of Pharmacology, First Faculty of Medicine, General University Hospital in Prague, Charles University, Prague, Czech Republic, ²Department of Chemical Engineering, Faculty of Chemical Engineering, University of Chemistry and Technology, Prague, Czech Republic, ³Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic

Introduction: Cannabinoids are a large group of pharmacologically active substances some of which are registered as medicinal products or currently tested as investigational compounds in the treatment of various neurological, psychiatric and immunological disorders. The oral bioavailability is usually low, but a proper measurement of the basic pharmacokinetic parameters is often not available. Furthermore, cannabinoids as highly lipophilic substances are expected to undergo lymphatic transport after intestinal absorption, but there are only very few studies addressing this topic. The aim of this work was to assess the pharmacokinetics of two selected important cannabinoids – cannabidiol (CBD) and cannabigerol (CBG) including the parameters of lymphatic transport.

Methods: A series of pharmacokinetic studies was performed in jugular vein cannulated rats. Anesthetized, mesenteric lymph duct cannulated rat model was used for the measurement of lymphatic transport parameters such as absolute bioavailability via lymph (FAL), absolute bioavailability via portal vein (FAP) and relative bioavailability via lymph (FRL). The tested compounds were administered in the form of an oil solution. The serum and lymph cannabinoid concentrations were measured using validated HPLC methods.

Results: The lymphatic transport has played a major role in the process of intestinal absorption in both compounds with mean \pm SD FRL of 49.6 \pm 11.6% for CBD and 51.2 \pm 23.8% for CBG (n=6 for both). The total absolute bioavailability (FAL + FAP) was rather low with 4.0 \pm 1.5% for CBD and 0.47 \pm 0.30 for CBG.

Conclusions: CBD and CBG display a poor bioavailability after oral administration in a basic oil solution with a significant proportion of substance being transported through the mesenteric lymphatic system.

This work was supported by GAUK no. 102322 and AZV NU22-08-00346 grants.

PP131. BP-2, a newly synthesized pan-PPAR agonist, emerges as a lead candidate for reducing liver fibrosis in non-alcoholic steatohepatitis

Sanz M^{1,2,3}, Marques P^{1,2,4}, Francisco V¹, Aparicio-Collado J¹, Domingo E¹, Gomez-Martin A¹, Descalzo I¹, Vila L¹, Piqueras L^{1,2,3}, Cortes D^{1,5}, Cabedo N^{1,5}

¹*INCLIVA Biomedical Research Institute, Valencia, Spain*, ²*Faculty of Medicine, University of Valencia, Valencia, Spain*, ³*CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Madrid, Spain*, ⁴*CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Madrid, Spain*, ⁵*Faculty of Pharmacy, University of Valencia, Valencia, Spain*

Introduction: Non-alcoholic steatohepatitis (NASH), a complication of metabolic syndrome, is a liver manifestation defined by the presence of steatosis and lobular inflammation often accompanied by liver fibrosis. The associated proinflammatory milieu seems to be the trigger for the development of related complications, some of which are irreversible processes. Importantly, no pharmacological approaches are currently available in this context. Additionally, a new pan-PPAR agonist, synthesized by our group (BP-2), demonstrated to normalize triglyceridemia and minimize the inflammation associated with metabolic dysfunction, especially the infiltration of T-lymphocytes and macrophages into the liver in a murine model of metabolic syndrome. Therefore, in this preliminary study, we evaluated the effects of BP-2 on steatosis, fibrosis, and inflammatory state in a murine model of NASH.

Methods: Six-week-old C57BL/6 mice (n=7/group) were fed a control diet, or subjected to a NASH-inducing diet (choline-deficient, high-fat diet) for 12 weeks. BP-2 was administered subcutaneously using osmotic pumps starting at week 6 for an additional 6 weeks (3 mg/kg/day). Then, mice were anesthetized and sacrificed, blood samples were collected into heparin-containing tubes, and complete blood counts were analyzed. Leukocyte subsets' activation (myeloid lineage: CD11b expression; lymphoid lineage: CD69 expression) was assessed by flow cytometry. Liver tissue was extracted for histological analysis, including hematoxylin-eosin staining for steatosis evaluation, and picrosirius red staining for fibrosis determination. The animal studies, approved by the ethics review board of the University of Valencia, were carried out in compliance with the guidelines of Directive 2010/63/EU of the European Parliament.

Results: BP-2 exerted no effects on steatosis development, which is in agreement with our previous results; nevertheless, BP-2 did reduce liver fibrosis in NASH animals (45.3% reduction). Although no significant differences in complete blood counts were observed, monocyte activation was increased by BP-2 treatment, which seems to be due to a shift towards a classical monocyte phenotype.

Conclusions: Our data suggest that BP-2 might emerge as a lead candidate for reducing liver fibrosis in NASH, a disease for which there is currently no pharmacological treatment. However, these are only preliminary results of an ongoing study, and further analysis needs to be done, including leukocyte infiltration into the liver, the characterization of the circulating cytokine profile, and the evaluation of the biochemical parameters.

This work was supported by the Spanish Ministry of Science and Innovation [PID2020-120336RB-I00]; the Carlos III Health Institute (ISCIII) and the European Regional Development Fund (FEDER) [PI21-00220]; the Generalitat Valenciana [APOTIP/2020/011, CIPROM/2022/45].

PP132. Misfolding of cystathione beta-synthase (CBS) in homocystinuria – A novel cell-based CBS folding reporter assay for the study of CBS folding and activity

Petrosino M¹, Ascencao K¹, Szabo C¹, Majtan T¹

¹*University of Fribourg, Fribourg, Switzerland*

Background: Cystathione beta-synthase (CBS) is a pivotal enzyme in sulfur amino acid metabolism, which deficiency results in classical homocystinuria (HCU) [1]. HCU is an inborn error of metabolism primarily caused by the presence of a missense mutation in the CBS gene [2]. Pathogenic CBS mutations often disrupt regulation or affect stability leading to protein misfolding and disposal. Pharmacological chaperones directly and specifically interact with the protein of interest to stabilize the active conformation and/or induce proper folding. As HCU is considered a conformational disorder, CBS-folding-reporter-assay would represent an invaluable tool in search for a potent and specific pharmacological chaperone, which would rescue folding, steady-state levels and enzymatic activity of pathogenic CBS variant.

Methods: We designed a self-complementing split-fluorescent protein CBS-folding-reporter-assay using an improved yellow-green split-mNeonGreen2 (mNG2) fluorescent. For the initial trial, the assay was constructed for the most common pathogenic CBS I278T variant using CBS WT as a positive control.

Results: We designed and prepared lentiviral vectors overexpressing non-fluorescent mNG2(1-10) fragment and mNG2(11) fragment N-terminally fused to CBS WT or I278T variant from a bicistronic IRES-mediated cassette. The vectors were used to generate stable cell lines using human HEK293 cells, which naturally express high levels of CBS, but the intrinsic CBS expression was knocked out by CRISPR-Cas9 approach. Using fluorescence microscopy, we confirmed that expression of CBS WT, which folds correctly, resulted in complementation of split-mNG2 fragments yielding strong localized fluorescent signal. On the other hand, expression of CBS I278T variant, which misfolds, produced cell-wide diffused signal equal to background fluorescent in comparison to CBS WT. Treatment with proteasome inhibitors known to rescue CBS I278T folding defects resulted in increased fluorescent signal intensity and localization similar to CBS WT as well as increased enzymatic activity of CBS I278T variant determined using hydrogen sulfide-sensing AzMC fluorescent probe. Furthermore, we evaluated performance of the CBS-folding-reporter-assay in high-throughput format using chemical library and characterized several hits in order to correlate rescue of CBS folding with the rescue of enzymatic activity.

Conclusion: Split-fluorescent protein complementation assay represents a powerful approach and novel opportunity in search for pharmacological chaperone able to correct folding and rescue activity of misfolded CBS variants.

- [1] Zuhra K, Augsburger F, Majtan T, Szabo C. (2020). Cystathione-β-synthase: molecular regulation and pharmacological inhibition. *Biomol.*, 10(5), 697, doi:10.3390/biom10050697.
- [2] Kraus JP, Janošík M, Kožich V, et al. (1999). Cystathione β-synthase mutations in homocystinuria. *Hum. Mutat.*, 13(5), 362-375, doi: 10.1002/(SICI)1098-1004(1999)13:5<362::AID-HUMU4>3.0.CO;2-K.

PP133. Novel identification of Glucocorticoid-induced leucine zipper in enteroendocrine cell subtypes in healthy and IBD gut

Rosati L¹, Cari L¹, Sette M¹, Leoncini G², Migliorati G¹, Ronchetti S¹

¹*Università Degli Studi di Perugia, Perugia, Italy*, ²*Fondazione IRCCS, Istituto Nazionale dei Tumori, Milano, Italy*

Introduction: Glucocorticoid (GC)-induced leucine zipper (GILZ) is a gene that mediates the anti-inflammatory effect of GCs. Besides immune cells, GILZ has been recently identified in goblet cells of the gut. Studies done on biopsies of inflammatory bowel disease (IBD) patients, including ulcerative colitis (UC), showed that GILZ was reduced in goblet cells in active disease, whereas it was restored in quiescent diseases [1]. GILZ was also observed highly expressed in cells morphologically identified as enteroendocrine cells (EEC). To determine the subtype of EEC expressing GILZ, we performed immunostaining on colon biopsies of healthy subjects and UC patients. We also used bioinformatics tools to confirm our observations.

Materials and Methods: FFPE biopsy specimens (5µm-thick) were deparaffinized and rehydrated. Antigen retrieval was performed with Sodium Citrate. Anti-GILZ (rabbit, 1:100 dilution), anti-MUC2 (mouse, 1:100), anti-5-HT (serotonin) (rat, 1:100) and anti-GLP-1 antibodies (mouse, 1:100 dilution) were incubated o.n. at 4°C. The slides were analyzed using a Nikon Eclipse Ti microscope equipped with Confocal Spinning Disk.

Gene expression profile analysis was performed using Gene Expression Omnibus (GEO) and ArrayExpress databases.

Results: The immunostaining revealed that GILZ was expressed not only in goblet cells co-expressing MUC2, but at high levels in cells morphologically identified as EEC. To determine this subtype of EEC, we started with the staining of enterochromaffin (EC) cells with an anti-5-HT antibody, which revealed a weak GILZ expression in EC cells. The alternative type of EEC cells expressed in the colon is the L-type, characterized by the expression of GLP-1, the hormone responsible for the release of insulin. Our results demonstrated that GILZ was co-expressed with GLP-1 in the majority of L-cells (GILZhight/GLP-1+, 80.6% of all GLP-1+ cells). Conversely, the analysis of GILZhight/GLP-1+ cells in UC revealed a significant reduction in these cells. Bioinformatics analysis found a significant correlation of GILZ with L-type specific genes, confirming our observations.

Conclusions: GILZ was here identified in L-type cells in the colon. Owing to the important role played by these cells in the stimulation of insulin, we believe that GILZ may play some role in the development of such cells, as confirmed by our bioinformatics analyses. Current studies are focused on the investigation of GILZ role in L-type EEC in health and disease.

- [1] Leoncini G, Gentili M, Lusenti E, et al. The novel role of glucocorticoid-induced leucine zipper as a marker of mucosal healing in inflammatory bowel diseases. Pharmacological Research. 2022;182:106353. doi:<https://doi.org/10.1016/j.phrs.2022.106353>

PP134. The Liver X receptor agonist GW3965 downregulates inflammatory cytokine expression and secretion in the muscular layers of the rat small intestine

Alfaqih M^{1,2}

¹Arabian Gulf University, Manama, Bahrain, ²Jordan University of Science and Technology, Irbid, Jordan

Introduction: Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the intestinal tract. Current treatment approaches are limited to alleviating symptoms [1]. Liver X receptors (LXRs) are nuclear receptors that modulate the expression of several cytokines involved in proinflammatory processes [2]. The role of these receptors in mediating an inflammatory process of the muscular layers of the small intestine was not previously investigated. This study thus represents a novel investigation of the anti-inflammatory effects of GW3965 (LXR agonist) in these tissues.

Methods: Smooth muscle layers of the small intestine were obtained from male Sprague Dawley rats. The mRNA and protein expression levels of LXR- α and LXR- β were evaluated using reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry techniques, respectively. Isolated muscular layers above were then subjected to different treatments: vehicle only, lipopolysaccharide (LPS) at a concentration of 1 μ M, LPS (1 μ M) along with GW 3965 at concentrations of 5 μ M or 10 μ M. Enzyme-linked immunosorbent assay (ELISA) was utilized to measure interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) levels in the muscular layer homogenate and conditioned media. Spectrophotometric methods were used to quantify nitric oxide (NO) metabolites and malondialdehyde (MDA) products.

Results: LXR- α and LXR- β were expressed in the isolated muscular layers at both the protein and mRNA levels, with LXR- β demonstrating comparatively higher mRNA and protein expression. Treatment with GW3965 significantly reduced LPS-induced expression and secretion of IL-1 β , IL-6, and TNF- α . This treatment also reduced the production of nitric oxide metabolites and the lipid peroxidation of intestinal smooth muscle layers.

Conclusion: This study is the first investigation to show that the LXR agonist GW3965 inhibited the production and secretion of proinflammatory cytokines and oxidative products involved in the inflammatory response of the smooth muscle layers of the small intestines. Our findings suggest that GW3965 could be of utility as a therapeutic agent in IBD and other inflammatory conditions of the small intestines.

[1] Body-Malapel M, Djouina M, Waxin C, et al. The RAGE signaling pathway is involved in intestinal inflammation and represents a promising therapeutic target for Inflammatory Bowel Diseases. *Mucosal Immunology*. 2019/03/01 2019;12(2):468-478. doi:10.1038/s41385-018-0119-z

[2] de la Aleja AG, Herrero C, Torres-Torresano M, et al. Inhibition of LXR controls the polarization of human inflammatory macrophages through upregulation of MAFB. *Cell Mol Life Sci*. Mar 17 2023;80(4):96. doi:10.1007/s00018-023-04745-4

PP135. Targeting the interaction of VEGFA with protein tyrosine phosphatase receptor zeta 1 as a novel anti-angiogenic approach

Choleva E¹, Ntenekou D¹, Kastana P¹, Tzoupis H^{2,3}, Katraki-Pavlou S⁴, Drakopoulou M¹, Kanelloupolou V¹, Enake M¹, Beis D^{4,5}, **Papadimitriou E¹**

¹Lab of Molecular Pharmacology, Dept. of Pharmacy, University of Patras, Patras, Greece,

²Department of Chemistry, University of Patras, Patras, Greece, ³Department of ChemoInformatics, NovaMechanics Ltd., Nicosia, Cyprus, ⁴Zebrafish Disease Models Lab, Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation Academy of Athens, Athens, Greece, ⁵Laboratory of Biological Chemistry, Faculty of Medicine, University of Ioannina, Ioannina, Greece

Introduction: Protein tyrosine phosphatase receptor zeta 1 (PTPRZ1) is a transmembrane tyrosine phosphatase (TP) that serves as a receptor for angiogenic growth factors, such as pleiotrophin (PTN) and vascular endothelial growth factor A (VEGFA), to regulate endothelial cell migration. In the present work, we studied whether disruption of the VEGFA binding to PTPRZ1 would affect VEGFA-induced endothelial cell activation and angiogenesis.

Methods: Endothelial cells from human umbilical veins and mouse lungs were stimulated by VEGFA165 in the presence or absence of the tested pharmacological inhibitors, followed by proliferation, migration, tube formation assays, and Western blot or proximity ligation assays for signaling molecules. In vivo, the zebrafish and the chick embryo chorioallantoic membrane (CAM) angiogenesis assays were employed. Molecular Dynamics and molecular docking simulations were performed to investigate the interactions between VEGFA and PTPRZ1.

Results: We identified a PTN peptide fragment (PTN97-110) that inhibits the interaction of VEGFA with PTPRZ1 but not with VEGF receptor 2 (VEGFR2). This peptide abolished the stimulatory effect of VEGFA165 on endothelial cell migration, tube formation on Matrigel, and Akt activation in vitro. It also partially inhibited VEGFA165-induced VEGFR2 activation but did not affect ERK1/2 activation and cell proliferation. In vivo, PTN97-110 inhibited or dysregulated angiogenesis in the CAM and the zebrafish assays, respectively. Finally, experimental and in silico evidence suggests that VEGFA binds to the extracellular fibronectin type-III (FNIII) domain of PTPRZ1 and inhibition of this binding by using an anti-FNIII antibody inhibited VEGFA165-induced HUVEC migration.

Conclusions: Our data highlight the VEGFA binding domain on PTPRZ1, strengthen the notion that PTPRZ1 is required for VEGFA-induced signaling, and identify a peptide that targets this interaction and can be exploited as a novel anti-angiogenic therapeutic approach.

Acknowledgments: This work has been financed by the State Scholarship Foundation in Greece (IKY) (Operational Program “Human Resources Development – Education and Lifelong Learning”, Partnership Agreement 2014-2020, scholarships to EC, DNt, PK, and SK-P), and a University of Patras “Andreas Mentzelopoulos” scholarship (to M-KE).

PP136. Casein kinase 2 (CK2) inhibition alleviates diet-induced harmful effects in a murine model of high fat diet consumption

Porchietto E¹, Aimaretti E², Ferreira Alves G¹, Einaudi G¹, Rubeo C², Collotta D³, Marzani E³, Mastrocola R², Aragno M², Cifani C¹, Collino M³

¹Pharmacology Unit, School of Pharmacy, University of Camerino, Camerino, Italy, ²Department of Clinical and Biological Sciences, University of Turin, Turin, Italy, ³Department of Neurosciences "Rita Levi Montalcini", University of Turin, Turin, Italy

Introduction: Casein kinase II (CK2) is a crucial enzyme implicated in the inflammatory crosstalk, whose upregulation has already been demonstrated in different pathologies [1]. TBB (4,5,6,7-tetrabromobenzotriazole) is a selective CK2 α inhibitor that has already been tested in vivo models of chronic inflammation[2]. Hence, we investigated the involvement of CK2 in metaflammation and the outcomes of the pharmacological inhibition of CK2 α in a murine model of HFHS consumption.

Methods: 45 male 4 weeks old male C57BL/6OlaHsd mice were fed with a standard diet (SD,10% fat, n=15) or a high-fat-high-sugar diet (HFHS, 58% fat, 26% sugar,n=15) for 12 weeks. A subgroup of HFHS were administered TBB (2,5mg/kg/day/p.o., HFHS+TBB,n=15) for the last 8 weeks. At the end of the protocol, oral glucose tolerance test was performed, and organ and plasma were collected for the ex-vivo analyses. Shapiro-Wilk test was used to verify data distribution. One way ANOVA followed by the analysis of variance and Bonferroni's test was assessed for establishing a standard of significance level ($p<0.05$). Data are expressed as mean \pm SEM.

Results: HFHS consumption induced glucose tolerance and alteration of lipid profile ($p<0.05$ vs SD): treatment with TBB reduced hyperglycaemia, restoring glucose and lipid homeostasis despite not being effective in reducing body weight gain ($p<0.05$ vs HFHS). HFHS fed mice presented increased plasmatic levels of transaminases (AST,SD=22,920 \pm 2.67 U/L,HFHS=64,270 \pm 13,99 U/L, n=11/group, $p<0.05$; ALT,SD=10,230 \pm 1,73U/L, HFHS=34,440 \pm 7,39U/L,n=11/group, $p<0.05$) and pro-inflammatory cytokines, i.e. TNF α (SD=2,368 \pm 0,58 μ g/ml, HFHS=9,883 \pm 2,318 μ g/ml, n=7/group, $p<0.05$): interestingly, TBB reduced these values AST(HFHS=64,270 \pm 13,99 U/L,HFHS+TBB=27,550 \pm 4,62U/L,n=11/group, $p<0.05$),ALT(HFHS=34,440 \pm 7,39U/L,HFHS+TBB=15,350 \pm 2,15U/L,n=11/group, $p<0.05$), TNF α (HFHS=9,883 \pm 2,318 μ g/ml,HFHS+TBB=1.653 \pm 0.634 μ g/ml,n=7/group, $p<0.05$). We recorded CK2 α hepatic upregulation in HFHS fed mice ($p<0.05$ vs SD) that was significantly reduced by TBB ($p<0.05$ vs HFHS). Furthermore, the drug simultaneously downregulated other inflammatory cascades involved in metaflammation, i.e. NF κ B, JAK2/STAT3, NLRP3($p<0.05$ vs SD), dramatically upregulated by HFHS chronic feeding ($p<0.05$ vs HFHS).

Conclusions: Pharmacological intervention with TBB alleviates diet-induced metabolic derangements, hence hinting at CK2 as a key player for counteracting metaflammation.

- [1] Borgo C, D'Amore C, Sarno S, Salvi M, Ruzzene M. Protein kinase CK2: a potential therapeutic target for diverse human diseases. Signal Transduct Target Ther. 2021;6(1):183. Published 2021 May 17. doi:10.1038/s41392-021-00567-7
- [2] Huang J, Chen Z, Li J, et al. Protein kinase CK2 α catalytic subunit ameliorates diabetic renal inflammatory fibrosis via NF- κ B signaling pathway. Biochem Pharmacol. 2017;132:102-117. doi:10.1016/j.bcp.2017.02.016

PP137. The Role of Store-Operated Calcium Entry in Sorafenib-Resistant Hepatocellular Carcinoma

Yurdacan Yasar B¹, Erac Y

¹Ege University, Bornova, Turkey

Introduction: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the leading cause of cancer-related deaths worldwide. Sorafenib (SOR) is used to suppress Ras/Raf/MEK/ERK signaling pathways in the treatment of HCC. Approximately 30% of HCC patients respond to SOR treatment, while the remainder develop drug resistance. Intracellular Ca²⁺ homeostasis regulates different aspects of cancer progression. Additionally, numerous studies demonstrate the role of store-operated calcium entry (SOCE) in tumor development, progression, and drug responses. The aim of this study is to investigate the contribution and potential therapeutic effects of SOCE and SOCE-related proteins to the development of resistance to SOR in SOR-resistant Huh7 cells.

Methods: We induced in vitro resistance in the Huh-7 cell line by exposing it to increasing doses of SOR ranging from 2 μM to 6 μM for 6 months. SOR resistance was confirmed using a real-time cellular analysis system (xCELLigence) in SOR-resistant Huh (SRHuh7) and Huh7 cells. RT-PCR was performed to determine mRNA levels of MDR1, N-cadherin, E-cadherin, TRPC1, TRPC6, STIM, and Orai1. Additionally, nuclear morphologies of Huh7 and SRHuh7 cells were monitored by DAPI staining. Migration levels were analyzed using wound healing assay, and changes in intracellular Ca²⁺ levels were determined by spectrofluorometry in Fura-2/AM-loaded Huh-7 and SRHuh7 cells.

Results: The half-maximum inhibitory concentration (IC50) of Huh7 and SRHuh7 cells was determined as 2.24 and 5.071μM, respectively. In SRHuh7 cells, MDR1 and N-cadherin mRNA expression increased significantly compared to Huh7 cells, while E-cadherin mRNA expression decreased ($p < 0.05$). No significant difference was observed in the nuclear morphology of Huh7 and SRHuh7 cells by DAPI staining. A significant increase in TRPC1, TRPC6, STIM1 and Orai1 mRNA levels was observed in SRHuh7 cells compared to Huh-7 cells. Real-time changes in [Ca²⁺]_i were monitored in Huh-7 and SRHuh7 cells. SOCE was significantly increased in SRHuh7 cells compared to Huh-7 cells ($p < 0.05$).

Conclusion: With this study, we showed for the first time that SOCE can play an important role in SOR resistance in HCC. Our findings suggest that pharmacologically inhibiting SOCE may be a novel strategy to overcome SOR resistance in HCC. However, further studies are needed to determine the effects of SOCE on resistance mechanisms. This project was supported by the Ege University Research Project (Project no: 23075) and The Scientific And Technological Research Council Of Türkiye (Project no: 223S649).

PP138. The Effects of Hyperforin in the Sorafenib-Resistance Hepatocellular Carcinoma

Yilmaz E¹, Yurdacan B², Getbogan D², Sen T³, Karstarli I³, Erac Y⁴

¹*Department of Biotechnology, The Institute of Natural and Applied Sciences, Ege University, Izmir, Turkey*, ²*Department of Pharmacology, The Institute of Health Sciences, Ege University, Izmir, Turkey*, ³*Faculty of Pharmacy, Ege University, Izmir, Turkey*, ⁴*Department of Pharmacology, Faculty of Pharmacy, Ege University, Izmir, Turkey*

Introduction: Hepatocellular carcinoma (HCC) is a major cause of cancer-related deaths and is often associated with chronic liver disease. Sorafenib, a multi-target tyrosine kinase inhibitor, is a frontline therapy for advanced HCC. However, the emergence of sorafenib resistance remains a significant challenge in HCC treatment. Calcium signaling through store-operated Ca²⁺ entry (SOCE) plays a significant role in HCC progression, with TRPC1 and TRPC6 channel proteins being involved in SOCE. Hyperforin, a component of Hypericum perforatum, is a TRPC6 activator that reduces cell proliferation and causes apoptotic death. This study aims to investigate the effects of hyperforin on sorafenib-resistant HCC cells and resistance mechanisms.

Methods: We established a sorafenib-resistant HCC cell line (SRHuh7) through prolonged exposure to increasing sorafenib concentrations. The resistance was confirmed using a real-time cellular analysis system (xCELLigence) and RT-PCR in SRHuh7. The effect of hyperforin on the cell proliferation rate was also monitored in real-time. The mRNA expression levels of MDR1, N-cadherin, E-cadherin, TRPC1, and TRPC6 were determined in SRHuh7 cells treated with 2.5 µM hyperforin using RT-PCR. Additionally, intracellular calcium levels were measured by spectrofluorometry in SRHuh7 cells, and survival analyses were conducted in HCC patients.

Results: Hyperforin effectively inhibited SOCE in SRHuh7 cells, leading to enhanced cytotoxicity. Furthermore, hyperforin demonstrated a significant capacity to overcome Multi-Drug Resistance (MDR) by downregulating key MDR and EMT-related genes. Kaplan-Meier survival analysis revealed a correlation between high TRPC6 expression and improved survival outcomes in HCC patients on sorafenib.

Conclusion: Our findings suggest a novel therapeutic strategy, with hyperforin emerging as a promising candidate for addressing sorafenib resistance in HCC by targeting TRPC6 and SOCE. The study provides insights into the molecular mechanisms underlying sorafenib resistance and proposes a novel approach to enhance the effectiveness of sorafenib in HCC treatment. Moreover, natural products like hyperforin with low side effects and high bioavailability potential can be an alternative treatment approach to prevent and treat chemoresistance in patients treated with SOR. Further investigations are needed to elucidate the detailed mechanisms of hyperforin-mediated SOCE responses.

This project was supported by The Scientific and Technological Research Council of Turkey (TUBITAK 2209 Project No: 1919B012309503) and Ege University Research Project (Project No: 23075).

PP139. First generation vanadium-based PTEN inhibitors: comparative study in vitro and in vivo and identification of a novel mechanism of action.

Premeti K¹, Nadalis A¹, Syropoulou V¹, Karagkiozeli D¹, Aggelis G¹, Tsipa D¹, Papanikolaou M², Kampanos T², Labrakakis C³, Pappas P¹, Antoniou K¹, Doulias P², Leondaritis G¹

¹*Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece*, ²*Department of Chemistry, University of Ioannina, Ioannina, Greece*, ³*Department of Biological Applications and Technology, School of Health Sciences, University of Ioannina, Ioannina, Greece*

Introduction: PTEN, a crucial lipid phosphatase in human physiology, is implicated in various human diseases and pathologies [1]. PTEN inhibitors have been widely used in both cell-based and animal experiments. However, their specificity, potency and mechanism of action remain unexplored. In the present study we report the comprehensive re-evaluation of first-generation PTEN inhibitors, including standard bisperoxo-vanadium (V) complex compounds (bpVs) by focusing on their inhibitory efficacy, selectivity, and mechanism of action in vitro and in vivo.

Methods: We synthesized established and novel vanadium-based and vanadium-free compounds and assessed inhibition of PTEN using water-soluble PI(3,4,5)P3. Phosphorylation of Akt at Ser473 and S6 at Ser233/235 were used as proxy markers of PTEN inhibition in PTEN WT and null cells or tissues. Oxidation of PTEN was assessed using a modified WB assay under non-reducing conditions, while organic mercury solid phase was used for chemoselective capture of S-nitrosylated PTEN. bpV(phen) was administered in Wistar rats (1mg/Kg) evaluating Akt/mTORC1 activity after 1h (n=4) or after 10 days (n=5) in CNS and peripheral tissues. Wistar rats treated with saline (n=6) or bpV(phen) (n=6) were subjected to Open Field test to score locomotion.

Results: All drugs inhibited PTEN at the low micromolar range in vitro (IC50 values: 0.2-0.8μM). bpV(phen), bpV(OHpic), and a phenanthroline-dione compound induced PTEN-dependent increase of pSer345Akt in PTEN WT but not PTEN null cells, suggesting specificity. bpV(phen), in addition, increased pSer345Akt in PTEN null cells after expression of exogenous PTEN. In vivo, bpV(phen) increased pS233/235-S6 levels in kidney and liver but not CNS tissues after both acute and subchronic administrations. Furthermore, bpV(phen) inhibited the locomotion and exploratory activity of Wistar rats. Notably, while PTEN inhibition by bpV(phen) and other drugs depended on reducing conditions in vitro, oxidative inhibition of PTEN was not confirmed in cells. On the contrary, bpV(phen) inhibits PTEN via a mechanism involving S-nitrosylation.

Conclusions: First-generation PTEN inhibitors, especially bpVs, have been used extensively in cellular and animal studies. We show here that although compounds such as bpV(phen) and bpV(OHpic) are potent PTEN inhibitors in vitro and in cells, they suffer from low specificity (bpV(OHpic)) and inhibit basal locomotion and exploratory behavior in vivo (bpV(phen)). Importantly, we found that bpV(phen) inhibits PTEN by S-nitrosylation, challenging the previously assumed oxidative inhibition.

[1] Kotzampasi DM, Premeti K, Papafotika A, et al. The orchestrated signaling by PI3Kα and PTEN at the membrane interface. Comput Struct Biotechnol J. 2022;20:5607-5621. Published 2022 Oct 7. doi:10.1016/j.csbj.2022.10.007

PP140. Effects of Broad-Spectrum UV Absorber Ecamsule on Epidermal Barrier Constituents in Human Keratinocytes

Huang Y¹, Chang K¹, Yang C¹

¹Providence University, Taichung, Taiwan

Introduction: Chronic ultraviolet (UV) irradiation can lead to skin photoaging and disturb skin compositions. Damage of skin barrier may cause skin problems, and even lead to chronic inflammatory skin diseases. Ecamsule as a water-soluble and broad-spectrum UV absorber is commonly used in sunscreens. In the present study, we intend to investigate the effects of ecamslule on cellular oxidative stress, skin hydration, and barrier constituents in human keratinocytes.

Methods: Cytotoxicity of ecamslule was examined with the MTT assay. Extracellular contents of hyaluronan (HA) were estimated for skin hydration. The state of oxidative stress was determined by measuring intracellular levels of reactive oxygen species (ROS) using the fluorescence dye 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Expression of skin barrier-related proteins, differentiation markers, and water channel aquaporin-3 (AQP3) were evaluated using real-time polymerase chain reaction and western blots, respectively.

Results: According to the results of DCFH-DA staining assay, it exhibited that ecamslule increased the intracellular levels of hydrogen peroxide (H₂O₂). In the meantime, ecamslule enhanced the mRNA expression of epidermal differentiation markers, keratin 10 and loricrin. Subsequently, ecamslule increased involucrin and transglutaminase as well as up-regulated protein expression of AQP3. Although HA is responsible for water retention capacity, human keratinocytes treated with ecamslule did not influence the extracellular HA contents.

Conclusions: These data demonstrate that UVA filter ecamslule increases intracellular ROS accumulation and subsequently promotes the expression of epidermal differentiation markers and aquaporin-3 in human HaCaT keratinocytes. It has been demonstrated that cell-surface NADPH oxidase enzymes drive the production of H₂O₂, and AQP3 can facilitate the uptake of H₂O₂ for intracellular signaling. It suggests that ecamslule act through the AQP3-mediated uptake of H₂O₂ to interfere with skin barrier, which is based on altering the components of cornified envelope such as loricrin, involucrin, and transglutaminase. In conclusion, the present study illustrates that ecamslule regulates the molecular compositions of skin barrier in human keratinocytes.

PP141. A human neuroinflammation platform for drug screening against neurological disorders.

Charou D¹, Chanoumidou K¹, Malliou F², Kakatsou P², **Charalampopoulos I¹**

¹*Dept. of Pharmacology, Medical School, University of Crete & Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, Greece*, ²*Institute of Pharmaceutical Research and Technology (IFET S.A.), Research Laboratory, Dept. of Research, Pallini, Greece*

Introduction: Neuroinflammation is considered a major pathological co-factor in many diseases of the Central Nervous System. Although inflammation represents a beneficial mechanism that initially protects the brain by removing pathogens, sustained secretion of pro-inflammatory factors by astrocytes and microglia, as observed under neuropathological conditions, has a detrimental effect on neuronal health. Therefore, various anti-inflammatory treatments have emerged as novel therapeutic strategies against neurodegenerative disorders like Alzheimer's Disease (AD). To date, most of the studies for developing new therapeutics that could target neuroinflammation have taken place in rodent models. However, most of the tested drugs on mice have no therapeutic outcome in humans. Thus, the need for developing human-based models for brain diseases is of high importance and value.

Methods: In the present project we use commercially available human induced Pluripotent Stem Cell (hiPSC) lines, which we specifically differentiate towards mature neuronal and glial cells and their precursors. Co-cultures of the aforementioned cell types have been developed in order to resemble human brain neuroinflammatory conditions.

Results: The present translational research aims to establish and exploit a human iPSC-based brain model imitating neuroinflammatory conditions, in order to be utilized as drug screening platform. The generated platform is comprised of hiPSC-derived neurons and astrocytes co-cultured in 2D and 3D conditions (using porous collagen scaffolds). Use of AD-derived iPSCs allows for personalized disease models and evaluation of inflammatory component in a disease-specific context.

Conclusions: Our efforts are focusing on the development and use of a the multi-cellular platform as a humanized disease model for testing the toxicity and efficacy of known and newly developed anti-inflammatory drugs against inflammatory diseases, visioning to accelerate preclinical drug development.

PP142. Combined impact of adolescent ethanol and cocaine exposure on hippocampal neurochemical markers in male and female rats

Colom-Rocha C^{1,2}, Garcia-Fuster J^{1,2}

¹IUNICS, University of the Balearic Islands, Palma, Spain, ²Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain

Introduction: Drug use is frequently initiated during adolescence, a vulnerable period of development with a great deal of neuro-remodeling characterized by a unique sensitivity to drug abuse, specially affecting hippocampal remodeling (i.e., neuroplasticity and cell fate regulation), which could potentially contribute to sustained addictive behaviors. In this context, previous data centered in evaluating the neurochemical effects exerted by either cocaine or ethanol alone in the adolescent brain, but since few studies measured the combined negative impact of both drugs, this will be the aim of the present study.

Methods: Groups of allocated Sprague-Dawley rats (n=44) were treated during adolescence with binge ethanol (2 g/kg, i.p., 2 consecutive days at 48-h intervals x 3 rounds, between PND 29-38), cocaine (15 mg/kg/day, i.p., from PND 33-38), the combination of both (ethanol + cocaine) or vehicle (0.9% NaCl, 1 ml/kg/day, between PND 29-38) (n=5-8 per group/sex). Rats were sacrificed 24 h after treatment (PND 39), when neurogenesis markers (Ki-67: cell proliferation; NeuroD: early neuronal survival) were evaluated by immunohistochemistry, and several hippocampal markers involved in the regulation of cell fate (BDNF, FADD, CDK-5, NF-H) were ascertained by Western blot analysis. Since no significant sex differences were observed in the parameters evaluated, we performed one-way ANOVAs combining all rats independently of sex (n=10-16 per group), and with treatment as the main variable.

Results: The main results showed that either the combination of ethanol + cocaine or just ethanol alone decreased hippocampal NeuroD +cells (**p<0.001 and *p=0.027 respectively) and FADD adaptor protein (*p=0.026 and *p=0.017 respectively, vs. vehicle-treated rats), with no other modulations observed. Moreover, cocaine alone did not alter any of the markers evaluated in hippocampus.

Conclusions: The findings indicate clear signs of neurotoxicity induced by ethanol in the hippocampus of adolescent rats, since cocaine did not induce any apparent damage, and therefore, the effects observed when combining both drugs are clearly driven by ethanol. Moreover, they demonstrate no differences in the neurochemical damage induced by the drugs by biological sex. Future studies will evaluate whether these effects persist over time and into adulthood.

Funded by Delegación del Gobierno para el Plan Nacional sobre Drogas (2020/001, Ministerio de Sanidad) to MJG-F. CC-R and MJG-F are members of RIAPAd (RD21/0009/0008; Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación).

PP143. IL-33 and IL-18 drive the pro-tumoral ILC2 functions in prostate cancer patients

Maresca D¹, La Civita E², Romano B¹, Ambrosio M², Somma F¹, Ianaro A¹, Terracciano D², **Ercolano G¹**

¹*Department of Pharmacy, University of Naples Federico II, Naples, Italy*, ²*Department of Translational Medical Sciences, University of Naples "Federico II", Naples, Italy*

Prostate cancer (PCa) is one of the most common cancer in males worldwide with a high mortality rate [1]. Different immune cell populations are associated with PCa progression, therapy resistance and establishment of a pro-tumoral microenvironment. Innate lymphoid cells (ILCs) represent a recently identified family of innate immune cells considered as the innate counterpart of T lymphocyte subpopulations [2]. In particular, type-2 ILCs (ILC2s) are defined as a primarily pro-tumorigenic subset through the production of type 2 cytokines (IL-5, IL-13) [3]. Nevertheless, nothing is known about the contribution of ILCs in PCa development and progression. In this study we characterized the role of ILCs in PCa in the Peripheral Blood Mononuclear Cells (PBMCs) of PCa patients.

PBMCs were isolated by Lymphoprep. PCa patients (n= 43) were classified in low-grade and high-grade (LG and HG respectively) according to the Gleason score and ILCs frequency was evaluated by flow cytometry and compared to healthy donors (HDs) (n= 21). The levels of ILC2-activating cytokines have been analyzed by multiplex assay in the serum of HDs and PCa patients (n=8 and n=24 respectively). For evaluating the crosstalk between ILC2s and cancer cells, PC3 human prostate cancer cells were used. Finally, by exploiting bioinformatical tools, we evaluated the impact of ILC2 in PCa patients survival as well as the contribution of IL-33 and IL-18 in sustaining the progression of PCa.

We found that the frequency of ILC subsets was dysregulated in PCa patients. In particular, ILC2s were increased in both LG and HG PCa patients. The highest frequency of ILC2 was correlated with the Prostate-Specific Antigen (PSA) levels in PCa patients and affected patients' survival. Moreover, the frequency of ILC2s in PCa patients was correlated to higher levels of ILC2-activating cytokines revealing that both IL-33 and IL-18 might play a potential role in PCa progression. Our results suggest that both ILC2s and their activating cytokines (IL-33 and IL-18) could represent a novel therapeutic target for the treatment of PCa.

[1] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *Cancer J Clin.* 2021;71(3):209-249.

[2] Eberl G, Colonna M, Di Santo JP, McKenzie AN. Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology. *Science.* 2015;348(6237):aaa6566.

[3] Wu L, Zhao W, Tang, Chen R, Ji M, Yang. Role of ILC2s in Solid Tumors: Facilitate or Inhibit?. *Front Immunol.* 2022;13:886045.

PP144. Oleoylethanolamide (OEA) restores skeletal muscle dysmetabolism in pre-obese young rats

Vari F¹, Friuli M¹, Stanca E¹, Romano A¹, Gaetani S¹, Vergara D¹, Giudetti A¹

¹*Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy, Rome, Italy,* ²*Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce, Italy, Lecce, Italy*

Obesity is a pathological condition whose incidence is constantly increasing among young people due to the prevalent consumption of foods rich in saturated fats and refined sugars. Skeletal muscle insulin resistance, related to obesity, is one of the causes of metabolic diseases onset [1]. Understanding the metabolic changes that precede obesity can help find strategies to avoid the onset of obesity-related diseases [2]. Oleoylethanolamine (OEA), a lipid mediator belonging to the N-acylethanolamine family, is widely studied for its positive effect on food intake and lipid metabolism [3]. We therefore investigated the effect of OEA on skeletal muscle metabolism in young pre-obese rats.

Young male Wistar rats were fed a high-fat diet (HFD) or a low-fat diet (LFD) for 7 weeks. Both groups were subsequently divided into two experimental groups and subjected to the following treatments for 14 days: HFD, or LFD ad libitum + daily intraperitoneal injection of vehicle (HV and LV groups respectively) and HFD, or LFD, ad libitum + intraperitoneal injection of OEA (10 mg/kg) (HO and LO groups, respectively).

Skeletal muscle from the HV group accumulated triacylglycerols and had lower activation of the insulin signaling compared to the LV group. Furthermore, compared to LV, the skeletal muscle of the HV group showed hypertrophy and fibrosis of myofibers and increased expression and activity of mitochondrial respiratory complexes. Additionally, lower levels of carnitine palmitoyltransferase-1 and ATP, with induction of mitochondrial biogenesis, were also measured in the skeletal muscle of the HV group, compared to LV. In all cases, OEA completely ameliorates changes induced by HFD. Overall, our findings suggest that short-term HFD induces early detrimental changes in skeletal muscle that are reversed by OEA supplementation.

- [1] Li Y, Xu S, Zhang X, Yi Z, Cichello S. Skeletal intramyocellular lipid metabolism and insulin resistance. *Biophys Rep.* 2015;1:90-98. doi: 10.1007/s41048-015-0013-0.
- [2] World Health Organization. Obesity and Overweight. <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>. April 2020.
- [3] Schwartz GJ, Fu J, Astarita G, Li X, Gaetani S, Campolongo P, et al. The lipid messenger OEA links dietary fat intake to satiety. *Cell Metab* (2008) 8:281–8. Doi: 10.1016/j.cmet.2008.08.005.

PP145. H₂S is involved in the relaxant effects of osthole on murine corpus cavernosum

Alan Albayrak E^{1,2}, Pinilla E², Comerma Steffensen S², Simonsen U², Sevin G¹

¹Department of Pharmacology, Faculty of Pharmacy, Ege University, Izmir, Türkiye, ²Department of Biomedicine, Pulmonary and Cardiovascular Pharmacology, Aarhus University, Aarhus, Denmark

Introduction: Phosphodiesterase type-5 inhibitors are ineffective in 40% of patients with erectile dysfunction(ED), highlighting the need for NO-independent therapies. Osthole relaxes the corpus cavernosum largely independently of the endothelium[1], although its mechanism of action remains elusive. H₂S also has an endothelium-independent relaxant effect, sharing some mechanisms with osthole. Hence, we aimed to investigate the potential contribution of H₂S to the beneficial effects of osthole on erectile function.

Methods: Swiss albino mouse corpus cavernosum(MCC) and Wistar male rat pudendal artery(RPA, the main source of blood flow to the corpora) were mounted on a strip and an H₂S microsensor-attached wire myograph, respectively. Osthole(10^{-7} - 10^{-4} M)-induced relaxations were obtained with/without aminoxyacetic acid(AOAA, H₂S synthesis inhibitor, 10 mM, 30 min). L-cysteine (Substrate of H₂S synthesis, MCC: 10^{-5} - 10^{-2} M, RPA: 10^{-3} - 3×10^{-2} M)- and Na₂S(H₂S donor, MCC: 10^{-6} - 3×10^{-3} M, RPA: 10^{-5} - 3×10^{-3} M)-induced relaxations were investigated in the presence/absence of osthole(MCC:20μM, RPA:5μM, 15 min) and AOAA. The H₂S microsensor was inserted into the lumen of the RPA and H₂S levels were measured simultaneously during these concentration-response curves[2]. The expression levels of cystathionine-γ-lyase(CSE) and cystathionine-β-synthase(CBS) and real-time H₂S production in MCC homogenates(-/+ Osthole:20 μM, 100 μM, 24 h.) were studied by Western blot and H₂S microsensor experiments, respectively.

Results: AOAA inhibited osthole-induced relaxations in MCC($P<0.001$)(n=7) and RPA($P<0.05$)(n=5). In RPA, AOAA also decreased endogenous H₂S production induced by osthole($P<0.05$)(n=5). Osthole augmented the relaxation responses to L-cysteine and Na₂S in MCC/RPA, which were inhibited by AOAA($P<0.001$)(n=5). Additionally, osthole enhanced Na₂S- and L-cysteine-induced increases in H₂S levels in the lumen of RPA($P<0.05$)(n=5). In MCC homogenate, 20μM($P<0.05$) and 100μM($P<0.01$) osthole increased the expression levels of CSE and CBS, and H₂S levels, H₂S production rate(n=6).

Conclusions: For the first time, endogenous H₂S formation in the arterial lumen and changes in tension in response to osthole were simultaneously measured in RPA, showing the involvement of H₂S in the vascular effects of osthole. Osthole relaxed MCC and RPA in an H₂S-dependent manner, which could be linked to an increase in H₂S synthesis. Further studies should address whether osthole is a potential drug candidate for the treatment of ED.

Acknowledgments: This project is supported by The Scientific and Technological Research Council of Türkiye(TUBITAK,2211-C,2214-A programs), and Ege University office of Scientific Research Projects(Grant number:TDK-2021-22681).

[1] Chen J, Chiou WF, Chen CC, Chen CF. Effect of the plant-extract osthole on the relaxation of rabbit corpus cavernosum tissue in vitro. *J Urol*.2000;163(6):1975-1980.doi:10.1016/S0022-5347(05)67613-6

[2] Nielsen ER, Winther AK, Simonsen U. Simultaneous measurements of tension and free H₂S in mesenteric arteries. *Methods Mol Biol*.2019;2007:125-136.doi:10.1007/978-1-4939-9528-8_9

PP146. Circulating MicroRNAs from Plasma Extracellular Vesicles in Patients with Pharmacoresistant Epilepsy

Palmisani M^{1,2}, Tartara E², Crema F¹, Rizzo B², Galimberti C², Dattrino F³, Fattore C², Fedele G⁴, Gagliardi S², **Franco V^{1,2}**

¹Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy, ²IRCCS Mondino Foundation, Pavia, Italy, ³Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy, ⁴Associazione Farmaceutici dell'Industria (AFI), Milan, Italy

Background: Extracellular vesicles (EVs) are small membranous particles present in all human body fluids and their features are affected by many pathophysiological conditions. The exchange of EVs from and to the brain has been demonstrated in recent years. These particles are involved in cell to cell communication and deliver different cargos such as proteins, lipids, DNA, and RNAs, including MicroRNAs (miRNAs), a set of molecules which have attracted attention as potential epilepsy biomarkers [1]. In order to determine whether differences in EVs content differentiate drug-resistant from drug-responsive epilepsy, we evaluated plasma EVs content in adult patients with focal epilepsy.

Methods: We compared EVs in 20 pharmacoresistant patients, 20 pharmacoresponsive patients and 20 healthy controls. Individuals across the three groups were matched for age and gender. Large and small EVs (LEVs and SEVs) were isolated from plasma of patients and healthy volunteers by differential centrifugation with a validated protocol [2], and studied with nanoparticle tracking analysis to investigate their size. MiRNAs were extracted and sequenced by Next Generation Sequencing analysis. Transcriptomic analysis was performed with Illumina NextSeq.

Results: For LEVs, mean size was 114.1 ± 32.5 nm in controls, 109.5 ± 26.4 nm in pharmacoresponsive patients and 124.5 ± 23.1 nm in pharmacoresistant patients. For SEVs, mean size was 70.1 ± 8.1 nm in controls, 73.1 ± 16.5 nm in pharmacoresponsive patients and 72.4 ± 13.1 nm in pharmacoresistant patients. Five miRNAs were found to be de-regulated in SEVs isolated from pharmacoresistant patients compared with healthy controls. Specifically, miR-145-5p was up-regulated, whereas miR-3128, miR-3150a-3p, miR-3925-5p, and miR-6772-5p were down-regulated in pharmacoresistant patients. Moreover four miRNAs were found to be de-regulated in LEVs obtained from pharmacoresistant patients compared with healthy controls: two up-regulated miRNAs (miR-183-5p, miR-541-3p) and two down-regulated miRNAs (miR-26a-2-3p, miR-190a-5p).

Conclusions: Our findings revealed a limited set of miRNAs exhibiting deregulation, as a rigorous bioinformatics analysis was conducted to identify specific miRNAs. Notably, certain miRNAs may undergo deregulation in individuals with epilepsy, and variations in miRNA expression could potentially distinguish patients with drug-refractory focal epilepsy from those with drug-responsive focal epilepsy.

- [1] Yan S, Zhang H, Xie W, Meng F, Zhang K, Jiang Y, Zhang X, Zhang J. Altered microRNA profiles in plasma exosomes from mesial temporal lobe epilepsy with hippocampal sclerosis. Oncotarget. 2017;8:4136-4146.
- [2] Théry C., Witwer K.W., Aikawa E., 2018. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7(1):1535750.

PP147. Dose-dependent effects of standardized nose-horned viper (*Vipera ammodytes ammodytes*) venom on vascular responsiveness in isolated rat aorta

Mudnić I¹, Karabuva S², Šušak Crnčević M³, Lukšić B^{2,4}, Boban M¹

¹*Department of Basic and Clinical Pharmacology University of Split School of Medicine, Split, Croatia,*

²*Clinical Department of Infectious Diseases, University Hospital of Split, Split, Croatia,* ³*MediDerm Clinic of Dermatology and Venerology, Split, Croatia,* ⁴*Department of Infectology, University of Split School of Medicine, Split, Croatia*

Introduction: Venom of the nose-horned viper (*Vipera ammodytes ammodytes*), the most venomous European snake, induces various clinical manifestations, ranging from local tissue damage to potentially life-threatening systemic effects, including cardiovascular and hemodynamic disturbances [1]. In contrast to cardiotoxic effects of the venom and its fractions, described in isolated perfused rat heart [2], studies on the vascular effects are lacking. Therefore, we examined the direct dose-dependent vasoactive activity of the standardized nose-horned viper venom and how it affects vascular responsiveness in isolated rat aorta.

Methods: Thoracic aortas isolated from male Sprague Dawley rats (N=20) were randomized for either venom vasoactive or vascular responsiveness protocol. In vasoactive protocol, venom was cumulatively applied to final concentration from 3 to 150 µg/L on intact (n=22), or denuded (n=18) rings, sub-maximally pre-contracted with 1 µM noradrenaline. In vascular responsiveness protocol, acetylcholine (n=21) or noradrenaline (n=14) were cumulatively applied to final concentrations from 1 nM to 10 µM on control rings and venom-incubated rings (150 µg/L). Maximal effect (Emax) and the concentration that produced 50% of maximal effect (EC50) for the venom, noradrenaline and acetylcholine were evaluated. The venom sample was previously biologically standardized and the median lethal, the minimum hemorrhagic, and the minimum necrotizing dose (LD50, MHD, and MND, respectively) were detected [2]. The study was approved by the institutional Ethics committee.

Results: Nose-horned viper venom with LD50 of 12.5 µg/mouse, MHD of 14 µg/rat and MND of 17 µg/rat, induced dose-dependent direct vasodilatory effect with Emax of 78.3±5.1% and 64.7±10.9%, and EC50 being 7 µg/L (95%CI: 4-12 µg/L) and 52 µg/L (95%CI: 31-87 µg/L) for intact and denuded rings, respectively. Exposure to the venom reduced acetylcholine Emax from 117.5±3.9% to 65.7±5.2% and increased EC50 from 24 nM (95%CI: 18-34 nM) to 340 nM (95%CI: 229-510 nM). Furthermore, contractile response to noradrenaline was also reduced following exposure to the venom with decreased Emax (110.7±12.7% vs. 98.8±9.1%) and increased EC50 (39 nM (95%CI: 24-63 nM) vs. 110 nM (95%CI: 76-170 nM)), respectively.

Conclusion: Standardized nose-horned viper venom is a strong direct vasodilator of isolated rat aorta, at least partially dependent on the endothelium. The venom affected both vasodilation and vasoconstriction capacity in the model of isolated rat aorta.

[1] Dobaja Borak M, et al. Viper envenomation in Central and Southeastern Europe: a multicentre study. *Clin Toxicol (Phila)*. 2023;61(9):656-664.

[2] Karabuva S, et al. Cardiotoxic effects of the *Vipera ammodytes ammodytes* venom fractions in the isolated perfused rat heart. *Toxicon*. 2016;121:98-104.

PP148. The Effect of Intra-Hypothalamic Ghrelin Administration on Cisplatin-Induced Side Effects in *Suncus murinus*

Yang L¹, Lu Z¹, Liu Y¹, Ngan M¹, Sakai T², Sakata I², Chan S³, Rudd J¹

¹School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, Hong Kong,

²Graduate School of Science and Engineering, Saitama University, Saitama City, Japan,

³School of Health Sciences, Caritas Institute of Higher Education, Hong Kong, Hong Kong

Previous studies showed that centrally administrated acyl-ghrelin (AG) could antagonize cisplatin-induced emesis in ferrets, and we speculated an involvement of the hypothalamus. AG acts at GHSR1A, but it can also be de-acylated to des-acyl-ghrelin (DAG). It has been speculated that AG may have low potency actions at a distinct DAG receptor, but the receptor has not been cloned.

In the present studies, we used *Suncus murinus* to investigate the effect of intra-hypothalamic infusion of AG and DAG on cisplatin-induced emesis and plasma substance P and vasopressin levels.

Male *Suncus murinus* (n=10 for each group) were implanted with radiotelemetry transmitters. Subsequently, the paraventricular hypothalamus (PVH) was cannulated, and vehicle (saline, 14 µl/day), AG (1.0 µg/kg/day), or DAG (1.0 µg/kg/day) was infused via osmotic minipumps. At 1-day post the start of infusion, animals were transferred to whole-body plethysmography chambers to record basal respiratory activity. After a further 3-days, animals were administered cisplatin (30 mg/kg, i.p.) and recordings continued for a further 4-h. At the end of the experiments, plasma levels of substance P and vasopressin were assayed by commercially available enzyme immunoassays.

Cisplatin induced 14.2±2.3 episodes of 86.1±12.2 retches and/or vomits (R+V) within a latency of 40.8±4.4 min. DAG reduced the R+V response by 46.5 % (P=0.049) without affecting the latency to emesis. AG had no effect on cisplatin-induced emesis. Compared to baseline, cisplatin increased the dominant frequency of gastric myoelectric activity (GMA) from 14±0.2 to 15.1±0.3 cpm (P=0.046) and dominant power of GMA from 2.1±0.6 to 6.4±1.9 × 10⁻³ mV (P=0.010); AG and DAG did not modify these effects. Cisplatin reduced respiratory expiratory time by ~8.9% (P=0.029) and increased inspiratory flow by ~48.3% (P=0.004) and expiratory flow by ~17.5% (P=0.020); AG and DAG were inactive to modify these effects. Cisplatin increased substance P levels from 165.9±12.1 to 406.7±96.1 pg/ml (P=0.014) and decreased vasopressin levels from 4847.3±1022.4 to 1630.8±216.5 pg/ml (P=0.047); the decrease in vasopressin levels was antagonized by DAG to 5100.1±1374.9 pg/ml (P=0.042). There were no significant differences in temperature, blood pressure, and heart rate between groups. However, DAG-treated animals had lower R-R interval and SDR-R levels than AG-treated animals before and after cisplatin (P<0.001).

This study indicates that DAG is more active than AG in modulating emesis following an intra-PVH infusion, opening the possibility that non-GHSR1A is involved in the mechanisms of action.

PP149. Chemo-Phototherapy with Carfilzomib-Encapsulated TiN Nanoshells Suppressing Tumor Growth and Lymphatic Metastasis

Zhu X¹, Wu X¹

¹State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Taipa, Macau SAR, China

Introduction: The design of nanomedicine for cancer therapy, especially the treatment of tumor metastasis has received great attention. Proteasome inhibition has been accepted as a new strategy for cancer therapy. Despite being a big breakthrough in multiple myeloma therapy, carfilzomib (CFZ), a second-in-class proteasome inhibitor is still unsatisfactory for solid tumor and metastasis therapy. In this study, we synthesized hollow titanium nitride (TiN) nanoshells as a drug carrier of CFZ.

Method: For the TiN nanoshell preparation, the SiO₂@TiO₂ nanostructures were firstly prepared and nitridized into SiO₂@TiN. The TiN nanoshells were then obtained by etching the SiO₂ template with NaOH. The TiN nanoshells were loaded with the proteasome inhibitor CFZ. The effect of TiN nanoshells on autophagy was studied in the human glioblastoma U-87 MG cells. In vitro and in vivo studies were finally performed to evaluate the anticancer effect of drug-loaded nanoparticles.

Results and discussion: The obtained porous TiN nanoshells have a diameter of 206 ± 9 nm, and the photothermal conversion efficiency of the TiN nanoshells was determined to be (70.1 ± 5.4)% under 1064-nm laser irradiation. The encapsulation efficiency and loading capacity of CFZ were calculated to be (65.7 ± 6.2)% and 5.7 wt.%. TiN nanoshells enhance the expression of LC3-II and p62, indicating autophagy inhibition. TiN nanoshells' intrinsic inhibitory effect on autophagy synergistically enhances the activity of CFZ. Due to an excellent photothermal conversion efficiency in the second near-infrared (NIR-II) region, TiN nanoshell-based photothermal therapy further induces a synergistic anticancer effect. In vivo study demonstrated that TiN nanoshells readily drain into the lymph nodes which are responsible for tumor lymphatic metastasis, upon being administered near the tumor. The CFZ-loaded TiN nanoshell-based chemo-photothermal therapy combined with surgery offers a remarkable therapeutic outcome in greatly inhibiting further metastatic spread of cancer cells.

Conclusion: These findings suggest that TiN nanoshells act as an efficient carrier of CFZ for realizing enhanced outcomes for proteasome inhibitor-based cancer therapy, and our work also presents a 'combined chemo-phototherapy assisted surgery' strategy, promising for future cancer treatment.

- [1] Wu X, Wang L, Xu Y-N, Chen J-L, Luo K Q, Yuan M-H, Li J, Yuan G, Gu Z-Y, Jia X-H, Chen XP, Zhu X-M, Jiang RB (2022) Chemo-phototherapy with carfilzomib-encapsulated TiN nanoshells suppressing tumor growth and lymphatic metastasis. *Small* 18(29): 2200522.

PP150. Role of estrogens on the antidepressant-like effects of electroconvulsive seizures in adolescent rats

Garcia-Cabrerizo R^{1,2}, Garau C^{1,2}, Ledesma-Corvi S^{1,2}, Garcia-Fuster J^{1,2}

¹IUNICS, University of the Balearic Islands, Palma, Spain, ²Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain

Introduction: The use of electroconvulsive seizures (ECS) is emerging as a secure therapeutic choice for treating adolescents with depression resistant to conventional treatments. However, due to variations in efficacy influenced by age and sex, it is imperative to analyze and define its impact during adolescence. The antidepressant-like effects of ECS were previously evaluated in our research group, demonstrating differences in the potential efficacy in a sex- and age-dependent manner [1,2]. In this line, we aimed to evaluate the role of estrogens in the potential antidepressant-like effects of ECS in adolescent male and female rats.

Methods: Adolescent Sprague-Dawley male (n=55) and female (n=45) rats were pretreated with different estrogens' modulators (i.e., tamoxifen, 1 mg/kg; clomiphene, 10 mg/kg and letrozole, 1 mg/kg) for 5-8 days (i.p.), and treated during the last 5 days with ECS (95 mA, 0.6 s, 100 Hz). Behavioral responses were measured in the forced-swim test basally before treatment and 24 hours post-ECS.

Results: The main results demonstrated an antidepressant-like effect of ECS in male adolescent rats (**p=0.001 vs. baseline), but not in females. Therefore, when evaluating the role of estrogens as potential modulators of ECS' response, males and females were evaluated separately. In male adolescent rats, a one-way ANOVA detected significant differences among groups (p=0.024), with post-hoc analysis revealing an increased efficacy for ECS with tamoxifen (*p=0.038 vs. veh). Similarly, in adolescent female rats (ANOVA: p=0.009) that were pretreated with tamoxifen (*p=0.044 vs. veh) or letrozole (*p=0.029 vs. veh), ECS induced an effective antidepressant-like response.

Conclusions: Taken together, our results demonstrate that the lack of antidepressant-like effectiveness of ECS in female adolescent rats, could be improved by the modulation of estrogens. These results underscore a crucial role of sex hormones in antidepressant-like efficacy, and deserve further studies.

Funded by Grant PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033) to MJG-F. RGC is funded by the Spanish Ministry of Universities and co-funded by the UIB through the Beatriz Galindo Program (BG22/00037). CG is funded by the Maria Zambrano program, MUNI and NextGenerationEU. SL-C is funded by the program JUNIOR-IdISBa.

[1] García-Cabrerizo R, Ledesma-Corvi S, Bis-Humbert C, García-Fuster MJ. Sex differences in the antidepressant-like potential of repeated electroconvulsive seizures in adolescent and adult rats: Regulation of the early stages of hippocampal neurogenesis. Eur Neuropsychopharmacol. 2020 Dec; 41:132-145.

[2] Ledesma-Corvi S, García-Fuster MJ. Comparing the antidepressant-like effects of electroconvulsive seizures in adolescent and adult female rats: an intensity dose-response study. Biol Sex Differ. 2023 Sep 30;14(1):67.

PP151. Lower expression of RAD52 in prostate cancer cell lines converts the antiandrogen bicalutamide from an antagonist into a partial agonist

Alfaqih M^{1,2}, Norris J², McDonnell D²

¹Arabian Gulf University, Manama, Bahrain, ²Duke University Medical Center, Durham, United States of America

Introduction: Prostate cancer (PC) is the most common cancer in men worldwide [1]. The Androgen receptor (AR), a nuclear hormone receptor, plays a well-established role in the development and progression of PC [2]. Radical Prostatectomy remains the standard of care for localized PC. Metastatic PC, however, requires more aggressive treatments such as gonadotropin-releasing hormone (GnRH) agonists combined with an AR antagonist (e.g., bicalutamide) [1]. Resistance to these treatment modalities often occurs (castrate resistance) and is a challenge. Mechanisms of castrate resistance include AR mutations, amplification, and local androgen production. Additionally, the deregulation of AR cofactors was proposed to enable weak androgens or antiandrogens to function as full AR agonists which contributes to castrate resistance. Herein, we present in vitro evidence that RAD52, a protein involved in DNA repair machinery, also functions as an AR cofactor. We also show that downregulation of RAD52 expression affects PC response to antiandrogens. Taken together, our findings provide insights into a tentative mechanism by which loss of RAD52 could be contributing to castrate resistance. This may have implications for developing novel therapeutic strategies for PC.

Methods: Using LNCaP PC cell line, RAD52 expression was knocked down with small interfering RNA (siRNA). Western blotting was performed to assess the protein levels of AR and RAD52 following knockdown. Luciferase reporter assay and qPCR of AR target genes were employed to evaluate the transcriptional activity of AR following the knockdown of RAD52 and treatment with an increasing dose of bicalutamide. Chromatin immunoprecipitation (ChIP) assay was used to analyze the recruitment of AR to specific target gene promoters following treatment with bicalutamide.

Results: RAD52 knockdown resulted in increased AR protein levels and enhanced AR transcriptional activity in the presence of low levels of androgens. Furthermore, RAD52 knockdown enhanced bicalutamide agonist activity and induced the recruitment of AR to target gene promoters.

Conclusion: These findings indicate that downregulation of RAD52 might mediate PC resistance to antiandrogen therapy.

[1] Lowrance W, Dreicer R, Jarrard David F, et al. Updates to Advanced Prostate Cancer: AUA/SUO Guideline (2023). Journal of Urology. 2023/06/01 2023;209(6):1082-1090. doi:10.1097/JU.0000000000003452

[2] Fujita K, Nonomura N. Role of Androgen Receptor in Prostate Cancer: A Review. World J Mens Health. Sep 2019;37(3):288-295. Doi:10.5534/wjmh.180040

PP152. Siramesine, a non-opioid σ 2 receptor agonist as a potential agent for the development of novel targeted treatments for pancreatic cancer

Koutsougianni F¹, Sereti E², Jovanovits N¹, Alexopoulou D¹, Dervenis C³, Zacharoulis D⁴, Pešić M⁵, Rankov D⁵, Ilkay-Armutak E⁶, Uvez A⁶, Tsezou A⁷, Dimas K¹

¹*University of Thessaly, School of Medicine, Laboratory of Pharmacology, Larisa, Greece,*

²*Department of Translational Medicine, Malmo, Sweden,* ³*Department of Surgery, Metropolitan Hospital, Greece, Athens,* ⁴*Department of Surgery, University Hospital of Larissa, Greece, Greece,*

⁵*Institute for Biological Research, "Siniša Stanković, Belgrade, Serbia,* ⁶*Istanbul University-Cerrahpasa Faculty of Veterinary Medicine, Department of Histology and Embryology, Turkey, Istanbul, Turkey,* ⁷*Department of Biology, University of Thessaly, Larisa, Greece*

Siramesine, is an agonist of σ 2 receptors that is reported to show a promising antiproliferative and cytotoxic activity in tumor cells in vitro as well as in vivo. The aim of this study is the investigation of the in vitro activity of Siramesine both in the established human pancreatic cancer cell line PANC-1 and in an ex vivo pancreatic cancer cell population named «Attached» which was isolated in our laboratory directly by a patient derived xenograft and in vitro in zebrafish and mice.

We used the methods of SRB cytotoxicity test to determine the GI50, TGI, and LC50 of siramesine against pancreatic cancer cells, the clonogenic assay, and the wound healing assay. These procedures took place in order to investigate the cytotoxic activity of siramesine, the ability of single cells to make clones, and the ability of the specific cells to migrate when they were exposed to siramesine, respectively. Furthermore, through flow cytometry, we analysed the viability of siramesine-treated cells preliminary and studied the effect of the compound on the cell cycle to investigate if the activity is cell cycle phase-specific in synchronized cells in G0/1 phase. Moreover, an important question that was needed to be clarified was if Siramesine induces DNA damage, as preliminary tests using the algorithm COMPARE showed us this possible action.

The data of this study, confirmed that siramesine has a strong antiproliferative and cytotoxic activity under the experimental conditions that have been tested. Moreover, siramesine was found to inhibit the ability of single cells to create colonies and to migrate in a time and dose-dependent manner. The flow cytometry data suggest that siramesine induces cell cycle arrest at the G0/1 phase of the cell cycle. Moreover, Siramesine indeed caused extended DNA damage in primary PDAC cells.

Moreover, It was found to exhibit anticancer activity against the PDX developed in our lab either as monotherapy or acting as a sensitizer to standard chemotherapies such as gemcitabine. Further studies of SRM are ongoing in our laboratories to optimize its anticancer efficacy and elucidate its underlying mechanism of action. Further studies on the mechanism by which the compound exhibits these effects are ongoing.

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3 rd Call for HFRI PhD Fellowships (Fellowship Number: 6182).

The research work was supported by DEKA of the University of Thessaly under the Call for DEKA PhD Fellowships.

PP153. The PDK-1 inhibitor GSK2334470 induces cell death and G1 cell cycle arrest in human pancreatic cancer cells

Alexopoulou D¹, **Koutsogianni F¹**, Kotsopoulos G¹, Polychronis A¹, Masoura C¹, Lafazanis K¹, Papathanasiou I², Trachana V², Dimas K¹

¹*University of Thessaly, School of Medicine, Laboratory of Pharmacology, Larisa, Greece,*

²*Department of Biology, Faculty of Medicine, University of Thessaly, Greece*

*The first two authors contributed equally.

The aim of this work was to investigate the in vitro anticancer activity of the small molecule GSK2334470, a reported PDK-1 inhibitor, against human pancreatic ductal adenocarcinoma (PDAC) cell lines. PDK1 (phosphoinositide-dependent kinase-1) is a constitutive serine/threonine kinase that acts as a master kinase, phosphorylating and activating a subset of the AGC family of protein kinases, such as the RSKs, key downstream kinases of the MAPK/ERK pathway and being involved in the regulation of the PI3K/AKT/mTOR pathway.

PANC-1 were used to study the in vitro activity of this small molecule. An SRB cytotoxicity assay was first performed to determine the in vitro anticancer properties of this compound. A clonogenic assay and a wound healing (scratch) assay were then performed to test the anti-proliferative and anti-migratory capacity of the inhibitor, both in terms of the ability of individual cells to form colonies and their ability to migrate. Flow cytometry was used to identify the cell cycle phase at which GSK2334470 acts in both 170 synchronized and non-synchronised cells in the G0/1 phase. WB was then used to elucidate the mechanisms by which this molecule induces cell cycle arrest. Finally, the effect of the inhibitor was also compared with the silencing of the kinase by specific siRNA.

The data obtained from the above experiments suggest that GSK2334470 has significant anti-proliferative effects against all PDAC cell lines tested, with PANC-1 being the most sensitive as the GI50 was found to be around 10µM. In addition, GSK2334470 was able to inhibit both colony formation at a concentration of 0.1µM and pancreatic cancer migration at concentrations close to 1µM. Finally, GSK2334470 was observed to act via a cell cycle phase-specific mechanism, as it was found to arrest the cell cycle at the G0/1/S transition. WB showed that GSK2334470 was able to downregulate the activation of PDK1 with subsequent activation of GSK3 and finally, the downregulation of cyclin D1, consistent with the G1 arrest observed.

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 6182)

The research work was supported by DEKA of the University of Thessaly under the Call for DEKA PhD Fellowships.

PP154. Gender-related differences of hepatic lipid metabolism and mitochondrial function in epileptic WAG/Rij rats: effect of early lipopolysaccharide exposure

Melini S¹, Pirozzi C¹, Trinchese G², Lama A¹, Del Piano F³, Comella F¹, Cimmino F², Opallo N¹, Mollica M², Mattace Raso G¹, Meli R¹

¹*Department of Pharmacy, University of Naples Federico II, Naples, Italy*, ²*Department of Biology, University of Naples Federico II, Naples, Italy*, ³*Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy*

Introduction: Epilepsy is the most common CNS disorder worldwide, characterized by abnormal brain activity with several peripheral implications [1]. Among all, in vivo studies have supported the crucial role of peripheral and brain inflammation in the relationship between seizure predisposition and NAFLD [2]. Otherwise, severe early-life infections leading to sepsis may result in hepatic and neuro-inflammation that can aggravate epilepsy [3]. Moreover, hepatic dysfunction can drastically complicate epilepsy management because most medications are metabolized by liver.

Here, we examined the effect of early LPS challenge (1 mg/kg, i.p. at PND3) in inducing hepatic damage in a genetic model of young adult WAG/Rij epileptic rats (PND45), evaluating the possible gender-related differences on hepatic lipid dysmetabolism and associated mitochondrial oxidative damage and enzyme activities.

Methods: For biochemical and molecular determinations we performed Real-Time PCR, Bio-Plex Multiplex Elisa Kit, polarographic/spectrofluorimetric analyses.

Results: Both male and female epileptic rats, exposed to LPS, showed hepatic damage as shown by a higher serum levels of hepatic enzymes as well as increased cholesterol and triglycerides. Early LPS challenge induced a major inflammatory and immune response in male epileptic rats than females in both serum and liver, as demonstrated by increased pro-inflammatory cytokine levels and hepatic immune cell recruitment. Conversely, LPS-insulted females showed a marked alteration in hepatic metabolic and lipid profile as well as the reduction in fatty acid oxidation, capacity of mitochondria (decreased carnitine palmitoyltransferase activity). Interestingly, the two different gender-related mechanisms of liver injury by LPS converge in liver mitochondrial oxidative damage with intensified ROS production in both sexes, that notably induced a compensatory increase in antioxidant defense (mitochondrial superoxide dismutase activity) only in females. Consistently, female epileptic rats, challenged with LPS, showed a compensative increase of defensive antioxidant system of NRF2 but notably a decrease in heme oxygenase 1, that can justify the increased oxidative damage. Moreover, LPS-challenged male rats showed an altered hepatic glutathione redox status (GSH/GSSG ratio) rather than females. (all data presented are obtained from n=6 animals each group)

Conclusions: Our study translationally points out that early post-natal infections can predispose epileptic patients to develop or exacerbate hepatic disorders also considering sex-related susceptibility.

[1] Devinsky O, et al. 2018, Epilepsy. Nat Rev Dis Primer doi: 10.1038/nrdp.2018.24

[2] Gursky JM et al. 2020, Epilepsia doi: 10.1111/epi.16437.

[3] Jirillo E. et al. 2002, J Endotoxin Res doi:10.1179/096805102125000641

PP155. Phytochemical Constituents and Biological Activity of Wild and Cultivated Rosmarinus officinalis Hydroalcoholic Extracts

Romano B¹, Maresca D¹, Somma F¹, Chianese G¹, Formisano C¹, De Feo V², Ercolano G¹, Ianaro A¹

¹*University of Naples Federico II, Naples, Europe*, ²*University of Salerno, Fisciano, Europe*

Introduction: Rosmarinus officinalis L. is an aromatic evergreen plant from the Lamiaceae family that displayed multiple bioactivities including antioxidant, antibacterial, hypoglycemic and anticancer activity [1,2]. In this study, we characterized the chemical composition of the hydroalcoholic extract from wild (WRO) and cultivated (CRO) Rosmarinus officinalis and investigated their potential anti-inflammatory effects.

Methods: The chemical composition of the extracts was evaluated via LC-MS analysis. The in vitro experiments were performed by using the LPS/IFN- γ -activated J774 murine macrophages. The cytotoxic effect of both WRO and CRO extracts was evaluated by MTT assay. qPCR analysis was performed to evaluate the modulation of different proinflammatory mediators (e.g., IL-6, TNF- α , NOS2) after treatment with both WRO and CRO (10 μ g/mL for 24h). Likewise, frequency of dead cells, ROS production and calcium fluxes were assessed by flow cytometry. Levels of NO and PGE2 were measured in the cell culture medium of J774 cells by performing Griess and ELISA assays respectively. Finally, western blot analysis was performed to evaluate the expression levels of NF- κ B, I κ B- α and Cox2.

Results: The LC-MS analysis revealed the presence of a wide range of phenolic compounds, including flavonoids, phenolic and terpenes. Both WRO and CRO significantly inhibited the secretion of proinflammatory cytokines and reduced iNOS expression and function in J774 macrophages. Moreover, we found that both WRO and CRO induced a significant decrease in ROS generation in J774 macrophages by inhibiting NF- κ B activation and its downstream mediator COX-2.

Conclusion: Our results demonstrated that both WRO and CRO exert anti-inflammatory effects modulating the activity of macrophages through inactivation of NF- κ B. Therefore, both WRO and CRO could represent a potential therapeutic strategy for the treatment of inflammation-based diseases.

[1] Huang L, Ding B, Zhang H, Kong B, Xiong YL. Textural and sensorial quality protection in frozen dumplings through the inhibition of lipid and protein oxidation with clove and rosemary extracts. *J Sci Food Agric.* 2019;99(10):4739-4747. doi:10.1002/jsfa.9716.

[2] Bozin B, Mimica-Dukic N, Samoilik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. *J Agric Food Chem.* 2007;55(19):7879-7885. doi:10.1021/jf0715323.

PP156. Quantitative structure-activity relationship (QSAR) used to determine the molecular properties responsible for the antibacterial activity of new gamma-lactams targeting *Staphylococcus aureus*: design, synthesis, and in silico and in vitro evaluation of the test compounds

Morán-Díaz J¹, Quintana-Zavala D¹, González-Albores S², Ocampo-Néstor A², Trujillo-Ferrara J², Guevara-Salazar J²

¹Laboratorio de Química Orgánica, Centro de Investigación en Ciencia y Tecnología Avanzada Unidad Legaria, Instituto Politécnico Nacional, México, ²Departamento de Bioquímica, Farmacología y Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, México, México

Introduction: On November 17, 2021, the World Health Organization set the priority of creating new molecules against methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* because they were responsible for multiple deaths from sepsis.[1] The current contribution aimed to identify, through a quantitative structure-activity relationship (QSAR) analysis, the molecular properties of oxazolidinones (e.g., linezolid and tedizolid) that provide antibacterial activity against *S. aureus*. The antibacterial effect is generated by inhibition of protein biosynthesis normally carried out by the target bacteria. Methods and

Results: A model composed of physicochemical descriptors was established, then validated internally (test set) and externally (training set) to design 80 new γ -lactams.[2] Subsequently, a search conducted with the smile code in Reaxys and ScinFinder to corroborate the existence of the molecules showed that 15 of the γ -lactams have not been previously reported. Molecular docking was performed with AutoDock 4 to define the amino acids responsible for the binding of the γ -lactams to the bacteria. The results of the γ -lactams were compared to those of positive controls. ADME pharmacokinetic parameters were employed to evaluate the molecules according to Lipinski's rule of 5, finding that the new γ -lactams can probably be administered orally. The compounds were synthesized through two reactions, aniline + dimethyl itaconate and aniline + dimethyl itaconate ester, obtaining 8 γ -lactams in yields greater than 30 %. They were characterized by physical and spectroscopic methods. The minimum inhibitory concentration (MIC) of the test compounds was determined with in vitro biological experiments against the *S. aureus* ATCC (American Type Culture Collection) 25923, 11632, 33592, and 33862 strains. Microdilutions were made and the results were compared to the MIC. The experimental MIC of linezolid on the Epsilon-test was 1 μ g/mL. Finally, gradual concentration-response curves were constructed, and the statistical analysis was made with simple linear regression using the least squares method. Significant differences were considered at $p < 0.05$.

Conclusion: Molar size and refractivity proved to be the most important descriptors for ascertaining which γ -lactams will present directed biological activity against *S. aureus*.

[1] Guevara-Salazar JA, Morán-Díaz JR, Ramírez-Segura E, Trujillo-Ferrara JG. What are the origins of growing microbial resistance? Both Lamarck and Darwin were right. Expert Rev Anti-Infect Ther. 2021;19(5):563-569.doi: 10.1080/14787210.2021.1839418

[2] Morán-Díaz JR, Neveros-Juárez F, Arellano-Mendoza MG, et al. QSAR analysis of five generations of cephalosporins to establish the structural basis of activity against methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. Mol Divers. 2023. doi: 10.1007/s11030-023-10730-7

PP157. Endomorphin-1 analogues containing non-protonable tyrosine derivatives as novel and highly selective opioid ligands: design and characterization of innovative compounds with distinct binding profiles and possibly improved pharmacological activity

Bedini A¹, Francescato M², Gentilucci L²

¹*Department of Pharmacy and Biotechnology - University of Bologna, Bologna, Italy*, ²*Department of Chemistry "G. Ciamician" – University of Bologna, Bologna, Italy*

Introduction: Opioid analgesics targeting mu opioid receptor (MOPr) are still among the most widely used pain killers, despite their limited efficacy in different chronic pain conditions, their relevant adverse effects and abuse liability. To overcome these issues, innovative opioids with improved pharmacological profiles have been sought by exploring multiple alternatives (e.g.: G protein-biased or peripherally-restricted agonists at MOPr, KOPr, DOPr). Ligands simultaneously modulating multiple opioid receptors have been recently attracting increasing interest for their potentially enhanced effectiveness, reduced side effects, uncomplicated PK/PD [1].

Here, we aimed to characterize a library of novel endomorphin-1 (EM-1) analogues containing non-protonable tyrosine derivatives to identify innovative opioid ligands with distinct binding profile to one or more opioid receptors and possibly improved pharmacology.

Methods: To assess compounds affinity profile, competition binding assays were carried out in HEK-293 cells selectively overexpressing human MOPr, DOPr or KOPr, as previously described [2]. Ligands activity at their target opioid receptor(s) was investigated by evaluating their ability to inhibit forskolin-induced cAMP accumulation both in HEK-293 cells overexpressing the receptors and in human cell lines endogenously expressing MOPr, DOPr and KOPr (e.g.: SH-SY5Y, U87-MG).

Results: Tetrapeptide DME36 displayed high affinity and selectivity to MOPr ($ki=0.18\pm0.08$ nM), without inhibiting adenylyl cyclase in HEK-293/hMOPr cells. Tetrapeptide DME49 showed high affinity to KOPr ($ki=0.38\pm0.05$ nM) and DOPr ($ki=0.11\pm0.03$ nM), but not to MOPr; moreover, DME49 inhibited adenylyl cyclase in HEK-293/hKOPr but not in HEK-293/hDOPr. DME36- and DME49-mediated effects in human cell models endogenously expressing opioid receptors are being carried out and will be presented at the conference.

Discussion: By modifying tyrosine residue on EM-1 sequence a library of novel analogues containing non-protonable tyrosine derivatives was designed and characterized. This way, we identified DME36, a novel MOPr selective antagonist with nanomolar affinity, and DME49, a high-affinity and potent KOPr agonist/DOPr antagonist. This latter in particular emerges as an innovative multi-target opioid with a promising and potentially improved pharmacological profile.

[1] Li M, Stevens DL, Arriaga M, Townsend EA, Mendez RE, Blajkevch NA, Selley DE, Banks ML, Negus SS, Dewey WL, Zhang Y. Characterization of a Potential KOR/DOR Dual Agonist with No Apparent Abuse Liability via a Complementary Structure-Activity Relationship Study on Nalfurafine Analogues. ACS Chem Neurosci. 2022 13(24):3608-3628.

[2] De Marco R, Bedini A, Spampinato S, Comellini L, Zhao J, Artali R, Gentilucci L. Constraining Endomorphin-1 by β,α -Hybrid Dipeptide/Heterocycle Scaffolds: Identification of a Novel κ -Opioid Receptor Selective Partial Agonist. J Med Chem. 2018 61(13):5751-5757

PP158. The C-terminal regions of the GLP-1 and GIP receptors modulate rate of receptor endocytosis.

Al-Zaid B¹, Al-Sabah S¹

¹Kuwait University, Kuwait

Introduction: Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are important regulators of metabolism and mediate the incretin effect. This glucose-dependent potentiation of insulin secretion is severely impaired in patients with type 2 diabetes mellitus. While supraphysiological levels of GLP-1 can overcome this impairment, the same is not true for GIP. The reasons for this are unclear; however, differences in the signalling profiles and mechanisms of desensitisation and internalisation of the GLP-1 and GIP receptors (GLP-1R and GIPR) may contribute. GLP-1R and GIPR are closely related members of the secretin class of G protein-coupled receptors and predominately couple to Gs. GLP-1R is also a robust recruiter of arrestin, whereas arrestin recruitment to GIPR is relatively poor. The aim of this study was to identify the role of the C-terminal region of the two receptors in their differing signalling and internalisation behaviours.

Methods: Chimeric receptors, where the C-terminal tail of one receptor was replaced with that of the other, were constructed (GLP-1/GIPR and GIP/GLP-1R). Bioluminescent Resonance Energy Transfer (BRET)-based assays were used to compare mini-Gs, Gq, and arrestin recruitment and internalization of the chimeric receptors with that of wild-type receptors expressed in HEK-293 cells. All BRET experiments were performed in triplicate, and the data were obtained from at least three independent experiments.

Results: Replacement of the C-terminal tail had no significant effect on either Gs or Gq to either GLP-1R or GIPR. This substitution had no effect on arrestin recruitment to GLP-1R, nor did it rescue arrestin recruitment to GIPR. GIP-stimulated internalisation of GIPR occurred at a significantly ($P<0.001$) slower rate than GLP-1-stimulated internalisation of GLP-1R. Replacement of the C-terminal tail of GIPR with that of GLP-1R significantly ($P<0.05$) increased the rate of internalisation but not to that of wild-type GLP-1R. Conversely, the replacement of GLP-1R's C-terminal tail with that of GIPR significantly ($P<0.005$) decreased the rate of internalization.

Conclusion: The C-terminal region of GLP-1R and GIPR is not the key determinant of their differing ability to recruit arrestin but modulates the rate of agonist-mediated receptor endocytosis.

PP159. Antitumoral role of the lipid mediators N-acyl taurines in colorectal cancer

De Cicco P¹, Amico R¹, Miraglia M¹, Cutignano A², Capasso R¹, Romano B¹, Pagano E¹, Izzo A¹, Borrelli F¹

¹Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Napoli, Italy, ²Institute of Biomolecular Chemistry, CNR, Pozzuoli, Italy

Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the second leading cause of cancer-related deaths [1]. Recently, the role of fatty acids (FAs) and their metabolism in CRC has attracted growing interest in the development of new biomarkers and innovative pharmacological therapies. Among FA derivatives, N-acyl taurines (NATs, taurine-conjugated fatty acids) represent a new group of endogenous bioactive lipids that play a role in metabolic regulation [2]. To date, the involvement of NATs in colorectal carcinogenesis is still unknown. Therefore, the aim of our study was to investigate the contribution of N-stearoyl taurine (NST) to the progression of CRC by in vitro and in vivo studies.

Methods: The levels of NATs were assessed in human colon biopsies from CRC patients using UPLC-MS/MS quantitative methods. In addition, human colon adenocarcinoma cells (HCT116) and healthy human colonic epithelial cells (HCEC) were used to evaluate the effect of NST. Subsequently, in vivo studies were performed using the CRC xenograft mouse model. NST (5 mg/kg) was administered peritumorally daily for a fortnight, and tumour size was measured throughout the duration of the experiment. Statistical significance was determined by t-test or ANOVA followed by Bonferroni post hoc test.

Results: Our results showed, for the first time, the presence of several NATs in human colon biopsies. Interestingly, all NATs showed remarkable increase in the tumoral counterpart. Therefore, to investigate the role of NATs in CRC progression we focused on the activity of NST on HCT116 cells. Our results showed that NST (0.3-10 µM) decreased the cell viability of HCT116 cells in a concentration-dependent manner ($p<0.001$, $n=5$) whereas it did not affect HCEC viability at all concentrations tested (0.3-10 µM), indicating a selective effect of NST on tumour cells. Importantly, NST induced apoptosis in HCT116 cells as demonstrated by the cytofluorimetric analysis with annexin V ($p<0.01$, $n=5$) and inhibited cell migration. Finally, in vivo, NST (5 mg/kg) significantly ($p<0.01$, $n=8$) reduced xenograft tumour volume by 48% compared to the vehicle group and inhibited the EMT phenomenon.

Conclusions: In conclusion, our findings show that NST reduces tumor growth and progression and could therefore be considered a promising anti-cancer drug for CRC.

[1] Xi Y., Xu P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl. Oncol.* 2021;14:101174. doi: 10.1016/j.tranon.2021.101174.

[2] Arul Prakash S, Kamlekar RK. Function and therapeutic potential of N-acyl amino acids. *Chem Phys Lipids.* 239:105114 (2021). Doi: 10.1016/j.chemphyslip.2021.105114.

PP160. Lipid nanoparticles for efficient delivery of siRNA in colorectal cancer

De Cicco P¹, Amico R¹, Ferillo T¹, Conte C¹, Quaglia F¹, Borrelli F¹

¹*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*

Introduction: Colorectal cancer (CRC) is the third most common cancer worldwide, with a high mortality rate (10%) [1]. The standard approaches for the treatment of CRC, such as chemotherapy and radiotherapy, have drawbacks, mainly due to systemic toxicity and unsatisfactory response rate [2]. In recent decades, RNA-based drugs have emerged as an exciting strategy to improve the effectiveness of cancer therapy, as they can specifically modulate the expression of disease-related genes and have a favourable safety profile. Unfortunately, RNA delivery is challenging due to limited cellular uptake, endosome degradation, and transient therapeutic effects. It is, therefore, crucial to develop novel strategies to improve the delivery of RNA drugs to target cells and maximize their effectiveness.

Methods: In this study, we evaluated the safety, efficiency, and efficacy of lipid-based nanoplatforms for the delivery of siRNA drugs in CRC. Specifically, we designed lipid nanoparticles (LNPs) consisting of the ionizable lipid DLin-MC3-DMA, DSPC, DMG-PEG 2000, and cholesterol entrapping a Firefly Luciferase siRNA (siRNALuc). The safety and efficiency of the LNPs were evaluated in human colorectal adenocarcinoma cells (HCT116) and healthy human colonic epithelial cells (HCEC) using MTT assay and flow cytometry. Subsequently, the efficacy of siRNALuc delivery on 2D luciferase-expressing cells (HCT116-Luc) was investigated.

Results: Our results demonstrated that the LNPs were biocompatible in all cell lines at all concentrations (12.5-50 ug/ml) and times (24-48-72 h) considered. Moreover, LNPs were efficiently internalized by HCT116 cells (about 100%) and released siRNA effectively, as demonstrated by luciferase silencing in HCT116-Luc at all time points considered (24-48-72 h) ($p<0.0001$, $n=2$).

Conclusions: In conclusion, these results show that LNPs might represent a promising strategy for efficient RNA drug delivery in cancer treatment.

We acknowledge the grant CN00000041 "National Center for Gene Therapy and Drugs based on RNA Technology" (concession number 1035 of 17 June 2022-PNRR MUR - M4C2 - Investment 1.4 Call "National Centers", financed by EU- NextGenerationEU), project code (CUP E63C22000940007)

[1] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249. doi:10.3322/caac.21660

[2] Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther. 2020;5(1):22. Published 2020 Mar 20. Doi:10.1038/s41392-020-0116-z

PP161. Inhibition of mitochondrial complex I reduces hepatic steatosis by modulating lipid metabolism, inflammation, and fibrosis in an MCD diet model

Trucchi M¹, Di Pasqua L¹, Protopapa F¹, Scotti E¹, Sun P², Kauschke S², Vairetti M¹, Croce A³, Ferrigno A¹

¹*Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy*, ²*Department of CardioMetabolic Diseases Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany*, ³*Institute of Molecular Genetics, Italian National Research Council (CNR), Pavia, Italy*

Introduction: non-alcoholic fatty liver disease (NAFLD) affects 25% of the global population. Inflammation, ROS and mitochondrial dysfunction promote the transition from simple steatosis to non-alcoholic steatohepatitis (NASH) and hepatic fibrosis. During NAFLD progression, mitochondrial complex I is the main ROS producer. Thus, this study aims to explore whether the selective inhibition of complex I could contribute to reducing lipid deposition, modulating lipid metabolism, and attenuating hepatic fibrosis.

Methods: male Wistar rats, fed a Methionine and Choline deficient (MCD) diet or a Control diet for 6 weeks, were orally administered, starting from the 4th week, with complex I modulator (CIM, Boehringer Ingelheim) at 10 mg/Kg/day or vehicle. Markers of oxidative stress as TBARS and ROS were evaluated, as along with Nitrate/Nitrite concentration. Total lipid content was measured using Nile Red dye. The area of lipid droplets and the rate of inflammatory cell infiltration were calculated on H&E stained liver sections. The expression of factors involved in lipid metabolism (PPAR- α , SREBP1c and mTOR) were assessed by Western Blot. Collagen deposition was calculated on Sirius Red stained liver sections.

Results: TBARS increased in MCD rats versus Controls, without changes after CIM administration. ROS increased in MCD groups, but a significant reduction in CIM-treated Controls was detected, compared with untreated ones. Nitrate/Nitrite concentration decreased in MCD rats administered with CIM, compared with untreated ones. CIM administration promoted a significant reduction of hepatic total lipid content in MCD rats compared with untreated ones. This reduction was accompanied by a significant decrease in lipid droplet areas in the same group. In addition, CIM treatment significantly reduced inflammatory cell infiltration in MCD rats, together with a significant reduction in collagen deposition both in Control rats and in MCD rats, compared with untreated groups. Nuclear expression of PPAR- α significantly increased in CIM-treated MCD rats, compared with untreated rats, while, although not significantly, SREBP1c nuclear expression decreased in CIM-treated Control and MCD rats. The same trend, significant for Controls, was observed also for the ratio P-mTOR/mTOR. Lastly, AST and ALT were decreased in CIM-treated Controls, compared to untreated ones.

Conclusion: at the best of our knowledge, these data suggest for the first time that the administration of CIM could be a promising strategy to counteract lipid accumulation, inflammation and collagen deposition and to modulate lipid metabolism in a MCD model of benign steatosis.

PP162. Indorenate, an antihypertensive compound with new hypoglycemic effects mediated by 5-HT1a and 5HT2a receptors: in vivo and in silico studies

Giacoman Martínez A^{1,2,3,4}, Huang F², Almanza Pérez J¹, Alarcón Aguilar F¹, Rosiles Alanís W⁵, Romero Nava R⁴, Aguayo Cerón K⁴, Hong E³

¹Laboratorio de Farmacología, Universidad Autónoma Metropolitana-Iztapalapa., Ciudad De México, Mexico, ²Laboratorio de Investigación en Obesidad y Asma, Hospital Infantil de México "Federico Gómez", Ciudad De México, México, ³Laboratorio de Farmacobiología, Centro de Investigación y de Estudios Avanzados del I.P.N., Ciudad De México, México, ⁴Escuela Superior de Medicina, Instituto Politécnico Nacional, Sección de Estudios de Posgrado e Investigación., Ciudad De México, México, ⁵Facultad de Estudios Superiores, Universidad Nacional Autónoma de México., Ciudad De México, México

Indorenate is an antihypertensive compound; anxiolytic, antidepressant anticonvulsant, neuroprotective properties it is also reported. Structurally, indorenate it is similar to serotonin (5-HT). Studies have shown that 5-HT has effects on glucose, however, the effects of indorenate on glycemia have not been studied. To clarify this, we evaluated indorenate (10 mg/Kg) with or without 5-HT2a (pelanserin, 2.5 mg/Kg i.p.) or 5-HT1a (WAY-100 635, 3mg/Kg i.p.) receptors antagonists and the combination of these through an oral glucose tolerance test (OGTT) in normal and experimental diabetic Wistar rats. We observed that the individual administration of indorenate in normal male rats has a hypoglycemic effect, and in the presence of pelanserin, the hypoglycemic effect is inhibited and glucose inclusive it is higher than CT. When indorenate is administered with pelanserin and WAY-100635 the hypoglycemic effect it is inhibited too and increase glucose but lower than indorenate with pelanserin only. After, we evaluated the indorenate on the serum levels of insulin in normal male rats with or without glucose, where we observed that the only administration of indorenate significantly increases insulin levels compared to CT ($p<0.05$) and with glucose increase 2-fold than CT ($p<0.01$), and not show effect when is administered together with pelanserin compared to CT. Respect to diabetic rats, individual administration of indorenate show an antihyperglycemic effect. A euglycemic clamp test showed that the infusion of indorenate (33.33 µg/kg/min, i.v.) increase the rate of glucose compared to CT, being knocked down by the infusion together with the antagonist pelanserin (8.3 µg/kg/min, i.v.). Our in silico findings show that indorenate has a higher affinity for 5-HT2a ($\Delta G: -7.32$ Kcal/mol) than 5-HT ($\Delta G: -6.52$ Kcal/mol) and 5HT1a receptor ($\Delta G: -7.29$ Kcal/mol) compared to 5-HT ($\Delta G: -5.78$ Kcal/mol), where indorenate interacts with the same H-bonds in a similar way as 5-HT, but higher and confirm the participation of 5-HT1a and 5-HT2a receptors when are antagonized with pelanserin or WAY-100635 respectively showed in vivo studies which are related with the hypoglycemic effect.

These findings show that indorenate has a hypoglycemic effect through promote insulin secretion, where the 5-HT1a and 5-HT2a receptors are involved. Finding drugs that have beneficial multimodal effects on blood pressure and glycemic control is relevant, since polypharmacy would be avoided, which is recurrent in diseases with same etiology, such as hypertension and type 2 diabetes.

PP163. Effect of TAK-242 on Lipopolysaccharide-induced Acute Kidney Injury in Mice

Almazmomi M¹, Esmat A, Alsieni M

¹Kau, Jeddah, Saudi Arabia

Introduction: Acute kidney injury (AKI) is caused by a sudden loss of renal function, resulting in the build-up of waste products and a significant increase in mortality and morbidity. It is commonly diagnosed in critically ill patients, with its occurrence estimated at up to 50% in patients hospitalized in the intensive critical unit (ICU). Thus, it is critical to investigate novel therapy options for preventing the epidemic. Toll-like receptor-4 (TLR-4) and inflammation play an important role in the pathogenesis of AKI.

Objective: The aim of the existing study was to evaluate the potential effect of TAK-242, which is a TLR-4 inhibitor, against lipopolysaccharide (LPS)-induced AKI in mice as well as elucidate the involved mechanisms with respect to inhibition of pro-inflammatory cytokines as well as TLR-4 expression.

Method: Thirty Swiss mice were divided into five groups, and the AKI group was injected intraperitoneally (i.p.) with LPS (5 mg/kg) to induce AKI. TAK-242 was given in groups three and four in doses (3, and 10 mg/kg/d, i.p., respectively) four hours after the LPS injection. Serum levels of renal function biomarkers like creatinine, cystatin C, blood urea nitrogen, and neutrophil gelatinase lipocalin will be assessed. In addition, oxidative stress markers, and pro-inflammatory cytokines like interleukin-1 β (IL-1 β), IL-6, tumor necrosis factor, and interferon-gamma will be assessed using Elisa kits. Also, the immunohistochemical profile of TLR-4 in renal homogenate will be assessed.

Results: TAK-242 significantly reduced renal inflammation and kidney dysfunction in mice, which was linked to the down-regulation of TLR-4.

Conclusion: The current finding shows that TAK-242 is a potential therapeutic agent to reduce the inflammatory response in AKI. TAK-242 could be a promising therapeutic option for treating AKI.

PP164. Direct-acting oral anticoagulant use during pregnancy: Experience from a teratology information service of a tertiary hospital

Vizdiklar C¹, Ulker G¹, Gultekin O¹, Akici A¹

¹*Department of Medical Pharmacology, School of Medicine, Marmara University, Istanbul, Turkey*

Introduction: Direct-acting oral anticoagulants (DOACs) are among the drug classes with an increasing utilization trend in the community [1]. Various concerns regarding the use of the drug in women of childbearing age accompany the lack of knowledge about the potential teratogenic effects of exposure during pregnancy. We aimed to evaluate outcomes of DOAC exposure in pregnant women referred to a teratology information service (TIS).

Methods: We examined the admissions to Marmara University TIS for DOAC use during pregnancy between 2017 and 2023. The DOACs used by the cases, the indications for using these drugs, the duration of drug exposure, the results of the teratology risk assessment reports for the consulted cases, and the relevant pregnancy outcomes were evaluated.

Results: Six pregnant women on DOACs were identified throughout the study period, five on rivaroxaban and one on dabigatran. The mean age at the time of admission was 33.3 ± 6.7 (range: 25-40 years). All except one of the cases (83.3%) were consulted to our service in the first trimester of the pregnancy, and the mean gestational age at admission was 7.8 ± 2.7 weeks (range: 6-13 weeks). The most common indication for DOAC use was pulmonary embolism, which was encountered in four cases (66.7%). The mean duration of DOAC exposure was 5.5 ± 1.9 weeks. Fifty percent of the teratology risk assessment reports included recommendation to "avoid use/change to another anticoagulant". Out of five cases with known pregnancy outcomes, two were terminated. One of the terminations was performed due to Tay-Sachs disease, which was also diagnosed in the siblings, and the other one was elective. Among the three live births, one was diagnosed with atypical autism spectrum disorder and speech delay, another had low birth weight and strabismus, and the third experienced hypoglycemia during the first week after birth.

Conclusions: This study revealed abnormal outcomes in the children of mothers exposed to DOACs, without any specific pattern. While these results suggest the potential role of underlying diseases in the occurrence of adverse pregnancy outcomes, they do not appear sufficient to conclusively rule out the potential contribution of DOAC exposure.

- [1] Costa LSD, Alsultan MM, Hincapie AL, Guo JJ. Trends in utilization, reimbursement, and price for DOACs and warfarin in the US Medicaid population from 2000 to 2020. J Thromb Thrombolysis. 2023;55(2):339-345.

PP165. The anti-inflammatory and anti-remodelling effect of inhaled ROCK inhibitor hydroxyfasudil in monotherapy and after simultaneous administration with budesonide in experimentally induced allergic asthma

Franova S¹, Gondas E¹, Smiesko L¹, Joskova M¹, Fedorova L¹, Sutovska M¹

¹*Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Department of Pharmacology, Martin, Slovakia*

Asthma is a chronic inflammatory disease with reversible airway obstruction, hyperreactivity, mucus production, and airway remodelling. The current concept of asthma treatment focuses on inflammatory characteristics but overlooks the critical aspect of airway remodelling. In addition, some current drugs used for asthma treatment primarily target airway inflammation and have limited efficacy in airway remodelling processes. For this reason, identifying new medications to treat asthma should focus on substances' ability to influence airway inflammation and remodelling simultaneously.

The results of experimental studies have shown that the activity of the RhoA/Rho kinase signalling pathway plays a role in influencing many processes of allergic inflammation [1], airway hyperreactivity, and even airway remodelling [2].

A study assessed the anti-inflammatory and anti-remodelling effectiveness of inhaled Rho-kinase (ROCK) inhibitor hydroxyfasudil and its combination with budesonide in experimental allergic asthma.

The airway allergic inflammation was induced by 28 days of ovalbumin sensitisation. Hydroxyfasudil and budesonide were inhaled in monotherapy or a half-dose combination for the last 14 days. The evaluated parameters were the concentrations of i) inflammatory cytokines; ii) remodelling growth factors in the lung homogenate; iii) the count of leukocytes in the bronchoalveolar lavage fluid (BALF); iv) airway defence mechanism sensitivity - *in vivo* specific airway resistance (sRaw) and ciliary beat frequency (CBF).

It was observed that hydroxyfasudil inhalation significantly reduced the levels of inflammatory cytokines (IL-13, IFN- γ , TNF- α and GM-CSF) in lung homogenate, eosinophils, and lymphocytes in BALF. It also reduced the concentration of growth factor TGF-beta1, EGF receptors, and collagen type III and V. Additionally, inhaled hydroxyfasudil reduced sRaw and had no adverse effects on CBF. The simultaneous administration of hydroxyfasudil with budesonide in half doses had a synergistic effect in reducing s Raw and specific inflammatory factors TNF- α , IFN- γ , and EGFR.

Our study findings indicate that ROCK inhibition impacts inflammatory and remodelling markers that trigger and regulate airway remodelling in the settings of experimental asthma. Inhaled hydroxyfasudil did not negatively interfere with budesonide and, in some cases, even showed synergistic effects on specific markers.

This work was supported by the Slovak Research and Development Agency [Grant No. APVV-19-0033] and the Slovak Scientific Grant Agency [Grant VEGA 1/0314/21].

[1] Franova S et al. The anti-asthmatic potential of Rho-kinase inhibitor hydroxyfasudil in the model of experimentally induced allergic airway inflammation. Eur J Pharmacol 2023; 938: 175450.

[2] Zhang Y et al. RhoA/Rho-kinases in asthma: from pathogenesis to therapeutic targets. Clin Transl Immunology 2020; 9(5): E01134.

PP166. Stress-induced mechanical and thermal pain sensitisation mediated through central nervous system cell signalling

Fülöp B¹, Kormos V¹, Rozmer K¹, Király Á¹, Dénes Á², Lénárt N², Borbély É¹, Helyes Z^{1,3,4,5}

¹*Department of Pharmacology and Pharmacotherapy, Medical School & Centre of Neuroscience, University of Pécs, Pécs, Hungary*, ²*"Momentum" Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary*, ³*National Laboratory for Drug Research and Development, Budapest, Hungary*, ⁴*PharmInVivo Ltd, Pécs, Hungary*, ⁵*Eotvos Lorand Research Network, Chronic Pain Research Group, University of Pécs, Pécs, Hungary*

Chronic psychosocial distress is known to play a role in the development/exacerbation of several painful diseases (e.g. fibromyalgia), where drug therapy is unresolved, so it is necessary to examine the pathomechanism to identify new therapeutic targets. The role of neuroinflammation and the microglia surface fractalkine receptor (CX3CR1) and activated microglia-released interleukine-1 (IL-1) cytokine has already been proven to be involved in stress and inflammatory pain. Based on our unpublished results, we were able to demonstrate the role of the receptor in CX3CR1 knockout (KO) and IL-1 KO mice in the development of pain caused by chronic restraint stress (CRS). In this research, we investigated the potential analgesic effect of the fractalkine receptor antagonist AZD8797, and the IL-1 receptor antagonist anakinra in a mouse model of stress-induced pain.

Male C57Bl/6J wild-type (WT) mice were exposed to CRS for 2 weeks[1]. From the beginning of the stress protocol, AZD8797 (1 mg/kg, n=8-9)[2] or anakinra (10mg/kg, n=7-9) or saline vehicle (n=6-8) was administered intraperitoneally daily. The mechanical pain threshold was measured with a dynamic plantar aesthesiometer, and the cold tolerance of the hind paw was measured weekly with the withdrawal latency from icy water.

CRS induced significant ($p<0,0001$) mechanical hyperalgesia developed for the second, cold hyperalgesia ($p=0,0003$) for the first week (two-way repeated measurement analysis of variance followed by Tukey's tests) compared to the non-stressed. Both AZD8797 ($p=0,0009$) and anakinra ($p=0,0007$) prevented the formation of mechanical sensitisation of the hind paw compared to the vehicle treatment. Cold hyperalgesia was similar in vehicle-, AZD8797- and anakinra-treated animals in response to stress.

Based on our results, the microglia surface fractalkine receptor microglia activation consequent IL-1 release may play an important mediating role in the development of chronic stress-induced pain. AZD8797 and anakinra successfully attenuated the mechanical sensitization caused by CRS, further strengthening the potential of CX3CR1 or IL-1 as a drug target.

Acknowledgment: OTKA K-138046, RRF-2.3.1-21-2022-00015, TKP2021-EGA-16, Eotvos Lorand Research Network, National Brain Research Program-3, 2017-1.2.1-NKP-2017-00002 (NAP-2; Chronic Pain Research Group, Phar-maLab, RRF-2.3.1-21-2022-00015, OTKA K138046, OTKA FK FK137951). Project no. TKP2021-EGA-13 and TKP2021-EGA-16, ÚNKP-23-3

[1] Fülöp B, Hunyady Á, Bencze N, et al. IL-1 Mediates Chronic Stress-Induced Hyperalgesia Accompanied by Microglia and Astroglia Morphological Changes in Pain-Related Brain Regions in Mice. *Int J Mol Sci.* 2023;24(6):5479. doi:10.3390/IJMS24065479

[2] Wollberg AR, Ericsson-Dahlstrand A, Juréus A, et al. Pharmacological inhibition of the chemokine receptor CX3CR1 attenuates disease in a chronic-relapsing rat model for multiple sclerosis. *Proc Natl Acad Sci U S A.* 2014;111(14):5409-5414. doi:10.1073/PNAS.1316510111/-/DCSUPPLEMENTAL

PP167. New inhaled gallium delivery system: a therapeutic strategy for pulmonary diseases

Esposito E¹, Recchiuti A², Indolfi C³, Mitidieri E¹, Ferri G², Costabile G¹, Frangipani E⁴, Visaggio D⁵, Visca P⁵, Ungaro F¹, d'Emmanuele di Villa Bianca R¹, Sorrentino R¹

¹Department of Pharmacy, School of Medicine, University of Napoli Federico II, Napoli, Italy, Naples, Italy, ²Department of Medical, Oral and Biotechnological Sciences, Laboratory of Molecular Medicine, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy, Chieti, Italy, ³Department of Molecular Medicine and Medical Biotechnology, School of Medicine, University of Napoli Federico II, Napoli, Italy, Naples, Italy, ⁴Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy, Urbino, Italy, ⁵Department of Science, University Roma Tre, Rome, Italy., Rome, Italy

Introduction: The FDA-approved iron mimetic metal gallium [Ga(III)] has been proposed as an antimicrobial drug for ESKAPE pathogens pulmonary infection [1]. Due to the Ga(III) short half-life, we developed a novel Ga(III) inhalable nano-embedded microparticles (Ga_Man_NEM) [2]. Here we investigated the in vivo toxicity and efficacy of Ga_Man_NEM, compared to Ga(NO₃)₃ solution (GaN), in healthy and infected mice, respectively.

Methods: To evaluate in vivo toxicity, Ga_Man_NEM or GaN [50 µL/mouse, containing an equivalent amount of 12.5 µmol of Ga(III)] was intratracheally (i.t.) administered in mice and compared with lipopolysaccharide (LPS-5 mg/kg i.t.; n=5). Cell infiltration, IL-6, IL-8, and IL-22 were measured in bronchoalveolar lavage (BAL) at 24 and 72h after treatments. The Ga_Man_NEM efficacy was evaluated in mice infected with *P. aeruginosa* strains (PAO1, PA14, LESB58) at a single dose (LD70: ca. 107 for *P. aeruginosa* PAO1 and PA14 or ca. 108 for *P. aeruginosa* LesB58). Mice were co-treated with GaN or Ga_Man_NEM [12.5, 2.5, and 0.5 µmol of Ga(III)] by i.t. administration. Ga(III) distribution was evaluated in BAL fluid (BALF), BAL cells (BALC), plasma, lung, kidney, and liver in mice after intratracheal administration of Ga_Man_NEM [12.5µmol of Ga(III)].

Results: The intratracheal administration of Ga_Man_NEM did not affect neutrophil infiltration and macrophage recruitment, showing a profile significantly lower compared to LPS (p<0.01) (24h, 72h). The administration of GaN at 24h provoked macrophage recruitment to a similar extent to LPS. IL-6, IL-8, and IL-22 levels were significantly lower after Ga_Man_NEM or GaN treatment compared to LPS (p<0.0001). Ga_Man_NEM almost totally protects mice from lethal infection of PAO1 and PA14 while GaN did not show a significant protection, at the same molar concentration of Ga(III). Ga_Man_NEM exhibited activity against the hyper-virulent LESB58 strain, albeit with reduced efficacy compared to PA14 and PAO1 strains. Lung bioavailability of Ga(III) favoured by the i.t Ga_Man_NEM administration, was confirmed in mice.

Conclusions: Gallium for inhalation therapy appears as a feasible and safe antibacterial option, and the new Ga_Man_NEM formulation could represent a viable candidate for the local treatment of lung infections.

[1] S. Hijazi, D. Visaggio, M. Pirolo, E. Frangipani, L. Bernstein, P. Visca. Antimicrobial Activity of Gallium Compounds on ESKAPE Pathogens. *Front Cell Infect Microbiol.* 2018, 10 (8) 316.

[2] Costabile G, Mitidieri E, Visaggio D, Provenzano R, Miro A, Quaglia F, et al. Boosting lung accumulation of gallium with inhalable nano-embedded microparticles for the treatment of bacterial pneumonia. *Int J Pharm.* 2022, 629:122400.

PP168. A novel selective HDAC6 inhibitor as an epigenetic modulator for TNBC treatment

Bello I¹, Barone S¹, Barile M¹, Esposito C¹, Summa V¹, Cirino G¹, Brindisi M¹, Panza E¹

¹*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*

Introduction: Triple-negative breast cancer (TNBC) is associated with a poor prognosis and a high mortality rate [1]. Since epigenetic changes are highly implicated in TNBC tumorigenesis, histone deacetylase inhibitors (HDACis) could represent a promising therapeutic strategy. To date, all approved drugs targeting HDACs are pan-inhibitors and have many side effects due to their lack of selectivity. Therefore, the development of selective HDACis is crucial [2]. We recently discovered a novel class of spirocyclic compounds behaving as selective HDAC6 inhibitors. In this study, we assessed the mechanism and potential of sHDAC6-229 in TNBC cells.

Methods: MDA-MB-231 cells were used as an in vitro model of TNBC. Acetylation of α -tubulin and acetyl-H3 was assessed through western blot after treatment with the HDAC pan-inhibitor (3 μ M) or sHDAC6-229 (3-30 μ M). Subsequently, the antiproliferative effect of sHDAC6-229 (1-30 μ M) was evaluated using MTT assay after 24 and 48 hours. The impact on apoptosis and cell cycle was assessed by cytofluorimetric analysis. RT-PCR and western blot analyses were performed to evaluate the expression of apoptotic, cell cycle, autophagy, and epithelial-mesenchymal transition markers. Wound healing and clonogenic assays were performed to evaluate the effect on cell metastasis formation. Data were expressed as mean \pm SEM of n = 3 experiments.

Results: We found that sHDAC6-229 reduced cell proliferation in a time- and concentration-dependent manner, concomitantly inducing apoptosis as revealed by cytofluorimetric assay and western blot for Caspase-3 and PARP. This effect resulted from a selective inhibition of HDA6. Indeed, sHDAC6-229 demonstrated the ability to acetylate α -tubulin with no impact on acetyl-H3. Next, we assessed its effect on cell cycle progression. sHDAC6-229 arrested the TNBC cell cycle and decreased the number of cells in the S phase by reducing the expression of cyclin D1, cyclin B, CDK4, and CDC25A. Moreover, sHDAC6-229 induced autophagy in MDA-MB-231 cells by increasing the expression of BECN1, BNIP3, and LC3-II. Additionally, sHDAC6-229 halted migration and colony formation by inhibiting the expression of canonical markers of epithelial-mesenchymal transition.

Conclusions: These results confirmed the epigenetic role of the HDAC6 enzyme in TNBC and revealed sHDAC6-229 as a promising novel HDAC6 inhibitor for TNBC treatment.

[1] Duranti S, Fabi A, Filetti M, et al. Breast cancer drug approvals issued by EMA: A review of clinical trials. *Cancers*. 2021;13(20):5198.

[2] Maccallini C, Ammazzalorso A, De Filippis B, Fantacuzzi M, Giampietro L, Amoroso R. HDAC inhibitors for the therapy of triple-negative breast cancer. *Pharmaceuticals*. 2022;15(6):667.

PP169. Anti-inflammatory effect of Cannabigerol in Collagen-induced arthritis

Šteigerová M, Sklenárová M, Jelínek P, Bobek D, Šoós M, Šíma M, Slanař O

¹1st Faculty of Medicine and General University Hospital in Prague (VFN), Prague, Czech Republic

Introduction: Cannabigerol (CBG), a non-psychoactive phytocannabinoid, is studied for potential anti-inflammatory effects in various indications. However, its therapeutic use in the treatment of Rheumatoid arthritis (RA) has not been properly investigated yet. Hence, we studied the effect of this promising agent on the most common in vivo model of RA: Collagen-induced arthritis model (CIA).

Methods: Thirty-two female Wistar rats were randomized into four groups: placebo (PCB), CBG, methylprednisolone (MP), and a control group. CIA was induced in 24 rats: the CBG group was treated orally with 30 mg of CBG once daily, MP group was treated orally with 0.15 mg of methylprednisolone once daily and PCB group was treated with saline solution. After 28 days of therapy, all animals were euthanized and their blood, ankles and synovial membranes were collected. Expression levels of cytokines and related molecules in blood and synovial membranes were evaluated by real-time polymerase chain reaction.

Results: Mean CIA score (\pm SD) of the MP group (4.44 ± 4.64) and the GBG group (7.36 ± 0.94) were significantly lower in comparison with the PCB group (11.25 ± 4.40); $p\leq0.05$. In addition, the administration of CBG and MP delayed the onset of symptoms of the CIA model. The CBG and MP treatment reduced expression of IL-1 β , IL-10, IL-17A, COX-2, STAT-3, Caspase-3, MMP-3, and GM-CSF in synovial membrane samples. Average relative quantification as $\log_2\pm$ SD values for CBG versus PCB were as follows: IL-1 β (-0.84 ± 0.78), IL-10 (-1.46 ± 1.34), IL-17A (-1.36 ± 0.44), COX-2 (-0.98 ± 0.61), STAT-3 (-0.68 ± 0.21), Caspase-3 (-1.25 ± 0.52), MMP-3 (-0.28 ± 0.24), and GM-CSF (-0.80 ± 0.73). Furthermore, a statistically significant difference in expression among groups was noted also in blood samples, where both CBG and MP administration attenuated expression of IL-1 β and STAT-3 [CBG versus PCB: IL-1 β (-0.48 ± 0.44), STAT-3 (-3.76 ± 0.23)].

Conclusion: CBG produced therapeutic response in CIA rats via decreased inflammatory response. This suggests that CBG may have beneficial effect in the treatment of rheumatoid arthritis.

Acknowledgements: This work was supported by AZV grant No. AZV NU22-08-00346 and by the Charles University project Cooperatio (research area PHAR).

PP170. Simultaneous PPAR γ Activation and SGLT2 Cotransporter Inhibition Modulate Apoptosis in Diabetes-induced Nephropathy

Čináková A¹, Křenek P¹, Klimas J¹, Král'ová E¹

¹*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia*

Introduction: Apoptotic processes, which are highly accelerated by macrophage infiltration, play a significant role in the pathomechanism of diabetic nephropathy. Dapagliflozin (Dapa) and pioglitazone (Pio) have been shown to modulate processes associated with apoptosis even independently of their hypoglycaemic effect [1,2]. Here, we studied how simultaneous PPAR- γ activation and SGLT2 inhibition affect apoptotic markers in kidneys under diabetic conditions.

Methods: Experimental type 1 diabetes mellitus was induced in Wistar rats by streptozotocin (55mg/kg). The control (n=10) and diabetic groups (STZ;n=10) received standard chow, while the treated diabetic groups received chow containing Dapa (10mg/kg;n=10), Pio (12mg/kg;n=10) or their combination (n=10). Six weeks after streptozotocin administration, the animals were sacrificed by asphyxiation (CO₂). In the excised kidneys, we observed the expression of selected molecules associated with apoptosis (Bax, Bcl2, p53, Procaspsase 3, Casp3) using RT-qPCR and Western blotting. To determine kidney injury, we estimated the expression of Kim1 and Nphs1 genes. Macrophage infiltration was quantified by immunohistochemical staining of CD68.

Results: In the kidney of STZ rats, the propagation of apoptotic processes was accompanied by elevated macrophage infiltration (+78%±17%; p<0.05) in comparison to the control group. Kidney injury was demonstrated by elevated Kim1 (vs control; +86%±19%; p<0.05) and reduced Nphs1 (vs control; -35%±8%; p<0.05) gene expression. Dapa treatment markedly reduced the protein expression of Kim1 (vs STZ; -76%±17%; p<0.05) and proapoptotic markers (vs STZ; Bax -77%±9%; p53 -34%±7%; Casp3 -35%±10%; Procaspsase 3 -33%±6%; all p<0.05) and simultaneously upregulated the protein expression of antiapoptotic Bcl2 (vs STZ; +40%±25%; p<0.05). A similar trend was observed in the Pio group (vs STZ; Bax -81%±5%; p53 -64%±7%; Casp3 -47%±8%; Procaspsase 3 -33%±8%; all p<0.05), however, Pio treatment did not affect Kim 1 and Bcl2 expression. The expression levels of Nphs1 were reverted to the normal values by the monotherapies (vs STZ; Dapa +38%±16%; Pio +33%±18%; both p<0.05). Importantly, both monotherapies, as well as combined therapy, resulted in a significant reduction in CD68 immunopositive areas (p<0.05). However, the combination treatment only reached the effect of monotherapies in all the measured parameters.

Conclusion: In conclusion, we report that the combined therapy of Dapa and Pio protects renal tissue from apoptotic processes, however, without exhibiting an expected additive effect.

- [1] Oraby MA et al.Dapagliflozin attenuates early markers of diabetic nephropathy in fructose-streptozotocin-induced diabetes in rats.Biomed Pharmacother.2019;109:910-920.
- [2] Chen W et al.Pioglitazone Protects Against Renal Ischemia-Reperfusion Injury via the AMP-Activated Protein Kinase-Regulated Autophagy Pathway.Front Pharmacol.2018;9:851.

PP171. Morphine-THC Analgesic Drug Combination: Molecular Pathway Analysis and MOPr-CB1 Heteromerization Quantification in Different Human and Rodent Cell Models

Baiula M¹, Cuna E¹, Cimetti C¹, Renault P², Giraldo J², Bedini A¹

¹University of Bologna, Bologna, Italy, ²Universitat Autònoma de Barcelona, Barcelona, Spain

Introduction: Chronic pain is a longstanding and debilitating condition, often inadequately treated [1]. To obtain an improved, opioid-sparing analgesic treatment for chronic pain, the combination of MOPr and CB₁ receptor agonists have been proposed, considering possible receptor cross-talk and heteromerization [2]. Therefore, we aimed to evaluate morphine-THC combinational effects on MOPr-mediated signaling in several human and rodent primary neuronal cells and in HEK293 cells co-expressing Flag-MOPr and HA-CB₁, both following acute and prolonged (morphine 1μM;24h) MOPr stimulation. Moreover, we aimed to quantify MOPr-CB₁ heteromerization after acute morphine+THC co-treatment in HEK293 cells expressing the wild-type receptors or their mutant variants.

Methods: MOPr levels were quantified via saturation binding assay. Morphine (10⁻⁴–10⁻¹²M, in ethanol) ability to inhibit forskolin-induced cAMP production was quantified by ELISA in PMA (16nM, 5days)-differentiated SH-SY5Y human neuroblastoma cells, rat and mouse primary neurons and HEK-293 cells, under basal conditions and co-administering THC (10-100nM) with morphine. cAMP production was also measured in HEK293 cells expressing MOPr and CB₁ mutant variants. Confocal microscopy was employed to quantify MOPr-CB₁ heteromerization in HEK-293 cells co-expressing wtMOPr and wtCB₁ or their mutant variants, obtained by site-directed mutagenesis.

Results: Under basal condition, THC co-administered with morphine was able to significantly potentiate morphine-mediated inhibition of adenylyl cyclase in rat cortical primary neurons (morphine IC₅₀=2.26±0.29nM vs morphine+THC IC₅₀=1.46±0.62, p<0.05). On the contrary, in rat striatal, rat DRG, mouse cortical, mouse striatal primary neurons THC significantly reduced morphine ability to inhibit cAMP production. Following prolonged MOPr activation, in rat cortical, rat striatal primary neurons and in human PMA-differentiated SH-SY5Y cells the administration of THC rescued the reduced response to morphine. Moreover, THC reverted the reduced wtMOPr/wtCB₁ colocalization (Pearson's coefficient=0.5341, p<0.001) induced by morphine (Pearson's coefficient=0.3795) in HEK293 cells. In V82A-MOPr/wtCB₁ HEK293 cells, morphine was not able to reduce heterodimerization (Pearson's coefficient=0.6066), while THC induced a greater receptor colocalization (Pearson's coefficient=0.7011, p<0.001).

Conclusions: Our results indicate that coadministration of THC improves morphine effects, both under basal conditions and following prolonged MOPr stimulation. Thus, suggesting that MOPr-CB₁ heteromerization might be the underlying molecular mechanism. Furthermore, our data will be employed to implement a QSP platform to predict more effective and safer combinational analgesic treatments.

- [1] Marcianò G, Vocca C, Evangelista M, et al. The Pharmacological Treatment of Chronic Pain: From Guidelines to Daily Clinical Practice. *Pharmaceutics*. 2023;15(4):1165. doi:10.3390/pharmaceutics15041165.
- [2] Nielsen S, Sabioni P, Trigo JM, et al. Opioid-Sparing Effect of Cannabinoids: A Systematic Review and Meta-Analysis. *Neuropsychopharmacology*. 2017;42(9):1752-1765. Doi:10.1038/npp.2017.51.

PP172. In-vitro Validation of Mu Opioid Receptor (MOPr) Interfacial Residues Predicted to Be Relevant for Opioid/Cannabinoid Receptor Heteromerization and Crosstalk Under Basal Conditions and in Response to Morphine

Cimetti C¹, Cuna E¹, Baiula M¹, Renault P², Giraldo J², Bedini A¹

¹*Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum – Università di Bologna, Bologna, Italy*, ²*Institut de Neurociències (Inc) – Universitat Autònoma de Barcelona, Barcelona, Spain*

Introduction: Chronic pain is a debilitating condition, with inadequate management options [1]. Combining agonists at mu-opioid-receptor (MOPr) and cannabinoid-receptor-type-1 (CB1) was proposed for an improvement in the treatment of chronic pain [2] since their physical proximity was detected and research was conducted on their crosstalk and heteromerization [2] but related molecular aspects are yet largely unknown. We aim at validating possible MOPr/CB1 heteromerization interfaces in cell models co-expressing the receptors, under basal conditions and following morphine administration, also to help develop a quantitative systems pharmacology platform aimed at ameliorating chronic pain treatment.

Methods: Site-directed mutagenesis was performed to mutate MOPr interfacial residues computationally predicted to be relevant for heteromerization (MOPr-F86A, MOPr-V82A, MOPr-F206A, MOPr-F223A). Confocal microscopy analysis investigated potential MOPr/CB1 heteromerization in HEK-293 cells transfected to express human MOPr and CB1. The ability of morphine (10^{-4} – 10^{-12} M, solved in H₂O) to inhibit forskolin-induced cAMP accumulation was also quantified in HEK-293 cells transfected to express human MOPr (either wild-type or mutated) and CB1.

Results: We successfully obtained MOPr-V82A and MOPr-F86A and we compared MOPr-V82A colocalization with CB1 and activation by morphine with those of wt-receptor. MOPr-V82A displayed a greater co-localization signal with CB1 in HEK-293 cells (Pearson's coefficient = 0.5844) compared to wt-MOPr (Pearson's coefficient = 0.5279). Morphine decreased wt-MOPr/CB1 but not MOPr-V82A/CB1 colocalization.

Morphine inhibited cyclic-AMP in a concentration-dependent way in HEK-293 cells expressing both MOPr-V82A/CB1 and wtMOPr/CB1, displaying similar potency (EC₅₀wtMOPr 1.28 ± 0.29 nM; EC₅₀MOPr-V82A 0.58 ± 0.04 , p<0.001, N=6). Morphine potency in inhibiting cyclic-AMP was increased in HEK-293 cells transfected to express MOPr-F86A/CB1 compared to wt-MOPr/CB1 (EC₅₀MOPr-F86A 0.11 ± 0.05 ; EC₅₀wtMOPr 1.28 ± 0.29 nM; p<0.001, N=6). Experiments on the other mutants are ongoing and the results will be presented at the conference.

Conclusion: We show that the computationally predicted interfaces for MOPr/CB1 colocalization play a role within this process and may also affect morphine ability to activate MOPr. Supporting the idea that optimal stimulation of CB1 in combination with morphine may improve chronic pain management. Collected data could help develop a quantitative systems pharmacology platform aimed to identify drug combinations with improved efficacy against chronic pain.

[1] Cohen SP, Vase L, Hooten WM. Chronic pain: an Update on burden, Best practices, and New Advances. *The Lancet*. 2021;397(10289):2082-2097. doi:[https://doi.org/10.1016/S0140-6736\(21\)00393-7](https://doi.org/10.1016/S0140-6736(21)00393-7)

[2] Nielsen S, Sabioni P, Trigo JM, et al. Opioid-Sparing Effect of Cannabinoids: A Systematic Review and Meta-Analysis. *Neuropsychopharmacology*. 2017;42(9):1752-1765. doi:<https://doi.org/10.1038/npp.2017.51>

PP173. Studying state-dependent compound effects using adaptive voltage-clamp on a high throughput automated patch clamp system

Goetze T¹, Hampl M¹, George M¹, Obergrussberger A¹, Rapedius M¹, Seibertz F¹, Brinkwirth N¹, Friis S¹, Haarmann C¹, Becker N¹, Fertig N¹

¹Nanion Technologies, Munich, Germany

Background: Voltage-gated sodium channels (Nav) represent clinically relevant targets for the development of new medical strategies. Nav channels display complex gating characteristics involving transitions between open -, closed – and inactivated states across the time course of an action potential. To find effective drugs specific for particular Nav channels, it is important to understand the molecular mechanisms of drug actions with respect to Nav gating transitions. For instance, it is well known that certain compounds preferentially bind to a particular conformational state of the channel, e.g. inactivated versus resting.

Aim/Methods: A new technical development allows for the automatic determination of each cell's individual half-maximal inactivation potential (V_{half}) during measurement using a high throughput automated patch clamp device. The precise V_{half} of each cell can then be fed back to the individual recording and used as a measurement potential for sequential pharmacological tests. Using this method under various recording temperatures (22°C and 35°C), we aim to determine compound affinity for NaV1.5 and NaV1.7 more efficiently and accurately compared to the traditional global application of the same V_{half} potential to all measurements.

Results: In both channel subtypes, a shift of the voltage dependence of activation to more hyperpolarized potentials at physiological temperature was detected. Furthermore, we found a reduced affinity for mexiletine at elevated temperature and an increased IC50 for tetracaine for activation from the inactivated state when compared with activation from resting state.

Conclusions: Our data is in line with previous results and the adaptive voltage clamp technique helps to reduce the variability of compound IC50 values for mexiletine, tetracaine, amitriptyline and flecainide and allows for a more precise and unbiased approach to study the effect of compounds on Nav channels.

PP174. Evaluation of New β -Lactam Derivatives' Effects on $\alpha_4\beta_1$ and $\alpha M\beta_2$ Leukocyte Integrin-Mediated Immune Cells Activation and Migration

Maurizio A¹, Giraldi V², Giacomini D², Baiula M¹

¹Department of Pharmacy and Biotechnology - University of Bologna, Bologna, Italy, ²Department of Chemistry "G. Ciamician" - University of Bologna, Bologna, Italy

Introduction: Integrins are cell adhesion receptors which play a relevant role in the modulation of leukocyte recruitment. Since many inflammatory diseases are characterized by a dysregulated migration of leukocytes, there is a keen interest in finding and testing compounds able to modulate these processes [1]. Therefore, the current study aimed to evaluate the in vitro ability of some β -lactam derivatives to modulate integrin-mediated immune cells activation and recruitment.

Methods: The pharmacological activity and selectivity profile of β -lactam derivatives was at first evaluated by adhesion assays [2] on Jurkat E6.1 (expressing $\alpha_4\beta_1$) and HL-60 (expressing $\alpha M\beta_2$, differentiated 5 days with DMSO 1.25%) cells. The most promising compounds have also been studied for their ability to modulate ERK, Akt and JNK phosphorylation levels by Western Blot [2]. Moreover, the most promising $\alpha M\beta_2$ antagonist was used to conduct qPCRs on LPS-treated (100 ng/mL) dHL-60 cells to evaluate a possible reduction in pro-inflammatory cytokine expression; as well as in Transendothelial Migration Assay to confirm its ability to reduce the number of immune cells recruited into an inflamed tissue.

Results: The results of adhesion assays showed promising antagonistic activity against $\alpha_4\beta_1$ integrin by compounds GO29 (EC_{50} 144 nM, n = 3), MC177 (EC_{50} 309.8 nM, n = 3) and MC183 (IC_{50} 268 nM, n = 3) [2]. Interestingly, these compounds were also able to modulate $\alpha M\beta_2$ integrin-mediated cell adhesion, showing both agonistic (GO29, EC_{50} 1.4 nM; MC177, EC_{50} 22.55 nM) and antagonistic activity ($MC183$, IC_{50} 34.01 nM). The aforementioned compounds were also able to modulate integrin-mediated signal transduction, showing different level of ERK, Akt and JNK phosphorylation. Concerning qPCR results, HL-60 cells co-treated with LPS and MC183 (10^{-6} M, 10^{-7} M) showed significantly reduced levels of pro-inflammatory cytokine expression, just as treatment with MC183 was shown to reduce the number of migrating neutrophils.

Conclusions: Based on these preliminary results, we have found selective and potent leukocyte integrins ligands, able to modulate cell adhesion and intracellular signaling. Also, MC183 showed an interesting efficacy in reducing pro-inflammatory cytokine expression as well as leukocyte transendothelial migration, representing a lead for the development of new therapeutic agents to be employed in inflammatory diseases.

- [1] Herter J, Zarbock A. Integrin Regulation during Leukocyte Recruitment. *The Journal of Immunology*. 2013;190(9):4451-4457. doi:<https://doi.org/10.4049/jimmunol.1203179>
- [2] Baiula M, Galletti P, Martelli G, et al. New β -Lactam Derivatives Modulate Cell Adhesion and Signaling Mediated by RGD-Binding and Leukocyte Integrins. *Journal of Medicinal Chemistry*. 2016;59(21):9721-9742. doi:<https://doi.org/10.1021/acs.jmedchem.6b00576>

PP175. Impact of 5-HT2 receptor blockade by sarpogrelate on the allodynia induced by diabetic neuropathy in male rats

Fernández González J^{1,2,3}, Pizaña Encarnación J⁴, Escoto-Rosales M⁴, García-Pedraza J^{1,2}, García-Domingo M^{1,2}, Villalón C³, Granados-Soto V⁴

¹*Pharmacology Laboratory, Physiology and Pharmacology Department, University of Salamanca, Salamanca, Spain*, ²*Salamanca Biomedical Research Institute (IBSAL), Salamanca, Spain*,

³*Cardiovascular Pharmacology Laboratory, Departamento de Farmacobiología, CINVESTAV, Mexico City, México*, ⁴*Neurobiology of Pain Laboratory, Departamento de Farmacobiología, CINVESTAV, Mexico City, Mexico*

Diabetic neuropathy is one common complication during diabetes and it is characterized by the presence of severe hyperalgesia and allodynia [1]. Moreover, serotonin (5-hydroxytryptamine; 5-HT), which is found in enterochromaffin cells, platelets and central nervous system, modulates many (patho)physiological functions, including inflammatory pain. Indeed, 5-HT2 receptor blockade by sarpogrelate attenuates pain in animal models [2]. Accordingly, this study evaluated the antinociceptive effect of sarpogrelate treatment in diabetic animals. Diabetes was induced in male Wistar rats by streptozotocin (50 mg/kg, i.p.) and maintained for 28 (short-term) or 56(long-term) days . In the short-term diabetic group, at day 28 and after inhaled anesthesia (isoflurane 2.5%), sarpogrelate (100 and 300 µg, 10 µl, n=6 each) was intrathecally administered. After anesthesia recovery, tactile allodynia was measured every 30 min for two hours. In long-term diabetic animals (n=6), sarpogrelate was chronically administered (30 mg/kg·day, p.o.) from day 14 until day 56. Age-matched control normoglycemic (citrate buffer, 1 ml/kg, i.p.) and diabetic rats were maintained under the same conditions (n=6 each). Tactile allodynia using Von Frey test (<4 g, 50% withdrawal threshold [3]) was measured periodically from days 0 to 56 in the different treatment groups. Statistical significance (ANOVA) was accepted at p<0.05. The hyperglycemic state appeared at day 2 (495 ± 18 mg/dL) and was maintained all along the experiments. In short-term diabetic animals, acute sarpogrelate (intrathecal treatment) originated a dose-dependent anti-allodynic effect from t=30 min until t=90 min post administration (p<0.05 vs t=0 min). In long-term diabetic animals, tactile allodynia appeared from day 21 post-diabetes induction and was maintained throughout the experiments (p<0.05 vs normoglycemic group). Chronic sarpogrelate (oral treatment) prevented the establishment of allodynia in diabetic animals (p<0.05 vs diabetic group). In summary, diabetic animals show allodynia from day 21 after diabetes induction. Moreover, 5-HT2 receptor blockade produced by: (i) acute sarpogrelate administration abolished pain responses in short-term diabetic rats; and (ii) chronic sarpogrelate prevented allodynia in long-term diabetic animals. Thus, sarpogrelate treatment may become a possible therapy in the prevention of pain caused by diabetic neuropathy.

- [1] Freeman R. Diabetic autonomic neuropathy. *Handb Clin Neurol.* 2014;126:63-79. doi:10.1016/B978-0-444-53480-4.00006-0
- [2] Sasaki M, Obata H, Kawahara K, Saito S, Goto F. Peripheral 5-HT2A receptor antagonism attenuates primary thermal hyperalgesia and secondary mechanical allodynia after thermal injury in rats. *Pain.* 2006;122(1-2):130-136. doi:10.1016/j.pain.2006.01.021
- [3] Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods.* 1994;53(1):55-63. Doi:10.1016/0165-0270(94)90144-9

PP176. Antifibrotic potential of kynurenic acid in renal fibrosis

Sykorova S¹, Hadova K¹, Krivy J¹, Vavrinec P¹, Vavrincova D¹

¹*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia*

Introduction: Kidney damage of heterogeneous etiology leads to inflammation, immune reaction and activation of transforming growth factor- β (TGF- β), which can cause severe fibrosis with limited effective therapeutic modalities[1]. Kynurenine (KYN) and kynurenic acid (KYNA) suppress the immune response[2], and may modulate course of renal fibrosis. Here we studied the effect of KYN and KYNA in a cellular and animal model of kidney fibrosis.

Methods: Mouse fibroblasts were stimulated with recombinant TGF- β 1 for 48 h. Cells were divided into 6 groups (n=6): control untreated cells (C), TGF- β 1 stimulated cells (5 ng/ml; TGF) and TGF- β 1-stimulated cells treated with KYN (3 μ mol/l; TK3 and 10 μ mol/l; TK10, respectively) or KYNA (50 μ mol/l; Tka50 and 150 μ mol/l; Tka150, respectively). KYN and KYNA treatment was added simultaneously with TGF- β 1 treatment and after 24 h. Cells were harvested for Western blot analysis after 48 h. Wistar rats were divided into 4 groups (n=10): sham-operated rats (SHAM), rats which underwent unilateral ureteral obstruction (UUO) and rats underwent UUO treated with KYNA (100mg/kg every 24 hours p.o.; KYNA100 and 200mg/kg every 12 hours p.o.; KYNA400, respectively) for 7 days. KYNA was dissolved in 1% methylcellulose solution. On the eighth day rats were anesthetized with 3% isoflurane, sacrificed and excised kidneys were used for Western blot and histology analysis.

Results: In cells, KYN and KYNA treatments markedly attenuated expression of TGF- β - fibrotic pathway proteins; the most significant decrease was found in TKa150 group (α SMA -76% \pm 14%; pSMAD2 -59% \pm 21%; pp38 -52% \pm 12%; pERK/ERK -58% \pm 15%; pJNK/JNK -69% \pm 13%; pMEK/MEK -84% \pm 21%; all vs. TGF, p<0.05). In animal model, we observed significant decrease of fibrotic area (-45% \pm 6%; vs.UUO; p<0.05) as well as reduction in dilatation of cortex distal tubules (-17% \pm 3%; vs.UUO; p<0.05) in KYNA100 group. KYNA significantly decreased expression of fibrotic cascade proteins such as pp38, pJNK/JNK and pERK/ERK. Moreover, we observed significant decrease in collagen III expression (-49% \pm 17%; vs.UUO; p<0.05), one of the main components of fibrotic tissue in KYNA400 group, while the expression of collagen I was not affected.

Conclusion: Our study demonstrates an antifibrotic potential of KYN and especially KYNA by reducing expression of both fibrotic markers and proteins in in vitro and in vivo fibrotic models.

- [1] Ong CH, et al. TGF- β -induced fibrosis: A review on the underlying mechanism and potential therapeutic strategies. European Journal of Pharmacology. 2021;911:174510.
- [2] Stone TW, Williams RO. Modulation of T cells by tryptophan metabolites in the kynurenine pathway. Trends in Pharmacological Sciences. 2023;44(7):442-456.

PP177. Effect of Atorvastatin on Angiogenesis-Related Genes and the Modulation of Transcripts in Bone-Marrow-Derived Mesenchymal Stem Cells (BM-MSCs)

Gazova A¹, Adamickova A², Chomanicova N⁴, Cervenak Z², Valaskova S⁴, Adamicka M³, Kyselovic J²

¹Institute of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Comenius University, Bratislava, Bratislava, Slovakia, ² 5th Department of Internal Medicine, Faculty of Medicine, Comenius University Bratislava, Bratislava, Slovakia, ³Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University Bratislava, Bratislava, Slovakia, ⁴International Laser Centre, Bratislava, Slovakia

Statins, inhibitors of HMG-CoA reductase, are a class of drugs that have been extensively studied for their efficacy in the prevention and treatment of cardiovascular diseases. One of the “pleiotropic” effects of statin-mediated mechanisms may be the impact on the function of Rho-kinases, which act as serine/threonine kinase AKT inhibitors. The phosphatidylinositol 3-kinase (PI3K) and the downstream target AKT belong to a conserved family of signal transduction enzymes with roles in cell proliferation, transformation, paracrine function and processes of angiogenesis and apoptosis.

The objective of the study was to evaluate the effect of atorvastatin on the characteristics and properties of BM-MSCs; mainly if atorvastatin affected the gene expression of BM-MSCs.

We found that pharmacological stimulation with atorvastatin did not significantly affect cell viability in our experiment. Viability averages in the control group vs atorvastatin pre-treatment were 98.6 ± 0.8 vs 98.9 ± 0.5 , 95.2 ± 1.5 vs 95.0 ± 2.1 , 97.5 ± 0.6 vs 96.3 ± 2.1 and $99.4 \pm 0.2\%$ vs $97.5 \pm 1.0\%$ in 2h, 24h, 48h and 96h intervals. The expression of MSC surface markers was not changed by atorvastatin stimulation.

Atorvastatin treatment significantly increased the VEGF-A mRNA level ($148.1 \pm 14\%$) at the 96h time point and for 24h significantly upregulated HGF mRNA expression in BM-MSCs ($171.4 \pm 120\%$). In contrast, FGF-1R mRNA expression remained unaffected at all time points. Atorvastatin pre-treatment significantly decreased the mRNA expression level of IGF-1 after 24h ($34.7 \pm 4.3\%$), 48h ($23 \pm 2.3\%$) and 96h ($13.3 \pm 1.3\%$). The gene expression of IGF-2 was increased in 48h of atorvastatin pre-treatment ($149.8 \pm 12.3\%$). The downregulation of IGF-1R mRNA expression was observed at a 24h time interval of atorvastatin pre-treatment ($77.1 \pm 13\%$). The RT-PCR analysis revealed an upregulation of AKT1 mRNA to $120.1 \pm 8.8\%$ at 48h pre-treatment. Atorvastatin treatment significantly increased PI3KCA mRNA in BM-MSCs to $179.9 \pm 35.7\%$ at 48h. Together, these results indicate that the PI3K/AKT pathway is modulated on the mRNA level in atorvastatin-treated BM-MSCs.

In summary, in our in vitro study, the atorvastatin pre-treatment of BM-MSC did not affect the cell morphology, viability or expression of MSC markers. Our data demonstrated a significant increase in the mRNA expression of angiogenic factors, VEGF-A and HGF in atorvastatin-treated BM-MSCs. In contrast, there was a significant reduction in IGF-1 transcripts. Moreover, the results implicate atorvastatin's role in the PI3K/AKT signalling pathway with the upregulation of mTOR transcription. We propose that atorvastatin benefits BM-MSC treatment for its ability to upregulate angiogenesis-related gene expression and transcripts of the PI3K/AKT/mTOR pathway.

PP178. Epac involvement in colon cancer, a possible new target for metabolic reprogramming of tumor cells

Cullotta C¹, Lauro R¹, Eid A², Irrera N¹, Bitto A¹

¹*University of Messina, Messina, Italy*, ²*Qatar University, Doha, Qatar*

Introduction: Colorectal cancer is one of the most spread tumor with an elevated rate of mortality. Despite treatment advancements, the challenges of chemoresistance and recurrent relapses may diminish the efficacy of these treatments, emphasizing the imperative for the development of innovative cancer therapies[1].

cAMP (Cyclic adenosine 3',5'-monophosphate) is a second messenger, synthesized by adenylate cyclase, essential in several cellular pathways involved in migration, proliferation and apoptosis through the activation of protein kinase A (PKA)[2].

Epac (exchange protein activated by cAMP) has been recently recognized as a new mediator of molecular pathways involved in proliferation, apoptosis, migration, adhesion, cytoskeletal remodeling, inflammation, and oxidative stress. In cancer, the role of Epac is still unclear because of its dual role in the regulation of cancer progression [3].

The aim of this study was to understand the effect of Epac on the pro-malignant behaviors, such as proliferation, migration, invasion and adhesion, in an in vitro model of colorectal cancer using HT29 cell line.

Methods: Epac was activated in HT29 cells by either 5 uM 8-pCPT-2-O-Me-cAMP-AM, (potent Epac activator) or 10 uM forskolin (cAMP activator) and to evaluate the specific role of this pathway the Epac antagonist ESI09 (10 uM) and PKA inhibitor H89 (5 uM), were added to activated cells for 24 hours. Migration, invasion and adhesion assays were performed at 24 and 48 hrs. At the end of the treatment period, cells were collected and used for molecular analyses, specifically p-ERK, p-FAK, p-Akt, CXC-43, iNOS, and COX-2.

Results: Epac modulates cancer cells proliferation ($p<0.001$) and survival ($p<0.001$) through the stimulation of key signaling pathways involved in cell mitogenesis, oxidative stress, and inflammation. Moreover, Epac affects several signaling pathways involved in tumor cell motility and invasion (p-FAK and CXC-43), as also demonstrated by the scratch assay with over 50% reduction in invasion when cells were treated with ESI09 (10 uM).

Conclusions: These preliminary data suggest that Epac could be a promising therapeutic target to limit cancer progression.

- [1] Weitz J, Koch M, Debus J, Höhler T, Galle PR, Büchler MW. Colorectal cancer. Lancet. 2005;365(9454):153-165. doi:10.1016/S0140-6736(05)17706-X
- [2] Rooij, J.; Rehmann, H.; van Triest, M.; Cool, R.H.; Wittinghofer, A.; Bos, J.L. Mechanism of regulation of the Epac family of cAMP-dependent RapGEFs. J. Biol. Chem. 2000, 275, 20829–20836.
- [3] Wehbe N, Slika H, Mesmar J, et al. The Role of Epac in Cancer Progression. Int J Mol Sci. 2020;21(18):6489. Published 2020 Sep 5. Doi:10.3390/ijms21186489

PP179. Use of graphene quantum dots as effective drug delivery system for cancer medicines

Bitto A¹, Imbesi C¹, **Cullotta C¹**, Lauro R¹, Irrera N¹

¹*University of Messina, Messina, Italy*

Innovative materials like graphene quantum dots (GQDs), graphene oxide nanosheets, and various carbon nanotubes demonstrate distinct capacities for drug loading, release rates, and targeting. This diversity elucidates the observed variations in their therapeutic effectiveness when employed as carriers for drugs.

In the effort of characterize the effectiveness of GQDs loaded with different anticancer drugs to improve cancer cell death at lower drug's doses, thus reducing side effects, we used GQD-loaded particles with either doxorubicin or bortezomib in cancer cell lines.

MCF7 cells represents the most common (70% of diagnosed cases) breast cancer phenotype, were used to investigate either the ability of doxorubicin-loaded GQDs (12.5 to 100 ug/ml) to enter the cells and their efficacy compared to doxorubicin alone (12.5 to 100 ug/ml).

In another setting hepatocellular carcinoma cells HepG2 have been tested with bortezomib-loaded GQDs (0.5 to 10 ug/ml) compared to bortezomib alone (0.5 to 10 ug/ml).

Cell vitality, apoptosis related genes (Bax and Bcl2), and cell cycle genes (Cyclin D1, CDK4, and CDK6) have been determined in either cell lines.

The results showed a significant reduction in cell vitality in MCF-7 cells starting with the lower dose of loaded GQDs (12.5 ug/ml) against the 50 ug/ml dose of doxorubicine alone needed to obtain a similar result. Real-time PCR data demonstrated that loaded GQDs significantly improved BAX expression starting from 50 ug/ml as compared to the 75 ng/ml of doxorubicine and at the dose of 75 ug/ml both formulations significantly reduced cyclin D1 gene expression.

In HepG2 cells the bortezomib-loaded GQDs showed similar residual vitality of bortezomib alone at every tested dose. Also in the case of the gene expression similar results have been obtained with loaded GQDs and the drug alone at every tested dose.

The hard point of these results is that despite the quantity of the tested compound was formally identical, the GQDs loading capacity allowed to bind only the 18-22% of drug, meaning that the obtained results derive from 1/5 of the effective dose as compared to the un-conjugated drug, representing a great improvement in possible therapeutic applications of these nanomaterials.

PP180. Cancer derived exosomes containing sialoglycans: A promising tool for immune modulation

Toledo Santamaría D¹, von Gunten S¹

¹University of Bern, Bern, Switzerland

Introduction: Aberrant glycosylation plays a fundamental role in key pathological steps of tumor initiation and progression. Exosomes are small intracellular membrane-based vesicles that are naturally released into the circulation by all eukaryotic cells and have been recovered from many biological fluids. Siglecs (sialic acid-binding immunoglobulin-type lectins) are a family of immunoregulatory receptors found predominantly on cells of the hematopoietic system. In cancer, aberrant glycosylation is a common phenomenon that contributes to carcinogenesis, cancer progression and metastasis. Our goal is to explore the nature of sialoglycans in cancer cell-derived exosomes and their role in immune cells in the tumor microenvironment.

Methods: Siglec-7 ligand expression was assessed by immunostaining of a melanoma tumor microarray. Exosomes were purified by differential centrifugation and characterized by FACS using exosome capture Dynabeads and by Western blot. Furthermore, glycosylation-based interactions between cancer cells and immune cells were investigated in functional experiments co-incubating healthy donors granulocytes with melanoma-derived exosomes or adding exosomes in a NK cell cytotoxicity assay.

Results: Siglec-7 ligands are overexpressed in melanoma patients with a consequent impairment in the overall survival. A reproducible and efficient method to purify and characterize exosomes from cancer cell lines has been established. This relevance can be linked to melanoma-derived exosomes containing sialic acids specifically different Siglecs ligands. Exosomes derived from melanoma cell lines induce cell death in healthy donor granulocytes and significantly reduce NK cell cytotoxicity.

Conclusions: High levels of Siglec-7 ligands in melanoma impair the overall survival of the patients at any stage or metastatic status. We also show that cell line-derived exosomes contain mainly terminal α2,3 sialic acids (Siglec ligands) as part of the membrane composition. Melanoma-derived exosomes are cytotoxic for healthy donors granulocytes and decrease the cytotoxic activity of NK cells.

- [1] Jandus C, Simon HU, & von Gunten S (2011) Targeting Siglecs a novel pharmacological strategy for immuno- and glycotherapy. Biochem Pharmacol 82:323-332.
- [2] Quentin Haas et al. Siglec-9 Regulates an Effector Memory CD8+ T-cell Subset That Congregates in the Melanoma Tumor Microenvironment. Cancer Immunol Res 1 May 2019; 7 (5): 707-718.
- [3] Boligan KF, Mesa C, Fernandez LE, & von Gunten S (2015) Cancer intelligence acquired (CIA): tumor glycosylation and sialylation codes dismantling antitumor defense. Cell Mol Life Sci 72:1231-1248.
- [4] Kojima R et al. (2018). Designer exosomes produced by implanted cells intracerebrally deliver therapeutic cargo for Parkinson's disease treatment. Nat Commun 9(1):1305 15.

PP181. Exploring the anti-inflammatory potential of N-palmitoyl taurine

Amico R¹, Iannotti F², Cutignano A², Ligresti A², Nanì M¹, Di Marzo V^{2,3,4,5}, Borrelli F¹, De Cicco P¹

¹*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*, ²*Institute of Biomolecular Chemistry, CNR, Pozzuoli, Italy*, ³*Centre de Recherche de l'Institut de Pneumologie et Cardiologie de l'Université Laval, Faculté de Médecine, Université Laval, Québec, Canada*, ⁴*Institut sur la Nutrition et les Aliments Fonctionnels, Centre NUTRISS, École de nutrition, Faculté des sciences de l'agriculture et de l'alimentation (FSAA), Université Laval, Québec, Canada*,

⁵*Canada Research Excellence Chair on the Microbiome-Endocannabinoidome Axis in Metabolic Health (CERC-MEND), Université Laval, Québec, Canada*

Introduction: N-acyl taurines (NATs, fatty acids conjugated with taurine) are newly discovered lipid messengers categorized within the endocannabinoidome. NATs are synthesized by the peroxisomal acyltransferases acyl-CoA: amino acid N-acyltransferase (ACNAT-1 and ACNAT-2) or by the enzyme bile acid-CoA: amino acid N-acyltransferase (BAAT) [1]. Despite the increasing number of reports on the diverse biological processes in which NATs are involved, their biological functions have not been fully elucidated. Our study aims to delineate the role of NATs in inflammatory bowel disease (IBD) pathology focusing our attention on N-palmitoyl taurine (NPT) in IBD.

Methods: In vivo studies were performed in C57BL6/J mice. Ulcerative colitis was induced by 3% w/v DSS in drinking water for five consecutive days followed by normal drinking water. NPT (0.1-6 mg/kg) was administered intraperitoneally once daily, starting two days after DSS. Inflammation was assessed by measuring changes in colon length and colon infiltration by monocytes (by cytofluorimetry). NAT levels and gene expression of ACNAT-1, ACNAT-2, and BAAT in the colon of mice were measured using RT-PCR and UPLC-MS/MS, respectively. The effects of NPT on the endocannabinoidome receptors were evaluated using the corresponding binding, luciferase reporter, and calcium assays. Finally, in vitro studies were conducted on murine bone marrow-derived macrophages (BMDMs) stimulated with lipopolysaccharide (LPS), to confirm the potential anti-inflammatory effects of NPT and to identify its mechanism of action.

Results: In mice treated with DSS, the expression of the NATs-synthesizing enzymes ACNAT-1 and ACNAT-2 was downregulated ($p<0.05-0.001$, $n=11$) leading to decreased levels of various NATs ($p<0.05-0.01$, $n=7$). In the DSS-induced mouse model of colitis, NPT administration (0.1-6 mg/kg) significantly decreased intestinal inflammation by normalizing colon length ($p<0.05$, $n=11$) and reducing colon monocyte accumulation ($p<0.01$, $n=10$). Moreover, in in vitro experiments on macrophages stimulated with LPS, NPT showed specific activation of the PPAR- α receptor and exerted anti-inflammatory properties (0.01-3 μ M) by reducing nitrite levels ($p<0.0001$, $n=3$) and iNOS expression ($p<0.01$, $n=3$) in a concentration-dependent manner.

Conclusions: Our results provide new insights into the role of NATs in the development of IBD. Among NATs, NPT shows great potential as a therapeutic agent for IBD.

- [1] Hunt MC, Siponen MI, Alexson SE. The emerging role of acyl-CoA thioesterases and acyltransferases in regulating peroxisomal lipid metabolism. *Biochim Biophys Acta*. 2012;1822(9):1397-1410. doi:10.1016/j.bbadi.2012.03.009

PP182. Surface-engineered nanoparticles for siRNA delivery in colorectal cancer

Amico R¹, De Cicco P¹, Longobardi G¹, Conte C¹, Quaglia F¹, Borrelli F¹

¹*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*

Introduction: Colorectal cancer (CRC) is the third leading cause of cancer deaths worldwide [1]. In most cases due to non-specific delivery, chemotherapeutic agents fail to exert the desired therapeutic effect, generating multiple side effects. In recent years there has been a growing focus on the improvement of the effectiveness of cancer treatment. RNA-based therapies have the potential to modulate the expression of genes associated with carcinogenesis [2]. Nevertheless, some bottlenecks remain, like inefficient delivery to target tissue and a trade-off between cargo protection before and unloading of nucleic acids after arriving inside cells. Our research aims to evaluate the safety, effectiveness, and efficiency of novel nanoplatforms for the precise delivery of siRNA into CRC cells overexpressing CD44 receptors.

Methods: For this purpose, we designed biodegradable nanoparticles (NPs) based on a core of poly(lactic-co-glycolic acid) (PLGA) and a polymer shell of hyaluronan (HA) to recognize CD44 receptor and polyethyleneimine (PEI) to entrap siRNA. The cytotoxicity of the NPs was evaluated by MTT assay and flow cytometry on human colorectal adenocarcinoma cells (HCT116), human healthy colonic epithelial cells (HCEC), and peripheral blood mononuclear cells (PBMC). Subsequently, the cellular uptake and intracellular localization of the NPs were quantified by flow cytometry and immunofluorescence.

Results: The NPs showed biocompatibility with HCT116, HCEC, and PBMC at all concentrations (12.5-100 µg/ml) and times (24-48-72 hours) considered. Cellular uptake of fluorescent NPs was observed in 100% of HCT116 cells and in 7.32% and 28.81% of PBMC from healthy and CRC patients, respectively. Finally, competition experiments with free HA confirmed the involvement of the CD44 receptor in the receptor-mediated cell uptake of NPs.

Conclusions: Our results demonstrate the safety and efficiency of polymeric biodegradable nanoparticles for efficient RNA drug delivery. The nanoparticles were biocompatible and demonstrated active targeting to CD44 receptor-overexpressing cells. These findings suggest the potential of this formulation for future advances in cancer treatment and RNA-based therapies.

We acknowledge the grant CN00000041 "National Center for Gene Therapy and Drugs based on RNA Technology" (concession number 1035 of 17 June 2022-PNRR MURM4C2 - Investment 1.4 Call "National Centers", financed by EU- NextGenerationEU), project code (CUP E63C22000940007)

[1] Wang K, Shen R, Meng T, Hu F, Yuan H. Nano-Drug Delivery Systems Based on Different Targeting Mechanisms in the Targeted Therapy of Colorectal Cancer. *Molecules*. 2022;27(9):2981. Published 2022 May 6. doi:10.3390/molecules27092981

[2] Liu J, Guo B. RNA-based therapeutics for colorectal cancer: Updates and future directions. *Pharmacol Res*. 2020;152:104550. doi:10.1016/j.phrs.2019.104550

PP183. Indazole Partial Agonists Targeting Peripheral Cannabinoid Receptors

Maitra R¹, Amato G¹, Lauder milk L¹, Vasukuttan V¹, Runyon S¹

¹RTI International, Research Triangle Park, United States of America

Introduction: Our goal is to develop peripheralized indazole based partial agonists of the cannabinoid receptors CB1 and CB2 that exhibit good drug-like properties without crossing the blood-brain barrier (BBB). Such compounds will not elicit psychoactive effects that are noted with Δ9-tetrahydrocannabinol (THC) and other brain penetrant cannabinergic ligands. While peripheralized full agonists have been reported, there is a paucity of partial agonists. This is important because full agonists rapidly induce tolerance and have limited utility.

Methods: Compounds based on an indazole core were designed and synthesized with a topological polar surface area (tPSA) of 80-140 Å, 2-4 hydrogen bond donors, a cLogP of <5, and a MW of 350-500 Da. These compounds were characterized using functional assays for CB1 and CB2 along with radioligand displacement analyses using radiolabeled CP55,940, which is a full synthetic agonist of both receptors. Stability in human liver microsomes (HLM) and the potential to induce certain cytochrome P450 isoforms were determined. Pharmacokinetic studies were performed in mice for select compounds.

Results: Structure activity relationship (SAR) studies led to the identification of several activators of CB1 and CB2. Of the more promising early leads, an indazole bearing a N- difluorobenzyl and a phenyl carboxamide in the 3-position with adequate potency at CB1 and CB2 along with partial agonism was identified. This compound was stable in HLM but induced CYP3A4. Pharmacokinetic studies showed the compound to be orally absorbed in mice with a half-life of ~7 hr. Brain accumulation of this compound was also limited with a plasma:brain maximum concentration (Cmax) ratio >10.

Conclusions: Structural modifications focusing on tPSA, H-bond donors and strategically placed lipophilic moieties produced early lead partial agonists of CB1 and CB2 with significant peripheral selectivity and good drug-likeness including oral absorption. These analogs are viable leads for optimization in therapies where partial agonism of cannabinoid receptors would be beneficial but without neuropsychiatric liabilities.

Support: We express our gratitude to the NIDA drug supply program for providing radiolabeled probes. This research was funded by grants R01DA040460 and R01DK124615 from NIH.

PP184. Recombinant IgG1 Fc hexamer and neutrophils: Kill or not to kill

Hevia Hernandez G¹, Velschoor D, von Gunten S¹

¹*University of Bern, Bern, Switzerland*

Neutrophils can play different functions at inflammatory sites, and once they have played their function, a cell death program starts reducing the number and abrogating the recruitment of more cells. If this process fails, leads to a non-resolving inflammation which has been associated with some pathological conditions such as autoimmune diseases or other conditions like sepsis. One of the current options to treat these conditions is intravenous immunoglobulin (IVIg). However, it has some limitations including dependence on the supply of human plasma and the large doses of product needed for therapy. Since Fc fragments are important for the immunomodulatory properties of IVIg, one of the current alternatives for these preparations, is the use of recombinant Fc (rFc) protein-based therapeutics such as rFc polyvalent molecules, as the rFc hexamer Fc- μ TP-L309C. Considering previous results about IVIg on resting and primed neutrophils, we wanted to evaluate the effect of the rFc hexamer Fc- μ TP-L309C in a similar setting. For this purpose, we treated healthy donor-derived neutrophils, resting or under different priming conditions with the rFc hexamer or IVIg. We evaluated cell death as well as some neutrophil functions after the treatment. Our results indicate that the rFc hexamer Fc- μ TP-L309C does not induce cell death but is able to induce NET release in the neutrophils.

PP185. Induction of miRNAs expression in adipose-derived mesenchymal pluripotent stem cells. Effects of magnesium, cholecalciferol and ascorbic acid.

Kyselovic J¹, Chomaničová N, Adamičková A, Gažová A

¹Clinical Research Unit, 5th Department of Internal Medicine, Faculty of Medicine, Comenius University Bratislava, Slovakia, Bratislava, Slovakia

Background/Aim: Adipose-derived mesenchymal pluripotent stem cells (ADMSCs) have the potential of multi-directional differentiation. This potential is regulated by a variety of cytokines, signalling molecules and other bioactive substances and external environment to promote tissue regeneration. There is growing evidence that miR-1, miR-29b, miR-133a and miR-499 should be modulators of a few mechanisms how ADMSCs can repair or regenerate tissues. Because these miRNAs are only partially able to affect this process, by adding additional specific factors/molecules that can drive cell transdifferentiation, this process can be improved and made more effective. Vitamin D3, magnesium and also vitamin C have pleiotropic effects on various cell types.

The aim of the study was to gain new insight into the impact of magnesium, vitamin D3 and C on expression characteristics of ADMSCs during the cultivation and differentiation.

Methods: A sample of human subcutaneous adipose tissue was collected by aseptic liposuction into the PBS + 5% Penicillin/Streptomycin solution. The cells were isolated by 0.2% collagenase, For the experiment, the sixteenth passage of ADMSCs was used and were cultured for 24 h in CCM supplemented with vitamin C and D3 and magnesium. Quantitative and qualitative characterization of the cells was made utilizing a flow cytometry method (markers CD90, CD105, and CD73, and negative staining for markers CD14, CD20, CD34 and CD45) and the expression of selected bone-associated miRNAs were determined using RT-PCR.

Results: In our experiment after 24 hours incubation of ADMSCs with different concentration vitamin D3, the expression of all tested miRNAs was significantly increased. Magnesium, a significantly modified miR-1, miR -29b, miR 133a and miR-499 expression. Vitamin C increased expression of miR-29 and miR-133a (in high concentration) and decreased expression miR-499. Only increased concentrations of vitamin C significantly affected the structure and morphology of ADMSCs.

Conclusion: Our study has confirmed the possibility of influencing isolated adipose derivate mesenchymal stem cells not only by altered conditions, but also by pre-treatment of incubated cells with drugs (vitamin D3 and C, and magnesium).

PP186. Anti-cholestatic activity of bile acids mediated by glutathione-dependent antioxidant system

Pavlovic N¹, Stanimirov B¹, Djanić M¹, Mikov M¹, Stankov K¹

¹*University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia*

Introduction: Ursodeoxycholic acid (UDCA) is widely used agent for the treatment of cholestatic liver disorders, while its epimer, chenodeoxycholic acid (CDCA), as hydrophobic compound may exert toxic effects, but can also activate the farnesoid X receptor (FXR). FXR is the main regulator of bile acid homeostasis and the development of FXR ligands represents a new approach for the treatment of cholestasis. The aim of our study was to determine the effects of low, non-toxic doses of CDCA on parameters of oxidative stress in cholestatic rats.

Methods: Rats with intrahepatic cholestasis induced by 17 α -ethynodiol (EE) were treated with 10 mg/kg of CDCA and 25 mg/kg of UDCA during 5 days. Gene expression analysis was performed by SybrGreen-based qRT-PCR assay and activities of glutathione-peroxidase (GPx) and glutathione-reductase (GR) were measured using kinetic spectrophotometric methods. The study was approved by the Ethical Committee of the University of Novi Sad.

Results: The induction of cholestatic damage by EE was associated with 5-fold and 2-fold increase of expression of GPx and GR genes, respectively. Activities of these enzymes were also significantly increased in the livers of EE-treated rats ($p<0.05$). Both CDCA and UDCA decreased expression of GPx gene 2-fold. GR expression was also significantly reduced by CDCA treatment ($p<0.05$), while UDCA did not have that effect. Treatment with bile acids exerted similar effects on activities of studied antioxidant enzymes, but UDCA was shown to be more effective than CDCA in reducing both GPx and GR activities, suggesting the involvement of post-translational modifications of these proteins induced by CDCA and UDCA.

Conclusions: Both UDCA and CDCA exhibit protection against estradiol-induced oxidative liver damage and cholestasis through modulation of glutathione-dependent pathways.

Acknowledgement: This research was funded by Provincial Secretariat for Higher Education and Science, Autonomous Province of Vojvodina, Serbia (project No. 142-451-3522/2023-01).

PP187. Bile acids upregulate carbonyl reductase and aldo-keto reductase expression in breast adenocarcinoma cell line following doxorubicin treatment

Stanimirov B¹, Đanić M¹, **Pavlovic N¹**, Lazarevic S¹, Mikov M¹, Stankov K¹

¹*University of Novi Sad, Medical Faculty, Novi Sad, Serbia*

Introduction: Carbonyl reductase (CBR) and aldo-keto reductase (AKR) are involved in the NADPH-dependent two-electron reduction of the anticancer drug doxorubicin into the less active and cardiotoxic metabolite doxorubicinol. As endogenous signaling molecules, bile acids have a propensity to modulate the gene expression of numerous drug-metabolizing enzymes. The aim of the study was to assess the influence of bile acids, ursodeoxycholic (UDCA) and chenodeoxycholic acid (CDCA) on the expression of CBR1 and AKR1A1 in the breast adenocarcinoma MCF7 cell line.

Methods: Human breast adenocarcinoma MCF7 cells were treated with 0.25 µM (D) or co-treated with doxorubicin and either 50 µM of ursodeoxycholic acid (DU) or 6 µM of chenodeoxycholic acid (DC). Following 24 hours, cells were harvested, and RNA was isolated and transcribed into cDNA. The expression of CBR and AKRA1 mRNA was assessed using RT-qPCR compared to β-actin as a reference gene. Gene expression was analyzed using the comparative 2-ΔΔCt method, and the data were analyzed by one-way Anova and Tukey's post-hoc test.

Results: The expression of AKR1A1 in D group increased 1.1 fold compared to untreated cells. Expression of AKR1A1 was upregulated in DU group (11.49 ± 1.83 fold vs. control $p < 0.001$; 10.65 ± 1.69 fold vs. D $p < 0.001$) and in DC group (27.91 ± 3.58 fold vs. control $p < 0.001$; 25.86 ± 3.31 fold vs. D $p < 0.001$). CBR1 expression in D group increased 2.78 ± 0.59 fold vs. the control ($p = 0.946$). Co-treatment with ursodeoxycholic acid reduced CBR1 expression compared to group D (-1.57 ± 0.34 $p = 0.56$) whereas CBR1 was upregulated in DC group (14.38 ± 5.72 vs. control, $p < 0.001$ and 5.23 ± 2.08 vs. D, $p < 0.001$).

Conclusions: By upregulating the expression of CBR1 and AKR1A1, chenodeoxycholic and ursodeoxycholic may affect the therapeutic efficacy of doxorubicin by metabolizing the parent compound into an inactive metabolite.

Acknowledgements: Supported by the Ministry of Education, Science, and Technological Development, Republic of Serbia, Grant III41012, and Project of Special Interest for Sustainable Development in the Autonomous Province of Vojvodina, Grant 142-451-3522/2023-01.

PP188. Investigation of the antidepressant and anti-anxiety effects of DMTS mediated by the TRPA1 ion channel

Göntér K¹, Pozsgai G^{1,2}

¹Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary, Pécs, Hungary, ²Department of Pharmacology, Faculty of Pharmacy, University of Pécs, Pécs, Hungary, Pécs, Magyarország

Introduction: Dimethyl trisulfide (DMTS) is a polysulfide found in garlic and used as food additive. In our previous experiments we showed that DMTS inhibited spontaneous motor activity and respiration in mice. We wanted to investigate the effect of DMTS on anxiety and depression-like behaviour caused by chronic unpredictable mild stress (CUMS). To explore the mechanism of action, we used gene knockout mice for the TRPA1 ion channel gene likely to be involved in the process 1.

Method: The three-week CUMS paradigm consisted of 4 mid-day stressors and 3 different overnight stressors to induce depression-like behaviour. We used 8–10-week-old male TRPA1 wild-type (WT) and knock out (KO) mice on C57B1/6 background. The animals were divided into 12 treatment groups.: stressed and non-stressed groups, within these untreated, vehicle-treated and DMTS-treated subgroups.

Five well-established behavioural tests were used to verify depression-like behaviour and to test the impact of DMTS: open field test (OFT), marble burying test (MBT), sucrose preference test (SPT), tail suspension test (TST) and forced swim test (FST).

Results: In wild type mice, stress exposure significantly reduced the time spent in open area in every group in the OFT. It increased the number of marbles hidden in the MBT. Inactive duration in the FST and TST was longer in naïve stressed animals. Sucrose preference was reduced in stressed animals. Relative adrenal weight was larger and thymus weight was reduced after exposure to chronic stress. In knock out mice, stress-exposure did not lead to depression-like behaviour and anxiety.

In wild type mice, DMTS treatment significantly reduced time spent in the open area and increased the number of marbles hidden in non-stressed animals. The treatment reduced immobility anhedonia in stressed animals. DMTS administration increased relative adrenal weight and relative weight of the thymus in stressed mice.

Conclusion: DMTS treatment seemed to relieve depression-like behaviour in stress-exposed wild type mice. DMTS administration increased anxiety in non-stressed wild type animals, but reduced anxiety in chronic stress-exposed animals. According to our results, DMTS might be an ideal candidate for further study as a dietary supplement for the complementary treatment of depression.

- [1] Pozsgai G, Payrits M, Sághy É, et al. Analgesic effect of dimethyl trisulfide in mice is mediated by TRPA1 and sst 4 receptors. Nitric Oxide. 2017;65:10-21.

PP189. Inflammatory macrophage infiltration reduces subcutaneous adipocyte MPST levels through TNF α and IL-1 β secretion

Salagiannis K^{1,2}, Papadopoulou G², Katsouda A^{1,2}, Papapetropoulos A^{1,2}

¹Clinical, Experimental Surgery and Translational Research Center, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, ²Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Adipose tissue macrophages (ATMs) constitute the most abundant infiltrating immune cell population cross-talking with adipocytes. During obesity, ATMs differentiate into inflammatory M1-like phenotype and sustain low-grade inflammation by secreting TNF α , IL-1 β and IL-6 cytokines¹. MPST sulfurtransferase, a sulfide-species generating enzyme, is downregulated in adipose tissue depots of obese mice and humans^{2,3}. Mpst genetic ablation in mice fed a high fat diet results in increased body weight, excessive inguinal white adipose tissue (iWAT) fat accumulation and upregulation of inflammatory pathways³. Our study aimed to characterize the adipose macrophage infiltration under Mpst deletion and examine its potential contribution to the observed MPST reduction.

Methods: WT and Mpst-/- C57BL/6 male mice were fed normal chow (10%kcal) or high fat (45%kcal) diet for 16 weeks. iWAT was collected and ATMs phenotypes were characterized using FACS analysis for CD11b, F4/80, CD11c and CD206 markers. Bone marrow monocytes and iWAT pre-adipocytes, isolated from WT or Mpst-/- mice, were differentiated into macrophages and mature adipocytes, respectively. Bone marrow-derived macrophages (BMDMs) were next polarized to M1-like phenotype by LPS (100ng/ml) and IFN γ (20ng/ml) for 24hr and iNOS gene expression was detected via RT-qPCR. iWAT-derived adipocytes were used to examine MPST protein levels by western blot analysis after 48hr treatment with BMDMs conditioned media or cytokines TNF α , IL-1 β , IL-6 (100ng/ml).

Results: In bone marrow, M1-like BMDMs exhibited 2.2 ± 0.2 -fold higher levels of iNOS in Mpst-/- relative to WT mice. In iWAT, total numbers of infiltrating CD11b+ F4/80+ ATMs increased during obesity, while the inflammatory CD11c+ M1-like ATMs percentages were upregulated in Mpst-/- compared to WT obese mice ($9.1 \pm 1.3\%$ vs $15.6 \pm 1.9\%$, * $p < 0.05$). MPST protein expression was downregulated in iWAT adipocytes after treatment with M1-like BMDMs conditioned media by $51.0 \pm 7.0\%$. In line with this observation, TNF α and IL-1 β also reduced MPST adipocyte levels by $35.1 \pm 7.6\%$ and $25.4 \pm 7.4\%$, respectively.

Conclusions: Our findings suggest that Mpst deletion potentiates macrophage inflammatory polarization in both bone marrow and iWAT. Furthermore, our data demonstrate that TNF α /IL-1 β secreted by infiltrating macrophages results in downregulation of MPST in adipose tissue, further contributing to our understanding of the complex mechanisms driving obesity pathogenesis and progression.

- [1] Liang et al. The Roles of Adipose Tissue Macrophages in Human Disease. *Frontiers in Immunology*. 2022;13:908749.
- [2] Katsouda et al. Reduced adipose tissue H2S in obesity. *Pharmacological research*. 2018;128:190-199.
- [3] Katsouda et al. MPST sulfurtransferase maintains mitochondrial protein import and cellular bioenergetics to attenuate obesity. *Journal of experimental medicine*. 2022;219(7):e20211894.

PP190. Dosing time-dependent effects of everolimus on circadian gene expression and circadian physiology in mice

Ozturk Seyhan N¹, Li X², Levi F², Okyar A¹

¹Department of Pharmacology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey,

²Chronotherapy, Cancer and Transplantation, Faculty of Medicine, CNRS Campus, University of Paris-Saclay, Villejuif, France

Introduction: Circadian clock controls many biological functions in mammals including drug metabolism and detoxification, cell cycle events, and may affect pharmacokinetics, toxicity and efficacy of drugs [1]. Everolimus is an immunosuppressant/anticancer agent whose pharmacokinetics depends on the efflux transporter P-glycoprotein (Mdr1a) [2]. We aimed to investigate dosing time-dependent toxicity of everolimus with respect to intestinal P-glycoprotein expression rhythms in Mdr1a:Luc mice using Real Time-Biolumicorder System.

Methods: Mdr1a:Luc male mice were synchronized with Light:Dark (12h:12h)-cycle, with Light onset at Zeitgeber-Time (ZT)-0. After baseline recordings, everolimus (5 mg/kg/dayX14 days) was administered orally at ZT1-resting period- and ZT13-activity period to Mdr1a:Luc mice singly housed in an innovative monitoring device, RT-BIO units, which let us monitor real-time and long-term gene expression in freely moving mice. D-luciferin (1.5 mg/mL) was dissolved in drinking water. Mouse Mdr1a:Luc oscillation profile reflecting P-glycoprotein gene expression and locomotor activity pattern were recorded each minute with the photomultiplier tube and infrared sensor, respectively. Clinical signs and body weight were monitored as an index of toxicity. Statistical evaluation was performed with ANOVA. Circadian rhythms were validated with Cosinor analysis.

Results: Everolimus toxicity changed as a function of drug timing, which was least following dosing at ZT13, near the onset of the activity span in mice. Mean body weight loss was nearly twice as large in ZT1-treated mice compared to ZT13 (8.9% vs. 5.4%; p<0.001, ANOVA). Mdr1a expression displayed 24-h rhythms before everolimus treatment and in both vehicle-treated controls (p<0.001, Cosinor). It remained rhythmic in everolimus-treated mice at ZT13, whereas circadian rhythm was altered in mice treated at ZT1, and down-regulation was observed.

Conclusions: Everolimus-treatment altered both rest-activity and Mdr1a expression rhythms at ZT1. It was not the case following dosing at ZT13. Everolimus toxicity/tolerability changed as a function of drug timing, which was least at ZT13-treated mice. This study identified the circadian pattern of P-gp expression with an unprecedented precision. Adjusting dosing time of everolimus according to P-glycoprotein expression rhythms may play a crucial role in minimizing the toxicity of this drug.

*The present work was supported by the Research Fund of Istanbul University. Project No. TSA-2022-38734.

[1] Lévi F, Okyar A, Dulong S, Innominate PF, Clairambault J. Circadian timing in cancer treatments. *Annu Rev Pharmacol Toxicol.* 2010;50:377-421.

[2] Laplante A, Demeule M, Murphy GF, Beliveau R. Interaction of immunosuppressive agents rapamycin and its analogue SDZ-RAD with endothelial P-gp. *Transplant Proc.* 2002;34:3393-3395.

PP191. Lactobacillus species inhibits pancreatic cancer progression by regulating immune response

Tan H¹, Chan Y², Wang N²

¹Centre for Chinese Herbal Medicine Drug Development, Hong Kong Baptist University, Shatin, Hong Kong, ²School of Chinese Medicine, The University of Hong Kong, Pokfulam, Hong Kong

Introduction: Pancreatic ductal adenocarcinoma (PDA) is the fourth death-leading cancer globally, with persistent surge in incidence and negligible adjustment of death rates [1]. In Hong Kong, a total of 855 new incidences and 711 related-mortalities of PDA was reported, which accounted for a mortality-to-incidence ratio of 0.83 in 2018. The prognosis of PDA is very poor, in which the 5-year survival after diagnosis is less than 9%. Accumulating evidence has revealed that gut commensals modulate the immune response and enhance the inhibitory effects in various cancers.

Methods: The orthotopic PDA implantation mouse model will be established and the fecal bacterial composition will be characterized in wild type and orthotopic PDA bearing mice using 16s rRNA gene sequencing. The identified bacteria species will be supplemented to the mice in vivo and the efficacy and immune profile of the orthotopic PDA bearing mice following bacteria supplementation will be investigated.

Results: We identified a type of lactic acid bacteria, *Lactobacillus gasseri*, was particularly repressed in PDA-bearing mice. Oral supplementation of *L. gasseri* decreased tumour growth and increased PDA mice survival. *L. gasseri*-colonized mice showed increase abundance of CD103+ type I conventional dendritic cells, the antigen presenting cells that responsible for the activation of CD8+ T lymphocytes, in pancreatic tumour and neighbouring intestine, as well as increase circulating IL-12 and IFN- γ protein expression.

Conclusions: Our works provide scientific evidence for the therapeutic potential of *Lactobacillus gasseri* in inhibiting PDA progression through modulating tumor immune response.

- [1] Pourshams, A., et al., The global, regional, and national burden of pancreatic cancer and its attributable risk factors in 195 countries and territories, 1990–2013;2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet Gastroenterology & Hepatology*, 2019. 4(12): p. 934-947.

PP192. CTH/MPST double ablation results in hepatic steatosis

Markou M^{1,2}, Katsouda A^{1,2}, Papapetropoulos A^{1,2}

¹Clinical, Experimental Surgery and Translational Research Center, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, ²Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Hydrogen sulfide (H₂S), a gasotransmitter with protective effect in non-alcoholic fatty liver disease (NAFLD) is endogenously generated by cystathione-γ lyase (CTH), cystathione-β synthase (CBS) and 3-mercaptopropruvate sulfurtransferase (MPST) enzymes. CTH, MPST and CBS are highly expressed in liver. Cth global or hepatocyte-specific deletion in mice leads to increased hepatic lipid deposition under high fat diet (HFD) conditions. HFD feeding or aging of Mpst-/ mice results in an enhanced fatty liver phenotype. To investigate the hypothesis that Cth/Mpst double ablation in mice could lead to an early steatosis phenotype under normal conditions, we generated and characterized a double Cth/Mpst knockout (Cth/Mpst-/-).

Methods: 8 weeks-old Cth/Mpst-/- mice were sacrificed, liver tissues were isolated and used for histological studies and gene/protein expression analysis. For liver mass measurements the left lateral lobe was used. To restore H₂S levels the SG1002 donor was added in chow diet (80mg/kg/day) for 4 weeks. For in vitro procedures, HepG2 cells were cultured in the presence or absence of CTH and MPST pharmacological inhibitors (propargylglycine, PAG, 1mM and IMST-3 50μM) for 48 hours. Lipid accumulation was determined by Oil RedO.

Results: Cth/Mpst-/- mice were viable, fertile and exhibited no gross abnormalities. Lack of both CTH and MPST did not affect the liver levels of CBS and H₂S-degrading enzymes. Young CTH/MPST-deficient mice exhibited increased liver mass under normal feeding conditions ($1,65 \pm 0,07$ vs $2,09 \pm 0,07$ (%), p=0,00103, n=6-7), H&E staining revealed enhanced hepatic lipid accumulation following ablation of both enzymes. Upregulation of glucose transporter 2 (GLUT2) was detected in liver tissues of Cth/Mpst-/- mice. To investigate the hepatocyte-autonomous effects of CTH/MPST, HepG2 cells were treated with PAG and IMST-3. CTH/MPST inhibition resulted in increased lipid deposition in HepG2 cells ($1 \pm 0,03$ vs $1,19 \pm 0,05$ (folds), p=0,0082, n=6). SG1002 donor treatment attenuated the fatty liver phenotype of CTH/MPST-deficient mice ($2,10 \pm 0,06$ vs $1,79 \pm 0,09$, (%), p= 0.015, n=6).

Conclusion: Our findings further confirm the importance of endogenously produced H₂S in the pathogenesis of NAFLD and introduce the Cth/Mpst-/- mouse as a new animal model of hepatic steatosis.

PP193. Pharmacology of Skeletal Muscle Chloride Channels (ClC-1): Implications in Restoring Neuromuscular Transmission in Neuromuscular Disorders

Morgen J¹, Skals M¹, Huus N¹, Skov M¹, Findsen A¹, Broch-Lips M¹, Pedersen T²

¹*NMD Pharma A/S, Aarhus, Denmark*, ²*Institute of Biomedicine, University of Aarhus, Denmark, Aarhus, Denmark*

ClC-1 is a chloride channel specifically expressed in skeletal muscle cells. The channel stabilizes the resting membrane potential and dampens muscle fibre excitability and is involved in regulating muscle fibre excitability during intense exercise. Recently, it was shown that in isolated rat muscle exposed to a neuromuscular blocking agent, a model mimicking neuromuscular transmission dysfunction, that inhibition of the ClC-1 channel improves neuromuscular transmission. This suggests that ClC-1 inhibition may improve neuromuscular transmission failure in neuromuscular diseases in humans. While neuromuscular transmission is reliable in healthy individuals, transmission failure causes weakness and fatigue in a range of neuromuscular diseases including myasthenia gravis (MG). In the present study we investigated the pharmacodynamic/pharmacokinetic effect of different ClC-1 inhibitors in pre-clinical rat models of neuromuscular dysfunction. Three rat models were used; a pharmacological model induced in healthy rats by infusion of a neuromuscular blocking agent, and in two MG rat models. Data are shown from both ex vivo experimentation with muscle-nerve preparations and in vivo studies. Our results show that pharmacological inhibition of skeletal muscle ClC-1 restores synaptic transmission and skeletal muscle function leading to marked improvements in muscle strength in the pharmacological model of neuromuscular dysfunction as well as both MG rat models. These results support a hypothesis that ClC-1 inhibitors having suitable pharmacokinetic properties could be used as a therapeutic approach to improve muscle function in disorders where neuromuscular transmission is compromised.

PP194. Multifactorial antioxidant activity of CEMTIRESTAT, inhibitor of aldose reductase, in prevention of development secondary diabetic complications

Šoltésová Prnová M¹, Bod'o P¹, Lepaček M¹, Jahelkova M¹

¹*Center of Experimental Medicine Slovak Academy of Science, Bratislava, Slovakia*

Cemtirestat, a bifunctional drug acting as an aldose reductase inhibitor with antioxidant ability, is considered a promising candidate for the treatment of diabetic complications [1,2]. In our previous studies, we have demonstrated the inhibitory effect of cemtirestat against aldose reductase, the first enzyme of the polyol pathway [1]. In the present study, we studied the antioxidant activity of cemtirestat at in vivo, ex vivo and in vitro levels.

The DPPH assay was used to demonstrate the scavenging ability of Cemtirestat (Apollo Scientific). Spectrophotometric methods were used to study the interaction of Cemtirestat with GSH. At the ex vivo level, the protective effect of Cemtirestat towards RBCs under the influence of peroxy radical damage was investigated. Finally, a 6-month in vivo experiment was performed in rats of the ZDF strain treated with a dose of cemtirestat 2.5 mg/kg/day. Animals was divided into six groups (C- control, CT- control treated, O – obese, D – diabetic, OT – obese treated and DT – diabetic rats treated). Behavioral tests were performed before the procedure was terminated. At the end of the experiment, the animals were sacrificed by puncturing blood from the aorta after general anesthesia with chlorhydrate and isoflurane. From blood and urine samples, where appropriate, selected parameters (insulin, HbA1c, NGAL, MMP-9, IL-10...) was determined.

The DPPH assay revealed significant antiradical activity. One molecule of cemtirestat quenched approximately 1.5 DPPH radicals. In the system of isolated erythrocytes, the compound was readily taken up by the cells followed by the protection against free radical-initiated hemolysis. Spectrophotometric analysis showed GSH was able to reduce cemtirestat dimer, however interaction of GSH and cemtirestat was not observed. Treatment of diabetic animals with cemtirestat (i) did not affect insulin, HbA1c and glucose level; (ii) reversed sorbitol accumulation in RBC, sorbitol in sciatic nerves was not affected; (iii) attenuated the symptoms of peripheral neuropathy; (iv) significantly reduced cortisol, MMP9, NGAL, TBAR's in plasma samples of the treated group of diabetic animals; (v) cemtirestat did not impact on IL-10 and IL-6.

The present results indicate multiple antioxidant ability of cemtirestat treatment. However, further studies are needed to reveal the exact antioxidant mechanisms.

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-20-0411 and VEGA 2/0008/22.

[1] Stefek M. et al.: J Med Chem. 2015 Mar 26;58(6):2649-57. Doi: 10.1021/jm5015814.

[2] Prnova S. M. et al.: Redox Rep. 2015;20(6):282-8. Doi: 10.1179/1351000215Y.0000000019

PP195. Unveiling Human Lung Fibroblast Exosomes as Curcumin and Doxorubicin Nanocarriers for Cancer Therapeutics

Saiti A¹, Giannopoulos-Dimitriou A¹, Panteris E², Malousi A³, Stamoula E⁴, Anagnostopoulos A⁴, Vatsellas G⁵, Al-Maghribi P⁶, Chatzopoulou F⁷, Müllertz A⁶, Tseti I⁸, Fatouros D⁹, Vizirianakis I^{1,10}

¹Laboratory of Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece,

²Department of Botany, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece,

³Laboratory of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece,

⁴Proteomics Research Unit, Center of Basic Research II, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, ⁵Greek Genome Center, Biomedical Research Foundation Academy of Athens, Athens, Greece, ⁶Laboratory of Pharmaceutical Technology, Department of Pharmaceutical Design and Drug Delivery,

School of Pharmacy, University of Copenhagen, Copenhagen, Denmark, ⁷Laboratory of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, ⁸Uni-Pharma Kleon Tsetis Pharmaceutical Laboratories S.A, Athens, Greece, ⁹Laboratory of Pharmaceutical Technology, Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece, ¹⁰Department of Health Sciences, School of Life and Health Sciences, University of Nicosia, Nicosia, Cyprus

Introduction: Exosomes, as bio-vesicles naturally released by all cell types, display advantageous biopharmaceutical properties as Drug Delivery Systems (DDS) in cancer therapy, compared to synthetic nanoparticles. The MRC-5 cell line (normal human lung fibroblasts) poses a cellular model approved by regulatory affairs for viral-vaccine development research, facilitating the clinical translation of MRC-5 cell-derived nanotherapeutics. This study aims to standardize MRC-5 cell-derived exosome isolation, characterization and drug-loading approaches as well as to pharmacologically assess the Curcumin and Doxorubicin-loaded MRC-5 cell-derived exosome-based DDS towards targeting “heterologous” lung adenocarcinoma A549 and tongue squamous carcinoma HSC-3 cell lines in-vitro.

Methods: Exosomes from the culture medium of MRC-5 cells were isolated following three different exosome isolation approaches based on: (a) Ultracentrifugation, (b) membrane-affinity binding, and, (c) a modified methodology combining ultrafiltration with successive membrane-based affinity binding. Exosomes isolated via each methodology were subsequently subjected to morphological and physicochemical evaluation utilizing Dynamic Light Scattering (DLS) and Cryogenic Transmission Electron Microscopy (cryo-TEM) techniques. To clarify the drug-loading capacity of exosomes the hydrophobic Curcumin was incorporated in exosomes following the incubation method, whereas the hydrophilic Doxorubicin HCl was loaded in exosomes using two different methodologies: (i) sonication and (ii) freeze-drying-based approach. Accordingly, the drug-loaded exosomes were analyzed morphologically and the drug-loading efficiencies were investigated fluorometrically. Cellular uptake studies based on Confocal Laser Scanning Microscopy (CLSM) and cell viability assays were also performed upon incubation of naïve or drug-loaded MRC-5 cell-derived exosomes with “autologous” normal MRC-5 and “heterologous” tumorigenic A549 and HSC-3 cells.

Results: The DLS and cryo-TEM analyses verified that the modified exosome isolation protocol yielded round-shaped lipid-bilayer enclosed exosomes (diameter approximately 40nm) with higher purification and reduced aggregational formations compared to the other investigated exosome isolation methods. Drug-loading studies highlighted a great association of the loading efficiency with the compound’s polarity as well as with the applied drug-loading method. Interestingly, CLSM demonstrated an extensive internalization of MRC-5 cell-derived exosomes in cancerous A549 and HSC-3 cells, suggesting a selective “targetability” of exosomes in “heterologous” cancerous cells. Furthermore, cell viability assays rendered Doxorubicin- and Curcumin-loaded exosomes as well as the dual administration of both exosomal formulations efficient to reduce the proliferation of cancerous A549 and HSC-3 cells, while ensuring a minimal cytotoxicity effect of naïve MRC-5 cell-derived exosomes.

Conclusions: Overall, this study provides a comprehensive morphological, physicochemical and pharmacological characterization of MRC-5 cell-derived exosomes facilitating their clinical “translatability” as Curcumin and Doxorubicin DDS in oncology.

PP196. Systems Pharmacology and Network Analysis to Advance Pharmacogenomics and Precision Medicine Decisions in Type-2 Diabetes Therapy

Giannopoulos-Dimitriou A¹, Saiti A¹, Kazakos I¹, Galatou E², Vizirianakis I^{1,2}

¹Laboratory of Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece, Thessaloniki, Greece, ²Department of Health Sciences, School of Life and Health Sciences, University of Nicosia, Nicosia, Cyprus

Introduction: Diabetes mellitus type-2 (DMT-2) molecular pathophysiology is still challenging since representing a complex, multifactorial, polygenic-based metabolic disease. Advances in multi-omics technologies facilitated the establishment of genes and microRNAs as potential biomarkers implicated in DMT-2 prognosis, diagnosis, and therapy. In this work, integrating systems pharmacology with bioinformatics network analysis, we comprehensively assessed the genomics data associated with DMT-2 to: (a) Enrich the existing knowledge of DMT-2 molecular pathophysiology; (b) Unveil important miRNA-drug and gene-drug pharmacogenomics associations; and, (c) Create connectivity maps of practical clinical utility to improve precision medicine approaches in DMT-2 management.

Methods: To better exploit the pharmacogenomics knowledge related to DMT-2, the genes/variants, miRNAs, and curated variant-drug pairs associated with DMT-2 were recovered from DisGeNET, HMDD and PharmGKB databases, respectively. The validated target genes (VTGs) of the DMT-2-associated miRNAs were recovered from the miRTarBase, miRecords, and TarBase databases. A targeted overrepresentation analysis for Gene Ontology (GO), Disease Ontology (DOSE), and REACTOME pathways enrichment was performed in the unique genes extracted by integrating molecular information derived from independent databases via matching the VTGs of DMT2-related miRNAs with the genes implicated in DMT-2 pathogenesis. Appropriate packages in R and Cytoscape software were used for data processing and drug-miRNA-VTGs network construction/visualization.

Results: The targeted bioinformatic analysis revealed 71 unique VTGs of the DMT-2-associated miRNAs that are also implicated in DMT2 pathophysiological mechanisms. The GO and Pathway enrichment analysis revealed that the shared genes are involved in biological processes and pathways related to oxidative stress, glucose transmembrane transport and apoptosis regulation. The DOSE enrichment analysis unveiled that the DMT2-related shared genes are implicated in 3 major disease clusters, including urinary system disease, obesity, and colorectal cancer. The network analysis also revealed DMT-2 related shared genes that co-involved in the disease clusters highlighting their role as key “molecular players” which associate DMT-2 pathophysiology with other diseases that coexist in patients with DMT-2. The pharmacogenomics-based network analysis also revealed 13 microRNAs (miR-802, miR-320a, miR-320d, miR-375, miR-665, miR-107, miR-133b, miR-4534, miR-4463, miR-451a, miR-217, miR-206, and miR-384) that are connected via six DMT2-related SNP variants (rs290487, rs4402960, rs1470579, rs12255372, rs1801278, and rs13431554) with antidiabetic drugs, including repaglindine, sulfonamides, clopidogrel and urea-derivatives.

Conclusions: Overall, the data obtained facilitate the analysis of the molecular landscape of DMT-2 by improving our knowledge of the disease's pathophysiology, unveiling molecular connection maps of practical clinical utility, and empowering the exploitation of pharmacogenomics-guided therapeutic decisions within the concept of precision medicine.

PP197. Characterization of the Effects of Jmv2894, a Synthetic Growth Hormone Secretagogue, in a Cellular Model of Duchenne Muscular Dystrophy

Bresciani E¹, Rizzi L¹, Meanti R¹, Cappellari O², Mantuano P², Conte E², Boccanegra B², Liantonio A², Denoyelle S³, Fehrentz J-A³, Locatelli V¹, De Luca A², Torsello A¹

¹*Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*, ²*Department of Pharmacy-Drug Sciences, University of Bari, Bari, Italy*, ³*IBMM, UMR 5247, CNRS, Université de Montpellier, ENSCM, Montpellier, France*

Aim: Growth hormone secretagogues (GHSs) are a class of synthetic compounds analogues of ghrelin, known for their various endocrine and extra-endocrine properties, including the control of inflammation and metabolism, enhancing GH/IGF-1 mediated myogenesis, and inhibiting angiotensin-converting enzyme (ACE), all pathways of interest in Duchenne muscular dystrophy (DMD). Chronic treatment with the GHS JMV2894 has demonstrated beneficial effects in preserving muscle function in the mdx mouse, a model of DMD. JMV2894 decreased inflammatory status and fibrotic tissue deposition, and induced positive modifications in muscle metabolism. However, JMV2894 mechanism of action on muscle cells is largely unknown.

Method: We investigated JMV2894 effects on muscle cell precursors involved in the DMD muscle regeneration process. H2K-SF1 are muscle cells characterized by the absence of dystrophin. Short-term, 48 and 96 hours (h), or long-term incubation (9 days), with 1 µM JMV2894 alone or in combination with 0.2 µM methyl-prednisolone (m-pdn), commonly used in DMD patients, were performed to assess the expression levels of specific myogenic markers (Pax7, Myf5, MyoD, desmin, myogenin, fast and slow myosin), the cell fusion index and the mRNA levels of RYR1, a receptor involved in Ca²⁺ homeostasis regulation.

Results: Short-term incubations with JMV2894 significantly altered Myf5 and myogenin mRNA expression and enhanced the cell fusion index, compared to control; long-term treatments significantly increased slow myosin mRNA levels. Similar effects were induced by m-pdn, although m-pdn stimulated slow myosin mRNA expression more effectively than JMV2894. The long-term co-administration of m-pdn with JMV2894 significantly increased the slow myosin mRNA levels compared to the JMV2894 and m-pdn groups. Interestingly, 9-day treatment with JMV2894 as well as m-pdn stimulated RYR1 mRNA levels.

Conclusions: Our results suggest that in vivo JMV2894 beneficial effects may likely depend on its capability to impact in the muscle cell regeneration efficiency and differentiation process, supporting that JMV2894 could be developed as a possible modulator of the pathology.

PP198. Semaglutide significantly increases cell viability and ameliorates lipid accumulation in an in vitro model of MASLD

Petrovic A¹, Hefer M¹, Omanovic Kolaric T¹, Kuna Roguljic L¹, Kizivat T², Matic A¹, Smolic R¹, Vcev A¹, Smolic M¹

¹*Faculty of Dental Medicine and Health Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia,*

²*Faculty of Medicine Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia*

Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a sub-category of steatotic liver disease, affecting 40% of the population globally. MASLD can range from mild steatosis to steatohepatitis, steatofibrosis, cirrhosis, liver malignancies and failure. No pharmacotherapy has been approved for this condition. GLP-1 receptor agonists (GLP-1RAs), such as semaglutide, have shown to affect dysregulated lipid metabolism pathways, becoming a promising therapeutic option for MASLD [1]. In this research, for the first time to our knowledge, we investigated effects of semaglutide in an in vitro cell culture model of MASLD.

Methods: Cell culture model of MASLD was established by incubating human hepatoma cell line Huh-7 with 1 mM oleic acid (OA) for 24 hours. Cells were cotreated with semaglutide, concentrations ranging from 2 nM to 10 nM. MTS assay was used to assess cell viability upon treatment. Steatotic changes in the hepatocytes were assessed visually by light microscopy with Oil-Red-O staining, while microplate reader was used for colorimetric quantification. Fetal bovine serum and ethanol mixture was used as a solvent and chosen as an appropriate negative control. Statistical analysis was done accordingly.

Results: Cell viability in MASLD model was significantly reduced by 41.58% ($p < 0.001$) compared to the negative control. Compared to MASLD model, cotreatment with 2 nM, 5 nM, and 10 nM of semaglutide increased cell viability by 20.51% ($p < 0.03$), 14.42% and 18.45%, respectively. The increases in the 5 nM and 10 nM groups, while notable, did not reach statistical significance. Oil-Red-O staining in the MASLD model revealed increase of lipid accumulation compared to the negative control by 115.89% ($p < 0.001$). Cotreatment with semaglutide, 2 nM, 5 nM, and 10 nM, reduced lipid content compared to negative control in a dose-dependant manner: 101.87% ($p < 0.001$), 88.79% ($p < 0.001$), and 46.26% ($p < 0.001$), respectively. Compared to the MASLD model, the cotreated groups also showed statistically significant decrease in lipid accumulation by 6.64% ($p < 0.001$), 12.36% ($p < 0.001$), and 32.41% ($p < 0.001$), respectively. Steatotic changes were also observed microscopically.

Conclusions: The results of our study present solid evidence of direct beneficial effects of semaglutide and its lipid accumulation ameliorating properties in an in vitro model of MASLD, providing a foundation for further research of GLP1Ras as a potential treatment of MASLD.

- [1] Nevola R, Epifani R, Imbriani S, et al. GLP-1 Receptor Agonists in Non-Alcoholic Fatty Liver Disease: Current Evidence and Future Perspectives. *Int J Mol Sci.* 2023;24(2):1703. Published 2023 Jan 15. Doi:10.3390/ijms24021703

PP199. Investigation of antialzheimer and antiangiogenic activities of donepezil hydrochloride loaded PLGA nanoparticle (NP) systems by in vitro AchE and BuChE inhibitor activity analyzes and in vivo CAM method

Kömür M¹, Kıyan H², **Öztürk A¹**

¹Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eskişehir, Turkey, ²Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Turkey

Introduction: NPs with unique physicochemical and biological properties, are taken more easily by the cells than larger molecules; therefore, they can be used effectively as a drug delivery system (DDS). The most important reasons for the use of NPs as a DDS include prolonged release of encapsulated drug, increasing drug's therapeutic efficacy and reducing side effects [1]. For this purpose, PLGA-NPs were prepared in this study to increase the effectiveness of donepezil hydrochloride (DNP).

Methods: DNP loaded PLGA-based-NPs were prepared by the 'Double Emulsification Solvent Evaporation' method. The I-DNP coded formulation was selected as optimum and solid-state characterizations were performed with DSC, FT-IR and ¹H-NMR [1]. After the characterization of I-DNP in terms of pharmaceutical technology (particle size, PDI, zeta potential, EE% and release) was completed, the Ellman Test was used to determine the acetylcholinesterase (AchE) and butyrylcholinesterase (BuChE) enzyme inhibition activity. In vivo CAM assay was used to study the effect of I-DNP on angiogenesis [1,2].

Results: Encapsulation was proven by DSC, FT-IR, and ¹H-NMR. According to the AChE and BuChE inhibitor activity results, I-DNP showed strong anti-AChE activity with $96.88 \pm 0.16\%$ inhibition at 400 µg/mL concentration and strong anti-BuChE activity with $82.96 \pm 0.57\%$ inhibition at a concentration of 400 µg/mL. According to the in vivo CAM results, I-DNP showed anti-angiogenic effect by significantly inhibiting capillary vessel development at a concentration of 10 µg/pellet, compared to the blank (2.5% agarose) and I-Blank coded formulation. Depending on the decrease in capillary area, the activity order was determined as I-DNP>DNP>Blank formulation. Total capillary area was determined as $14.702\% \pm 3.395$, $17.982\% \pm 2.595$, and $20.362\% \pm 3.353$ for I-DNP, DNP, and blank formulation, respectively.

Conclusion: According to the anti-alzheimer activity results, I-DNP showed strong anti-AChE and anti-BuChE activities compared to DNP, which is an acetylcholinesterase inhibitor. Within vivo CAM experiments, it was determined that the I-DNP coded formulation attenuated (reduced/inhibited) angiogenesis and it was concluded that the prepared NP system could be a new anti-angiogenic treatment by stimulating the cholinergic system. This study was financed by Anadolu University Scientific Research Project Foundation (No:2304S027).

- [1] Öztürk AA, Kıyan HT. Treatment of oxidative stress-induced pain and inflammation with dexketoprofen trometamol loaded different molecular weight chitosan nanoparticles: Formulation, characterization and anti-inflammatory activity by using in vivo HET-CAM assay. *Microvasc Res.* 2020;128:103961.
- [2] Kıyan HT, Demirci B, Başer KHC, Demirci F. The in vivo evaluation of anti-angiogenic effects of Hypericum essential oils using the chorioallantoic membrane assay. *Pharm Biol.* 2014;52(1):44-50.

PP200. Effect of DMTS via the TRPA1 ion channel in acute stress in mouse model

Göntér K¹, **Pozsgai G**^{1,2}

¹Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary, Pécs, Hungary, ² Department of Pharmacology, Faculty of Pharmacy, University of Pécs, Pécs, Hungary, Pécs, Magyarország

Introduction: Although there is active research on the role of sulphide in the central nervous system, not much is known about its effects in anxiety and depression. Inorganic polysulfide is not an ideal candidate for drug development. Dimethyltrisulphide (DMTS), found in garlic, is more stable and has favourable pharmacokinetics. Several effects of DMTS are mediated by TRPA1 ion channel activation¹. In our study, we wanted to investigate the effect of DMTS in a mouse model of acute stress and the role of the TRPA1 ion channel in the process.

Methods: Male, 6–8-week-old TRPA1 gene-knockout (KO) and wild-type (WT) mice were used. We set the appropriate dose of DMTS via open field test. We used forced swim test to investigate the depression-like behavior and anxiety. Animals either received no treatment, vehicle (1.5% m/v polysorbate 80 in physiological saline) or DMTS (50 mg/kg i.p.). At the end of the experiments, c-Fos immunohistochemistry was carried out in brain areas involved in stress. The statistical evaluation of our results was thus performed using GraphPad Prism 8. One-way ANOVA followed by Dunnett's post hoc test was performed to compare the results of each treatment group.

Results: Dose of 50 mg/kg DMTS was the highest which did not diminish the natural activity of the mice. In the FST DMTS treatment reduced inactive duration, increased highly active duration and activity frequency in TRPA1 WT animals compared to either naïve mice or vehicle treated group. DMTS did not alter parameters in TRPA1 KO animals relative to naïve mice or vehicle treatment. Compared to the untreated groups, DMTS-treated mice showed significant c-Fos signal in some brain areas involved in stress processes, namely Lateral Septum, Dorsal Raphe nucleus, Paraventricular nucleus of the Thalamus, Locus Coeruleus, Paraventricular nucleus of the Hypothalamus, Edinger-Whesthal nucleus.

Conclusion: Since the beneficial effects of DMTS were not presented in TRPA1 KO mice, but we experienced them in TRPA1 WT mice, we concluded that DMTS reduces depression-like behavior in the forced swim test mediated by TRPA1 ion channels. It also activated brain areas that play an important role in stress. Therefore, DMTS might offer an alternative for the complementary treatment of depression.

- [1] Pozsgai G, Payrits M, Sághy É, et al. Analgesic effect of dimethyl trisulfide in mice is mediated by TRPA1 and sst 4 receptors. Nitric Oxide. 2017;65:10-21.

PP201. Melatonin Boosts Anti-Cancer Immunity in Pancreatic Adenocarcinoma by Modulating Tumor-Related Neutrophil Infiltration and NETosis

Wang N¹, Tan H²

¹*The University of Hong Kong, Hong Kong, ²Hong Kong Baptist University, Hong Kong*

Pancreatic adenocarcinoma (PAAD) is a primary cause of cancer-related fatalities due to its extremely unfavorable prognosis and limited curative options. Melatonin, mainly produced in the pineal gland and found in various extrapineal sources including the pancreas, has anti-inflammatory and anti-cancer properties aside from its well-known circadian effects. Melatonin inhibits tumor development and enhances the response to chemotherapy drugs. Abnormal regulation of endogenous melatonin levels is linked to tumor formation. Research emphasizes the importance of providing exogenous melatonin to suppress malignancies, but the specific mechanism of melatonin's anti-tumor effect in PAAD remains uncertain. This study reveals that melatonin levels are decreased in the pancreas of PAAD patients. Melatonin exerts an anti-tumor effect by recruiting neutrophils into the tumor environment. This infiltration is indirectly induced by the CXCL2 secreted from tumor cells under melatonin's influence. The recruited neutrophils exhibit an N1-like anti-tumor phenotype, with improved tumor-killing abilities via NETs formation through cell-to-cell contact. We show that NETosis is driven by a ROS-mediated pathway fueled by fatty acid oxidation, and then confirm that CXCL2 and NETosis activation markers are predictive indicators of PAAD patient survival. Our research highlights the potential application of melatonin in managing pancreatic cancer clinically.

PP202. Novel synthesized Coumarin derivate in airway smooth muscle

Elshani A¹, Sopi R², Haziri A², Thaći Q², Sylqa A¹, Daci A³, Zogu M³

¹*Faculty of Medicine, University of Prishtina, Prishtine, Kosovo*, ²*Department of Chemistry, University of Prishtina, Prishtine, Kosovo*

Background: Coumarins are a group of plant-derived polyphenolic compounds that belong to the benzopyrone family. Extensive pharmacological activities of coumarins were demonstrated, including anticoagulant, antimicrobial, anti-inflammatory, neuroprotective, antidiabetic, anticonvulsant, antiproliferative, vasorelaxant and smooth muscle relaxation.

There has been a study conducted in (coumarin derivatives) which offers intriguing airway relaxant capabilities that can be used to build novel therapeutic alternatives for the treatment of asthma. However, there is a lack of information covering the direct effect of this compound in the airway smooth muscle tone, or more precisely on smooth muscle of trachea.

Aims: Therefore, in this study we aimed to evaluate the relaxation effect of our newly synthesized Coumarin derivate(Compound 4 MW:352 g/mol)in the tracheal smooth muscle cell isolated from rats.

Methods: Tracheal cylinders were obtained from newborn Wistar rat pups weighing 180-280 g and was prepared for the tissue organ bath apparatus. . The direct relaxant effect of Compound 4 was tested in the rat trachea rings precontracted by metacholin(MCh,10-6 M).In addition,the pretreatment with Coumarin Compound 4 was tested on the tracheal reactivity induced by methacholine (MCh, 10-8- 10-4 M).

Results: Our findings demonstrated that C4 induced a concentration-dependent relaxation effects(E_{max}= 33.62%and E_{max}=97.46%) and a significant reduction of tracheal reactivity to MCh(Control: E_{max}=2.36+-0.23g,C4 low dose:E_{max}=2.28+-0.23g,p>0.05;pEC50,Control:5.75+-0.17,C4 lower dose:5.23+-0.17,p<0.001) and (Control:E_{max}=1.93+-0.14g,C4 higher dose:E_{max}=1.57+-0.11g,p<0.001;pEC50,Control:5.96+-0.11,C4 higher dose:5.13+-0.14,p<0.001).

Conclusion: C4 displayed relaxant and inhibit reactivity in the in vitro airway smooth muscles.Taken together,our findings indicate the potentiale role of this novel synthesized Coumarin derivate in the treatment of respiratory diseases with limited airflow. Even though there are still further studies needed to better understand its mechanistic effects.

PP203. The soluble form of PD-L1 is a valuable biomarker in COVID-19

Conti V^{1,4}, Francesco S^{1,2}, Pagliano P^{1,3}, Carmine S⁴, Berenice S⁵, Graziamaria C⁵, Valentina M⁴, Emanuela D⁶, Liguori L⁷, Stefano P^{1,2}, Amelia F^{1,4}

¹Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi, Italy, ²Oncology Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, Salerno, Italy, ³Infectious Disease Unit, San Giovanni di Dio e Ruggi D'Aragona University Hospital, Salerno, Italy, ⁴Clinical Pharmacology Unit, San Giovanni di Dio e Ruggi d'Aragona University Hospital, Salerno, Italy, ⁵Department of Translational Medical Sciences, University of Naples "Federico II", Naples, Italy, ⁶PhD School "Clinical and Translational Oncology (CTO)", Scuola Superiore Meridionale, University of Naples "Federico II", Naples, Italy, ⁷Department of Clinical Medicine and Surgery, University of Naples "Federico II", Naples, Italy

Introduction: COVID-19 continues to cause hospitalizations and deaths [1]. Therefore, understanding the link between patients' immune characteristics and COVID-19 manifestations is crucial. The immune checkpoints programmed death-1 (PD-1) and its ligand programmed death-ligand 1 (PD-L1) play a role in the immune response, so much so that the study of the PD-1/PD-L1 axis has led to the availability of immune checkpoint inhibitors, essential to treat some cancers. High expression levels of PD-L1 soluble forms (sPD-L1) have been reported not only in cancer but also in autoimmune and viral diseases [2]. This study investigated sPD-L1 as a biomarker of COVID-19 prognosis.

Methods: COVID-19 patients were enrolled at the University Hospital of Salerno-Italy. Peripheral blood samples were collected during routine venipuncture within 6 days from the admission (T0) and after 12–14 days (T1), at the same time at which biochemical and clinical parameters were recorded. sPD-L1 levels were measured by enzyme-linked immunosorbent assay. The study followed the Declaration of Helsinki and was approved by the local ethics committee.

Results: Thirty-one COVID-19 patients were enrolled. At T0, the number of lymphocytes tended to be higher in surviving than in dead patients. CRP and LDH levels were higher, while the PaO₂/FIO₂ ratio was lower in dead than in discharged patients. Patients with lower lymphocyte numbers showed longer length of stay (LOS) ($p=0.032$, $r^2=0.205$). Lymphocyte number was negatively correlated with CRP ($p=0.004$, $r^2=0.297$). Patients with higher levels of sPD-L1 had a longer LOS ($p=0.015$, $r^2=0.278$). Considering the differences measured at different times, a shorter LOS was found in patients with increased sPD-L1 and lymphocyte number compared with those in whom sPD-L1 and lymphocyte number decreased ($p=0.038$) and those with increased sPD-L1 and decreased lymphocyte number ($p=0.025$). A shorter LOS was found in patients with increased sPD-L1 and decreased CRP compared with those with increased sPD-L1 and CRP ($p=0.034$) and those with decreased sPD-L1 and CRP ($p=0.048$).

Conclusions: While at an early stage of COVID-19, sPD-L1 promotes an immune escape, it later can inhibit an excessive immune response. These results suggest a prognostic role of sPD-L1 and provide a rationale to implement clinical trials with PD-1/PD-L1 inhibitors in COVID-19 patients.

[1] Abbasi J. What to Know About EG.5, the Latest SARS-CoV-2 "Variant of Interest". JAMA. 2023;330(10):900-901. doi:10.1001/jama.2023.16498.

[2] Bailly C, Thuru X, Quesnel B. Soluble Programmed Death Ligand-1 (sPD-L1): A Pool of Circulating Proteins Implicated in Health and Diseases. Cancers (Basel). 2021;13(12):3034. Published 2021 Jun 17. doi:10.3390/cancers13123034.

PP204. Receptor Protein Tyrosine Phosphatase zeta 1 (RPTPZ1) pharmacological inhibition or deletion regulates osteoblasts activities in vitro and bone homeostasis in vivo

Xanthopoulos A¹, Lamprou M¹, Kastana P¹, Giannoutsou P², Barounis F², Mourkogianni E¹, **Orkoula M²**, Papadimitriou E¹

¹Laboratory of Molecular Pharmacology, Department of Pharmacy, School of Health Sciences, University of Patras, Rio, Patras, Greece, ²Laboratory of Instrumental Analysis, Department of Pharmacy, School of Health Sciences, University of Patras, Rio, Patras, Greece

Introduction: Protein tyrosine phosphatase receptor zeta 1 (PTPRZ1) belongs to the type V subfamily of receptor-type protein tyrosine phosphatases (RPTPs) and is primarily expressed in the brain, although it is also found in endothelial cells, cancer cells, and differentiated osteoblasts. A DNA microarray analysis of primary osteoblasts in vitro revealed that the Ptprz1 gene was the most significantly induced gene during differentiation and could be used as a marker for terminally differentiated osteoblasts. In the present work, we studied how PTPRZ1 expression and tyrosine phosphatase activity affect different functions of osteoblasts in vitro and bone formation and maintenance in vivo.

Methods: Primary calvaria osteoblasts were isolated from Ptprz1+/+ and Ptprz1-/- mice. Proliferation was studied by direct counting of cells. Osteoblast differentiation was estimated by ALP activity measurement using PNPP. Activation of signaling pathways was estimated by Western blot analysis or PLA. Mitochondria were visualized by Mitotracker staining. Gene expression was measured by RT-PCR. Bone mineralization was assessed employing Raman micro-spectroscopy. Three-dimensional bone and cartilage structures were visualized using X-ray micro-computed tomography.

Results: Ptprz1-/- osteoblasts demonstrated a slightly enhanced proliferation rate, significantly decreased differentiation potential, elongated mitochondria, increased activity of c-Src and ERK1/2 kinases, decreased activity of VEGFR2 and c-Met, and increased osteoprotegerin levels when compared to Ptprz1+/+ osteoblasts. Pharmacological inhibition of PTPRZ1 tyrosine phosphatase activity significantly decreased osteoblasts differentiation in Ptprz1+/+ but not in Ptprz1-/- osteoblasts. In vivo, bone maturation of the Ptprz1-/- mice occurred earlier (6 weeks) compared with the Ptprz1+/+ mice (12 weeks). Bone structure quality also declined earlier in Ptprz1-/- (36 weeks) compared with the Ptprz1+/+ mice (56 weeks) but ended up being the same at later stages.

Conclusions: Collectively, our data suggest that PTPRZ1 affects osteoblasts signaling and is involved in osteoblasts differentiation in vitro and the rate of bone maturation and remodeling in vivo. The latter may also be due to its known involvement in the regulation of angiogenesis. The potential to exploit our data therapeutically will be discussed.

Acknowledgments: The Ptprz1-/- mice were a generous gift from Drs. H. Himburg and J.P. Chute, UCLA, USA. The authors wish to thank the groups of Prof. Beatriz de Pascual-Teresa, Prof. Ana Ramos, and Prof. Gonzalo Herradon (Universidad San Pablo-CEU, Spain) for providing the selective PTPRZ1 tyrosine phosphatase inhibitor and the Advanced Light Microscopy facilities of the School of Health Sciences, University of Patras, for using the Leica SP5 confocal microscope.

PP205. Esculeoside A prevents retinal damage in diabetic rats by suppressing REDD1-dependent degradation of Nrf2

Alkhateeb M¹, Alsabaani N², Aldhaban W², Alshaikhli H¹

¹*Qatar University, Doha, Qatar*, ²*King Khalid University, Abha, King Khalid University*

Introduction: Esculeoside A (ESA) is a tomato-derived glycoside with antioxidant and anti-inflammatory properties. The protective effect of ESA against diabetic retinopathy is not well investigated and was the core objective of this study. In addition, we tested if such protection involves the activation of Nrf2 signaling.

Methods: Type 1 diabetes mellitus (T1DM) was induced in adult Wistar male rats by a single intraperitoneal injection of streptozotocin (STZ) (65 mg/kg). ESA was isolated using column chromatography. Non-diabetic and T1DM rats were divided into two subgroup groups given either the vehicle or ESA (100 mg/kg). An additional T1DM group was given ESA (100 mg/kg) and an Nrf2 inhibitor (2 mg/kg) (n=20 each). Treatments continued for 12 weeks. At the end of the treatment period, anesthesia was performed using a ketamine/xylazine mixture, and blood samples were collected for biochemical analysis of glucose, insulin, and lipids. The rats were then euthanized by cervical dislocation and their retinas were collected. Parts of the retinas were used for histological evaluation using hematoxylin and eosin. Other parts were used to prepare total and nuclear extracts which were then used to measure the levels of certain markers of oxidative stress, inflammation, and apoptosis. Other parts were used to isolate RNA, which was used to measure the expression levels of the nuclear factor erythroid factor-2 (Nrf2), the Kelch-like ECH-associated protein 1 (keap-1), and the stress response protein regulated in development and DNA damage 1 (REDD1) by real-time PCR.

Results: According to the histological features, ESA improved the structure of ganglionic cells, increased the number of cells in the inner nuclear and plexiform layers, and prevented intra-retinal bleeding in the retinas of T1DM rats. Concomitantly, it reduced the retinal levels of malondialdehyde (lipid peroxides), vascular endothelial growth factor, interleukin-6, tumor necrosis factor- α , Bax, and caspase-3. In the retinas of the control and diabetic rats, ESA boosted the levels of total glutathione, superoxide dismutase, heme-oxygenase-1, and Bcl2, reduced the mRNA levels of REDD1, and enhanced cytoplasmic and nuclear levels of Nrf2. However, ESA failed to alter the mRNA levels of Nrf2 and keap1, protein levels of keap1, plasma glucose, plasma insulin, serum triglycerides, cholesterol, and LDL-c in both the control and T1DM rats.

Conclusion: ESA alleviates retinopathy in T1DM rats by suppressing REDD1-associated degradation and inhibiting of Nrf2/antioxidant axis.

PP206. Inhibition of glucagon like peptide -1 signalization leads to brain region dependent changes in the energy metabolism and apoptosis

Osmanovic Barilar J¹, Kolaric Đ², Knezovic A¹, Babic Perhoc A¹, Homolak j¹, **Salkovic-Petrisic M¹**

¹*School of Medicine, University of Zagreb, Zagreb, Croatia*, ²*Medical University of Graz, Graz, Austria*

Introduction: The activation of the central GLP-1 receptors (GLP-1R) plays a role in neuroprotection but the exact signalization pathways of GLP-1 in the different brain region are still not fully understood. We aimed to determine changes in the hippocampal (HPC) and hypothalamus (HPT) expression of c-fos, insulin receptor (IR), extracellular signal-regulated kinase (ERK), phosphorylated form (p-ERK), ribosomal protein S6 kinase beta-1 (p70S6k) and protein kinase 1 (PDK1) after the intracerebroventricular (icv) administration of different doses of GLP-1R inhibitor exendin 9 (Ex9) in healthy rats.

Methods: Male Wistar rats were icv injected with Ex9 (60, 85, and 125 µg/kg) or vehicle (controls) and sacrificed 20 minutes after the treatment. Protein expression of c-fos, IR, ERK, pERK and p70S6k, PDK1 in HPC and HPT was measured by Western blot. Data were analysed by Kruskal-Wallis and Mann-Whitney U test ($p<0.05$).

Results: The dose of 60 µg/kg Ex 9 had no effects on the expression of investigated proteins in HPC but in HPT the decrement of ERK/pERK (-55%, $p<0.05$, vs CTRL) was seen. In the HPC the dose of 85µg/kg decreased the expression of c-fos (-58%) and IR (-56%) while the dose of 125µg/kg Ex9 increased the ratio of pERK/tERK (+85%) in comparison to control. In the HPT dose of 85 µg/kg increased the expression of PDK1 (+51%)

Conclusion: The icv administration of GLP-1R inhibitor Ex9 induces dose and region dependent changes in neuronal signalling pathways. Medium dose induced effects indicates a reduction of both energy metabolism and neuronal activation (decreased IR and c-fos respectively), while even higher inhibition of GLP-1R (125µg/kg of Ex9) seems to point to the increased apoptosis (increased pERK/tERK ratio) in HPT. Further research is needed to elucidate the dose and region dependent metabolic and apoptotic consequences of GLP-1R inhibition in the rat brain.

Supported by University of Zagreb, HRZZ-IP-2018-01-8938. Co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project “Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain”; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).

PP207. Neuroprotective effects of Psoralidin: potential mechanisms of action associated with PDE4A inhibition

Uzunhisarcıklı E¹

¹Erciyes University, Faculty of Pharmacy, Department of Pharmacology, Kayseri, Turkey

Background and Objectives: In this study, it was aimed to investigate the effect of Psoralidin, a natural phenolic coumarin compound, on MK-801-induced neurotoxicity that may cause Alzheimer's Disease and to determine the phosphodiesterase (PDE) - related molecular mechanism of action.

Methods: In this study, neurotoxicity was performed using the MK-801 in HT-22 cell line. The effects of compounds on the proliferation of HT-22 cells was determined by Real-time cell analysis (RTCA). After total protein concentration was measured using the BCA protein assay kit then PDE4A protein level was determined by western blot method. Sensitized chemiluminescent substrate based ECL reagents were used for reactive band visualization under the imaging system. The intensities of the bands were calculated with ImageJ 1.42 q software.

Results: Psoralidin (100, 200, 400 μ M) has been indicated to has a neuroprotective effect in MK-801 induced neurotoxicity by the Real Time Cell Analysis. In HT-22 cells, EC50 value of Psoralidin was calculated to be 230.4 μ M, IC50 value of MK-801 was calculated to be 62.4 μ M at 24 hours. It has been determined that Psoralidin (200, 400 μ M) inhibits PDE4A by western blot method.

Conclusion: As a result of this research, it was found that Psoralidin, has neuroprotective effects in MK801-associated accumulation of the excitatory amino acid glutamate neurodegeneration and Alzheimer's disease.

PP208. Diuretic activity of crude ethanol and saponin-rich extracts of *Solanum sisymbriifolium* Lam. in rats

Arrúa Báez W¹, Ibarrola D¹, Hellón-Ibarrola M¹, Duarte Santa Cruz J¹

¹*Universidad Nacional de Asunción, San Lorenzo, Paraguay*

Introduction: *Solanum sisymbriifolium* Lam. is used in Paraguayan traditional medicine for its diuretic and antihypertensive activities [1]. For this reason, the study aimed to evaluate the effect of acute oral administration of doses of the ethanol and saponin-rich extracts obtained from the root of *S. sisymbriifolium* Lam. on the diuresis profile of rats.

Methods: Both extracts were prepared using maceration. The saponins-rich extract was obtained using a selective liquid-liquid extraction method with organic solvents. The extracts used were characterized by phytochemical analysis and high-performance liquid chromatography. Male Wistar rats were randomly distributed in six groups (six animals each), which received single doses orally of saline solution 0.1mL/100g of body weight; 20mg/kg of furosemide; 50 and 100mg/kg of ethanolic extract of *S. sisymbriifolium*; and 1 and 10mg/Kg of saponins-rich extract of *S. sisymbriifolium*. Animals were placed in metabolic cages individually for 24 hours. The urine output, electrolyte concentration, pH, and glomerular filtration rate were measured. Additionally, extracts' saluretic, natriuretic, and carbonic anhydrase inhibition indexes were calculated [2]. Data were analyzed by one-way ANOVA followed by Dunnett's test. The work protocol was approved by the Institutional Ethics Committee according to CEI 869/2022.

Results: The purified saponin was analyzed by UPLC-ESI-MS in full scan mode. Nuatigenoside was identified by its protonated molecular ion $[M+H]^+$ with m/z 885.895. The findings indicated that both doses of ethanol and saponin-rich extracts of *S. sisymbriifolium* significantly increase diuresis after 24 hours of treatment compared with the control group. Urine volumes of 17.05 ± 4.31 and 18.20 ± 5.57 mL were obtained with the highest doses of the extracts, respectively. Na^+ and Cl^- urinary excretion was also significantly increased with all doses used. Were recorded at 1.23 ± 0.24 and 1.02 ± 0.38 mEq of urinary Na^+ ; and 2.60 ± 0.26 and 2.11 ± 0.71 mEq of urinary Cl^- with the highest doses of the ethanolic and saponin-rich extracts, respectively. The calculations indicated an increase in natriuretic, saluretic and carbonic anhydrase inhibitory activities with the administration of the extracts. Urinary pH was not affected by treatment. On the other hand, a significant increase in the glomerular filtration rate was evidenced.

Conclusion: This study confirms the diuretic activity of *S. sisymbriifolium* Lam. Both the ethanolic and saponin-rich extracts (nuatigenoside) presented natriuretic and saluretic effects with a possible mechanism of action mediated, at least partially, by the inhibition of carbonic anhydrase.

[1] Ibarrola DA, J Ethnopharmacol., v.298, pag.115605, 2022

[2] Hakim EM, J Ethnopharmacol., v.273, pag.113942, 2021

PP209. Assessment of sun protecting factor and in vitro cytotoxicity of methanolic pulp extracts from Serbian *Cucurbita maxima* in human keratinocytes

Dekanski D¹, Jovanović A¹, Miljić M^{2,3}, Krstić S⁴, Pirković A¹

¹*University of Belgrade, Institute for the Application of Nuclear Energy-INEP, Belgrade, Serbia,*

²*University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia,* ³*Institute of Food Technology (FINS), Novi Sad, Serbia,*

⁴*University of Graz, Institute of Pharmaceutical Sciences, Graz, Austria*

Ultraviolet radiation from sun exposure causes harmful skin effects, such as dermal photoaging and DNA damage, mediated by oxidative stress. Pumpkin (*Cucurbita maxima*) is abundant in carotenoids, polyphenols, and tocopherols which have antioxidant ability and protect cells from damage making them attractive as potential natural photo-protectants for skin applications. Pumpkin pulp, considered as by-product of the food industry, is the least explored in terms of its biological activities, although it contains considerable amounts of bioactive compounds. The aim of this study was to evaluate in vitro sun protecting factor (SPF) and cytotoxicity of methanolic pumpkin pulp extracts (MPE). They were prepared from the material of 4 accessions MAX 113, MAX 118-1, MAX 117, and MAX 1 from the breeding collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. These accessions were selected according to the highest carotenoid content and antioxidant capacity. The effect of MPE on human keratinocytes (HaCaT) viability was determined by crystal violet assay. In vitro SPF based on the Mansur equation and the absorbance measurements was chosen to screen the photoprotective potential of MPE. The results show that SPF values for extracts MAX 113, MAX 118-1, MAX 117, and MAX 1 were 2.351, 1.875, 4.573, and 3.812, respectively. The most pronounced in vitro SPF values were in extracts MAX 117 and MAX 1 which were previously shown to contain the high amounts of carotenoids zeaxanthin and β -carotene. In vitro study of cell viability in keratinocytes showed that MAX 118-1, MAX 117, and MAX 1 did not reduce the number of viable cells up to the concentration of 1000 $\mu\text{g}/\text{mL}$ and thus might be considered as non-toxic. Among the analyzed extracts, extract MAX 113 reduced HaCaT cells viability after 24 h incubation in a concentration-dependent manner, where the highest concentration of 1000 $\mu\text{g}/\text{mL}$ significantly reduced number of viable cells compared to the non-treated control. On the other hand, the treatment of keratinocytes using extract MAX 117 led to a significant increase in the number of viable cells at 1000 $\mu\text{g}/\text{mL}$ concentration. That was the same extract that exhibited the highest SPF value. These data demonstrate that bioactive compounds from MPE could have potential as anti-photoaging agents and can be considered as non-toxic for the skin cells. Additionally, findings suggest MPE of *C. maxima* Duchesne cultivar MAX 117 from Serbian accession has the best photoprotective potential and could be useful as component in natural cosmetic products.

PP210. Oleuropein treatment attenuates ROS generation, inflammation, and H₂O₂-induced damage in human trophoblast cells

Pirković A¹, Vilotić A¹, Nacka-Aleksić M¹, Bojić-Trbojević Ž¹, Jovanović Krivokuća M¹, **Dekanski D¹**

¹*University of Belgrade, Institute for the Application of Nuclear Energy-INEP, Belgrade, Serbia*

Higher energy demands during pregnancy lead to enhanced oxidative phosphorylation and increased production of reactive oxygen species (ROS) which have been shown to act as important regulators of angiogenesis, proliferation, differentiation and invasion of placental cells - trophoblast, autophagy and other important physiological processes and tissue adaptations. This controlled placental oxidative stress and inflammatory response are essential for successful early pregnancy. However, unbalanced, excessive ROS production has adverse effects on pregnancy outcome, causing trophoblast cell damage and dysfunction. Antioxidant supplements have been proposed as a possible approach in the prevention and treatment of such disorders. Secoiridoid oleuropein (OLE) is the most abundant phenolic compound found in olive leaves and drupes and has been shown to display profound antioxidant and anti-inflammatory activities. Its effects on trophoblast cells remain unexplored. The aim of our study was to investigate the impact of OLE on HTR-8/SVneo extravillous trophoblast cell line against hydrogen peroxide (H₂O₂)-induced oxidative damage. Results have shown that treatment with OLE at selected concentrations (10 and 100 µM) for 24h prevented a decrease in cell viability compromised by H₂O₂. Levels of lipid peroxidation indicators (MDA and LDH) were markedly reduced, and protein carbonylation and nitrosilation were significantly attenuated in trophoblast cells treated with OLE compared to untreated control cells. Further, OLE treatment decreased intracellular ROS production at both concentrations and significantly reduced the activity of antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase as well as restored glutathione levels in H₂O₂-exposed HTR-8/SVneo cells. OLE reduced the protein expression of inflammatory factor iNOS and decreased mRNA expression of pro-inflammatory cytokines IL-6 and TNFα in H₂O₂-treated cells. Our results indicate that OLE may ameliorate cellular oxidative damage, reduce inflammation and increase cellular antioxidant capacity in human trophoblast cells. It should also be emphasized that this olive-derived bioactive compound per se did not lead to any adverse effects in HTR-8/SVneo trophoblast cells under the described conditions, confirming its safety in vitro.

PP211. Fumaria officinalis L. as a novel source of biologically active compounds

Dekanski D¹, Pirković A¹, Jovanović A¹

¹*University of Belgrade, Institute for the Application of Nuclear Energy-INEP, Belgrade, Serbia*

Fumaria officinalis L. (Fumariaceae) is a scrambling annual plant, distributed and cultivated throughout Europe, and represents a component of various phytotherapeutic formulations in the European ethnobotany used in hepatobiliary dysfunction, illnesses of gastrointestinal and urogenital tracts, cancer, rheumatism, high blood pressure, and skin disorders.

The study aimed to determine the total polyphenol and flavonoid contents (TPC and TFC, respectively) in *F. officinalis* extracts prepared using maceration and heat-assisted extraction (HAE), as well as to investigate their cytotoxicity on human keratinocyte cells (HaCaT), antioxidant potential, and spectrophotometric sun protecting factor (SPFs).

The influence of extracts on the viability of keratinocytes was investigated in the MTT assay, while antioxidant capacity was tested using ABTS and DPPH assays. SPFs value was calculated using the Mansur equation and the absorbance measurements (290-320 nm) with the aim of expressing the photoprotective activity of the extracts.

The polyphenol concentration in lyophilized extracts was 165.5 ± 1.1 mg/g (maceration) and 171.8 ± 1.4 mg/g (HAE), whereas the flavonoid content amounted to 71.6 ± 1.9 mg/g (maceration) and 80.4 ± 1.6 mg/g (HAE). The results show that ABTS and DPPH radical scavenging capacity of lyophilized extracts was 11.5 ± 1.0 mmol Trolox/g and IC₅₀DPPH 0.36 ± 0.03 mg/mL (maceration) and 12.9 ± 0.8 mmol Trolox/g and IC₅₀DPPH 0.28 ± 0.02 mg/mL (HAE). SPFs values for the extracts at the concentration of 100 µg/mL were 1.162 ± 0.005 (maceration) and 1.255 ± 0.015 (HAE). The higher antioxidant potential and SPFs of HAE extracts can be explained by the higher polyphenol and flavonoid contents. In vitro cytotoxicity assay in HaCaT cells showed that both types of extracts at all tested concentrations (25-100 µg/mL) did not reduce the number of viable cells. Additionally, HAE extract significantly increased the number of viable keratinocytes at the concentration of 100 µg/mL.

The presented study provides evidence of the polyphenol and flavonoid yields in *F. officinalis* extracts and their biological/pharmacological potential. Due to its proven antioxidant potential and positive impact on human keratinocytes, HAE extract can add value and/or improve the quality of the existing pharmaceutical and cosmetic products for the skin.

PP212. Ircinia ramosa Sponge Extract (iSP) Induces Apoptosis in Human Melanoma Cells and Inhibits Melanoma Cell Migration and Invasiveness

Maresca D¹, Romano B¹, Somma F¹, Chianese G¹, Formisano C¹, Ianaro A¹, Ercolano G¹

¹*Università degli Studi di Napoli "Federico II", Napoli, Italy*

Introduction: Melanoma is considered the most lethal skin cancer worldwide, and it rapidly penetrates deep layers of the skin and infiltrates contiguous tissues, leading to metastases development. Many patients show resistance to the current therapies and experience tumor recurrence, with a fatal prognosis [1]. Marine compounds represent a varied source of new drugs with potential anticancer effects [2]. Among these, sponges, including those belonging to the Irciniidae family, demonstrated to exert cytotoxic effects on different cancer cell lines [3]. Here, we investigated the therapeutic effect of an extract (referred as iSP) from the sponge *Ircinia ramosa* on human melanoma cells.

Methods: The metabolic profile of the iSP was evaluated by HPLC-MS analysis. The antiproliferative effect and the IC50 value of iSP was defined by MTT assay by using concentration from 0-100µg/mL for 48h. The contribution of iSP on melanoma cancer cell apoptosis and cell cycle arrest has been investigated by both flow cytometry and qPCR analysis (30µg/mL, respectively for 48h and 24h). Likewise, the expression of the epithelial to mesenchymal transition (EMT) markers (1µg/mL for 48h) while the ROS production and mitochondrial parameters (30µg/mL for 24h) were assessed by flow cytometry. Finally, different in vitro assays were performed to evaluate the capacity of iSP to modulate melanoma cells migration, invasion and clonogenic abilities (1µg/mL for 48h).

Results: The LC-MS analysis showed the presence of different metabolites including glycerides, sterols and terpenoids. iSP affected A375 proliferation rate, promoted apoptosis and induced cell cycle arrest. In addition, iSP modulated the expression of cadherins and the EMT-related transcription factors and induced ROS production. Finally, iSP inhibited melanoma cell migration, colony formation and invasion.

Conclusions: Collectively, this study provides the first evidence to support the role of *Ircinia ramosa* sponge extracts as a potential therapeutic resource for the treatment of human melanoma.

- [1] Pires da Silva I, Lo S, Quek C, et al. Site-specific response patterns, pseudoprogression, and acquired resistance in patients with melanoma treated with ipilimumab combined with anti-PD-1 therapy. *Cancer*. 2020;126(1):86-97. doi:10.1002/cncr.32522
- [2] Ercolano G, De Cicco P, Ianaro A. New Drugs from the Sea: Pro-Apoptotic Activity of Sponges and Algae Derived Compounds. *Mar Drugs*. 2019;17(1):31. Published 2019 Jan 7. doi:10.3390/md17010031
- [3] Calcabrini C, Catanzaro E, Bishayee A, Turrini E, Fimognari C. Marine Sponge Natural Products with Anticancer Potential: An Updated Review. *Mar Drugs*. 2017;15(10):310. Published 2017 Oct 13. doi:10.3390/md15100310

PP213. Cafeteria diet abstinence induces depression and disrupts endocannabinoid signaling in dopaminergic areas: a preclinical study

Friuli M¹, de Ceglia M^{1,2}, Romano A¹, Micioni Di Bonaventura M³, Gavito A², Botticelli L³, Micioni Di Bonaventura E³, Cifani C³, Rodriguez de Fonseca F², Gaetani S¹

¹*Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy*, ²*UGC de Salud Mental y Unidad Clínica de Neurología, Grupo de Neuropsicofarmacología, Instituto de Investigación Biomédica de Málaga (IBIMA), Universidad de Málaga-Hospital Universitario Regional de Málaga, Malaga, Spain*, ³*School of Pharmacy, Pharmacology Unit, University of Camerino, Camerino, Italy*

Obesity is a polygenic and multifactorial condition that represents a very concerning public health issue affecting both developing and developed countries [1]. It is characterized by alterations in dopamine (DA) transmission in brain reward system leading to the establishment of an addicted-like state, defined as food addiction (FA). FA is associated with mood disorders, including depression, sustained by alterations of DA signalling [2]. The endocannabinoid system (ECS) is a major regulator of DA activity, and it has been investigated as a main target involved in processes regulating obesity, addiction, and depression [3]. In the present study we investigated the potential treatment of FA-associated comorbidities improving DA transmission through the pharmacological inhibition of the fatty acid amine hydrolase (FAAH) enzyme, by PF-3845 treatment which enhances endogenous cannabinoid and paracannabinoid signalling.

Adult male Wistar rats were exposed (40 days) and then abstained (4 weeks) from a palatable cafeteria diet. During abstinence, animals were treated every other day either with vehicle (ethanol/tween 80/saline in a proportion 5/5/90 v/v/v) or with PF-3845 (10 mg/kg; intraperitoneal administration). At the end of the treatment rats underwent a behavioral test for the evaluation of depressive-like behavior; the brains were extracted and analyzed for monoamine levels and markers of the endocannabinoid system by using high-performance liquid chromatography and western blot analyses respectively.

Four weeks after the withdrawal of palatable diet, animals displayed depressive-like behavior, coupled to significant variations in the concentration of brain monoamine and in the expression of proteins of endocannabinoid signalling machinery in DA-enriched brain regions including ventral tegmental area, dorsolateral striatum, substantia nigra and medial prefrontal cortex. Treatment with PF-3845 exerted an antidepressant-like effect and restored part of the alterations in monoaminergic and endocannabinoid systems.

Overall, our results suggest that abstinence from cafeteria diet provokes emotional disturbances linked to neuroadaptive changes in monoamines and endocannabinoid signalling in brain areas partaking to DA transmission. These diet-imposed neuroadaptations can be partially restored by the enhancement of endocannabinoid signalling through FAAH inhibition.

- [1] Kopelman PG. Obesity as a medical problem. *Nature* 2000. doi: 10.1038/35007508
- [2] Mills JG, Thomas SJ, Larkin TA, Deng C. Overeating and food addiction in Major Depressive Disorder: Links to peripheral dopamine. *Appetite* 2020. doi: 10.1016/j.appet.2020.104586
- [3] de Ceglia M, Decara J, Gaetani S, Rodríguez de Fonseca F. Obesity as a Condition Determined by Food Addiction: Should Brain Endocannabinoid System Alterations Be the Cause and Its Modulation the Solution? *Pharmaceuticals (Basel)* 2021. doi: 10.3390/ph14101002

PP214. Cytotoxic Effect of Apitoxin and Melittin in Bladder Cancer Cells: Investigation of their Antitumoral Potential

Poiazo G¹, Almeida T¹, **Boaventura De Oliveira A¹**, Marques Porto R¹, Nicoli da Silva G², Picolo G¹

¹*Butantan Institute, São Paulo, Brazil*, ²*Federal University of Ouro Preto, Ouro Preto, Brazil*

Bladder cancer ranks as the 11th most commonly diagnosed cancer globally, with two primary variations: non-muscle-invasive and muscle-invasive, the latter being the most aggressive form. Current treatments significantly impact the patient's quality of life, in addition to a high risk of cancer recurrence and toxicity. Given these circumstances, to explore new antineoplastic treatment strategies is essential. Over the years, natural products have become a valuable source of bioactive compounds for discovering new drugs. Melittin, an amphiphilic peptide, stands out as the main component of the *Apis mellifera* bee venom. It has demonstrated promising pharmacological properties, including antitumor effects against different types of cancer. Thus, the aim of this study was to investigate the cytotoxic potential of melittin and its underlying mechanisms of action in human bladder carcinoma cells. Venom of *Apis mellifera* was obtained through electric stimulation extraction (Brapi-Exp company, Guarapari, Brazil). Melittin was isolated using high-performance liquid chromatography. Cell viability of UM-UC-3 and T24 bladder cancer cells was assessed using the MTT assay after exposure to different concentrations of apitoxin (1.25-10 µg/ml) or melittin (1.25-10 µg/ml) for 24, 48, and 72 hours [1]. The next assays were performed exclusively with the UM-UC-3 cell line. The antimetastatic potential of melittin was evaluated using wound healing and Transwell assays [2]. To long-term effects of melittin evaluation, clonogenic survival assay was conducted [2]. The impact of melittin on the cell cycle progression and the confirmation of cell death caused by the treatments were analyzed by flow cytometry [1,2]. All experiments used n=3/triplicate, p<0,05. Data demonstrated that apitoxin and melittin reduced cell viability in the tested tumor cell lines, with melittin exhibiting higher cytotoxicity. Specifically in UM-UC-3 cells, melittin reduced cell migration (45% and 75% for treatments with 3.57 and 4.0 µg/ml, respectively), cellular invasion (12% and 47%) and long-term survival. Moreover, melittin induced cell cycle arrest, preventing cells from entering mitosis, and inducing cell death through apoptosis and necrosis. These findings confirmed the antitumoral potential of melittin in human bladder cancer and encourage the exploration of the mechanisms involved in this effect.

[1] Kong GM, Tao WH, Diao YL, et al. Melittin induces human gastric cancer cell apoptosis via activation of mitochondrial pathway. *World J Gastroenterol.*, doi:10.3748/wjg.v22.i11.3186

[2] Pereira IOA, da Silva GN, Almeida TC, et al. LncRNA JHDM1D-AS1 Is a Key Biomarker for Progression and Modulation of Gemcitabine Sensitivity in Bladder Cancer Cells. *Molecules*; <https://doi.org/10.3390/molecules28052412>

PP215. Early and Late Pharmacoacupuncture with Apitoxin or Melittin Induced Prolonged Analgesia and Reduced Neuroinflammation Markers in Rat Neuropathic Pain Model

Boaventura De Oliveira A¹, Silva D¹, Sant'anna M¹, Almeida T¹, Assis G¹, Marques-Porto R², Silva J³, Picolo G¹

¹Laboratory of Pain and Signaling, Butantan Institute, São Paulo, Brazil, ²Laboratory of Development and Innovation, Butantan Institute, São Paulo, Brazil, ³Laboratory of Neuroscience, Neuromodulation and Study of Pain, Federal University of Alfenas, Alfenas, Brazil

Introduction: Chronic pain is a complex condition that affects approximately 20% of the global population, making it a significant public health concern. Among the different forms of chronic pain, neuropathic pain, which arises from damage of somatosensory system, is particularly challenging to manage. Despite the availability of pharmacological treatments, none of them offers complete relief, and most of them induce undesirable effects after chronic administration. One such alternative approach is the use of integrative and complementary practices like acupuncture. Additionally, the practice of pharmacoacupuncture, involving the application of drugs at acupoints, has gained attention for its potential to amplify the effects of medications and toxins, such as Apitoxin. However, limited research has explored its effectiveness. Our aim was to assess the analgesic effect of apitoxin injected at the E36 acupoint for the control of neuropathic pain and whether Melittin, the major component in apitoxin, could promote analgesia. We also explored the ability of both toxins to control and prevent the development of chronic pain.

Methods: Neuropathy was induced in Wistar male rats (CEUA 7835170522) by the chronic constriction of sciatic nerve (1). On the 1st or 15th day after surgery pharmacoacupuncture was performed with either Apitoxin or Melittin in a single application to determine its lasting or in repeated applications after neuropathy onset, to assess its preventive potential. Allodynia was evaluated before and after the treatment (N=6 per group). Tissue samples were collected on the 19th or 20th day to analyze the expression of ATF3, IBA-1, and GFAP markers, which play a crucial role in neuroinflammation (N=6 per group).

Results: Pharmacoacupuncture with Apitoxin or Melittin increased in 71,28% the nociceptive threshold of the animals, whether applied early or later periods ($p<0,0001$ N=6). Remarkably, the analgesia was longer lasting than traditional acupuncture or the application of Apitoxin or Melittin outside of acupoints. Furthermore, treatment led to a reduction of 80% in the expression of ATF3, GFAP, and IBA-1 proteins ($p<0,0058$ N=6).

Conclusion: Pharmacoacupuncture with Apitoxin or Melittin emerges as an attractive therapy for chronic pain control, owing to its efficacy and prolonged duration of pain relief and to its ability to reduce important markers of inflammation at the central nervous system.

- [1] Bennett, GJ; Xie, Y.-K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33(1):p 87-107, April 1988. | DOI: 10.1016/0304-3959(88)90209-6

PP216. Antiseizure medication and direct oral anticoagulants: overview

Cancellerini C¹, Esposito R², Caravelli A², Vignatelli L², Licchetta L², Bisulli F^{1,2}

¹*Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy*, ²*IRCCS Istituto delle Scienze Neurologiche di Bologna, Full member of the European Reference Network EpiCARE, Bologna, Italy*

Introduction: Antiseizure medications (ASMs) are primary drugs for treating persons with epilepsy (PWE) to prevent epileptic seizures[1]. In PWE, it is not rare that ASMs are concomitantly prescribed with direct-oral-anticoagulants (DOACs). Post-stroke epilepsy (PSE) is a common cause of seizures in older adults (3-25%) and it requires chronic ASMs-DOACs treatment to prevent thromboembolism, particularly if the stroke is due to atrial fibrillation (AF)[2]. ASMs can affect DOACs metabolism, posing risks of bleeding or reduced antithrombotic efficacy. Major interactions involve cytochrome (CYP) P450 induction or inhibition and P-glycoprotein (P-gp) activity, making the ASMs-DOACs combination clinically concern[2].

Method: We used the PubMed database to find clinical series and case reports concerning specific interactions between ASMs (n=24) and DOACs (n=4).

Results: ASMs such as carbamazepine (CBZ), phenytoin (PHT), phenobarbital (PB), primidone (PRM), valproate (VPA), oxcarbazepine (OXC), and topiramate (TPM) are both CYP and/or P-gp inducers. The interaction between these ASMs and rivaroxaban or dabigatran are the most cited result in decreased DOACs levels, potentially compromising anticoagulant effectiveness. Regarding apixaban two interaction cases were found with CBZ. Levetiracetam (LEV) with potential induction of CYP and P-gp, two cases reported interaction with rivaroxaban and a case with dabigatran was found. Moreover, PWE follow-up dates from these case reports are lacking.

Conclusion: The European Society of Cardiology published in 2021 a practical guide for DOACs management in patients with AF[3]. They advise against co-administration of CBZ, PB, PHT, and VPA with DOACs and recommend caution with LEV, OXC, and TPM also considering renal function, age, and comorbidities. Indeed, the decreases in plasma level of DOACs co-administrated with LEV have an unclear mechanism. It would also be interesting to evaluate possible DDIs of brivaracetam, belonging to the same family as LEV and perampanel whose use in clinical practice is increasing in patients with multiple comorbidities. The co-administration of DOACs with inductor ASMs poses significant risks of reduced anticoagulant efficacy. Given this evidence, additional DDI trials are needed, and DOACs therapeutic drug monitoring may guide in ASMs therapy management.

- [1] Hanaya R, et al. The New Antiepileptic Drugs: Their Neuropharmacology and Clinical Indications. *Neurol Med Chir (Tokyo)*. 2016;56(5):205-220.
- [2] Galgani A, et al. Pharmacokinetic Interactions of Clinical Interest Between Direct Oral Anticoagulants and Antiepileptic Drugs. *Front Neurol*. 2018;9(December):1-10.
- [3] Steffel J, et al. 2021 European Heart Rhythm Association Practical Guide on the Use of Non-Vitamin K Antagonist Oral Anticoagulants in Patients with Atrial Fibrillation. *Europace*. 2021 Oct 9;23(10):1612-1676.

PP217. Tanshinones: promising lead compounds for treating various diseases

Chen X¹

¹*University of Macau, Macao, China*

Tanshinones are the major lipid-soluble ingredients isolated from *Salvia Miltiorrhiza* Bunge (Danshen), a famous traditional Chinese herb used in China for thousands of years. Chemically, tanshinones are a group of compounds belonging to abietane diterpene. To date, more than 60 tanshinones have been identified from Danshen and related species, but only a few of them have been systematically evaluated. Among the most studied tanshinones are tanshinone IIA and cryptotanshinone. The former has been approved in China for the treatment of cardiovascular disease. Accumulated data show that tanshinones exhibit significant biological activities, including anti-inflammatory activity, cardiovascular effects, and anticancer activity, etc. Our laboratory has focused on the pharmacological evaluation of tanshinones for more than 10 years. Our results showed that total tanshinones exhibit both anti-inflammatory and anticancer effects mediated by blocking TLR4 dimerization and reactive oxygen species (ROS), respectively. For the anti-inflammatory effect, we found that neocryptotanshinone and dihydronortanshinone exhibited significant anti-inflammatory activity in LPS-stimulated RAW264.7 macrophages. Cryptotanshinone and danshenol A inhibit TNF- α /ox-LDL-induced endothelial inflammation. Dihydrotanshinone I and cryptotanshinone ameliorate DSS-induced experimental ulcerative colitis in mice. In particular, dihydrotanshinone I shows significant anti-atherosclerotic effect by decreasing plasma lipid levels, protecting endothelial cells, inhibiting atherosclerosis formation, attenuating plaque vulnerability possibly through various mechanisms. It also protects against ischemic stroke by inhibiting ferroptosis. For anticancer activity, isocryptotanshinone is a potent STAT3 inhibitor, while tanshinol A significantly activates MLKL. Interestingly, both cryptotanshinone and tanshindiol B induces NQO1-mediated necrosis in cancer cells but show different effect on NQO1. Collectively, our results showed that tanshinones exhibit significant pharmacological effects and may be the promising lead compounds for the treatment of colitis, atherosclerosis, and cancer.

Acknowledgments: This work was supported by the Science and Technology Development Fund, Macao S.A.R (FDCT) (0070/2022/A2, 0081/2021/A2, SKLQRCM(UM)-2023-2025), the Research Fund of University of Macau (MYRG-GRG2023-00072-ICMS-UMDF), and the Ministry of Education Frontiers Science Centre for Precision Oncology, University of Macau (FSCPO).

PP218. RGH-397 inhibits the function of recombinant and native α 5 GABAA receptors and shows efficacy in animal models of cognitive impairment

Bódi V¹, Thán M¹, Fodor L¹, Bobok A¹, Visegrády A¹, Soukupné Kedves R¹, Kapus G¹

¹Gedeon Richter Plc., Budapest, Hungary

Excessive activity of α 5 subunit containing GABAA receptors in the hippocampus might play an important role in cognitive impairments and memory deficits. Selective reduction of the function of α 5 GABAAR could restore the normal neuronal activity and alleviate the symptoms. Chemical optimization of novel naphthyridine derivatives resulted in 1-(2-{[3-(4-fluorophenyl)-5-methyl-1,2-oxazol-4-yl]-methoxy}-5,6,7,8-tetrahydro-1,6-naphthyridin-6-yl)-2-methanesulfonylethan-1-one (RGH-397) a subtype-selective negative allosteric modulator (NAM) of α 5 GABAARs [1]. The biological characterisation of this molecule is presented here.

Displacement assays on human α 5 β 3 γ 2, α 1 β 3 γ 2 and α 2 β 3 γ 2 GABAARs were performed using the allosteric radioligand [³H]Ro15-1788. Receptor function was investigated using automated and manual whole-cell patch clamp in human α 1 β 3 γ 2, α 2 β 3 γ 2 and α 5 β 3 γ 2 GABAAR-expressing HEK-293 cell lines. Additionally, conventional voltage clamp experiments were carried out to measure the inhibition of tonic GABAA currents in rat hippocampal brain slices. The compound was tested at concentrations ranging from 10 nM to 10 μ M. Finally, RGH-397 (1, 3 and 10 mg/kg) was tested in two models of cognitive impairment in schizophrenia, the phencyclidine (PCP) (2 mg/kg, i. p. or 10 mg/kg s.c.) induced impairment of novel object recognition (NOR) and social recognition (SI) in rats.

RGH-397 had high affinity to human α 5 GABAARs and moderate affinity to α 1 and α 2 GABAARs (Ki values of 4.1, 221 and 161 nM (all n = 3), respectively. The compound inhibited α 5 GABAAR-mediated current with an IC₅₀ of 330 nM and an efficacy of 41% with relatively fast on-kinetics and slow off-kinetics when compared to the reference α 5 NAM compound basmisanil. RGH-397 showed no significant inhibition of α 1 and α 2 GABAAR currents up to 30 μ M. In hippocampal interneurons RGH-397 partially blocked the α 5 GABAAR-mediated tonic currents in rat brain slices at the concentration of 100 nM and above (all n = 83). In the cognitive models RGH-397 reversed the PCP-induced cognitive deficit at the effective doses of 3 mg/kg (SI) and 10 mg/kg (NOR).

RGH-397 is a potent and selective NAM of α 5 GABAARs. The compound inhibited tonic current at the minimal effective concentration of 100 nM which is in accordance with its inhibitory potency on human recombinant α 5 β 3 γ 2 GABAARs. Altogether, the efficacy of RGH-397 was proven on human recombinant receptors, in rat brain tissue and in animal models of cognitive impairment as well.

[1] Szabó G, Éliás O, Erdélyi P, et al. Multiparameter optimization of naphthyridine derivatives as selective α 5-GABAA receptor negative allosteric modulators. J Med Chem. 2022; 65: 7876–7895

PP219. Investigation of the effects of pregabalin on experimental schizophrenia models

Kilic Tatlici C¹, Kaygisiz B¹, Yazici Z¹, Erdogan Erden E¹, Ay H², Bal C³, Yildirim E¹, Kilic F¹

¹Eskisehir Osmangazi University Faculty of Medicine Department of Medical Pharmacology, Eskisehir, Turkey, ²Eskisehir Osmangazi University Faculty of Medicine Department of Anatomy, Eskisehir, Turkey, ³Eskisehir Osmangazi University Faculty of Medicine Department of Biostatistics, Eskisehir, Turkey

Introduction: Increased dopaminergic and decreased glutamatergic neurotransmissions have been suggested as the main hypotheses in schizophrenia pathophysiology. However, recent data suggests the role of excessive glutamatergic transmission[1]. Also, it has been suggested that disorders of dopaminergic and glutamatergic transmission in prefrontal cortex(PFC), nucleus accumbens(NA) and hippocampus(HC) contribute to pathophysiology of schizophrenia[2]. Pregabalin is approved for epilepsy, postherpetic neuralgia, diabetic neuropathy, fibromyalgia and acts by binding to alpha-2-delta subunit of voltage-gated calcium channels located presynaptically in central nervous system and suggested to reduce the synaptic release of excitatory neurotransmitters[3].

We aimed to investigate the effects of acute pregabalin administration on experimental schizophrenia models and brain dopamine and glutamate levels in rats.

Materials-Methods: MK-801(0.3mg/kg;i.p.)(nonspecific antagonist of glutamate NMDA receptors)-induced hyperlocomotion and disruption of PPI of the acoustic startle reflex were used as experimental schizophrenia models in Wistar-albino male rats(200-250g). Pregabalin(30,100mg/kg;i.p.) were administered acutely 1 hour before the tests. The effect of pregabalin was compared with the antipsychotics haloperidol(1mg/kg;i.p.) and olanzapine(3mg/kg;i.p.). Dopamine and glutamate levels in PFC, NA and HC were detected by ELISA. Statistical analysis were performed by using One-way ANOVA.

Results: In hyperlocomotion test, MK-801 significantly increased the distance traveled compared to control($p<0.01$). Pregabalin(30,100mg/kg), haloperidol and olanzapine significantly reduced the distance traveled compared to MK-801, similarly($p=0.001$; $p<0.001$; $p<0.001$; $p<0.01$ respectively). In PPI test, MK-801 significantly reduced PPI% at prepulse levels of 74,78,86dB compared to control($p<0.05$; $p<0.01$; $p<0.01$, respectively). Pregabalin(30,100mg/kg) and haloperidol significantly increased PPI% compared to MK-801, at 86dB($p<0.05$) similarly. Olanzapine increased PPI% compared to MK-801 at 86dB, however, this wasn't statistically significant($p>0.05$). Pregabalin(30,100mg/kg) didn't alter dopamine levels in PFC and NA while pregabalin 100mg/kg reduced dopamine levels in HC compared to control. There was no significant difference between groups in glutamate levels in PFC and NA while pregabalin 100mg/kg significantly reduced glutamate levels compared to control in HC.

Conclusions: We suggest that pregabalin may have an antipsychotic-like effect mediated by glutamatergic transmission. Further studies are needed to elucidate the exact mechanism of this action in terms of dopaminergic and glutamatergic interaction.

This study was supported by Eskişehir Osmangazi University Scientific Research Projects Coordination Unit(Project number:2586;Ethical approval number:902-1)

- [1] Yang AC, Tsai SJ. New Targets for Schizophrenia Treatment beyond the Dopamine Hypothesis. *Int J Mol Sci.* 2017;18(8):1689. Published 2017 Aug 3. doi:10.3390/ijms18081689
- [2] Grace AA. Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. *Nat Rev Neurosci.* 2016;17(8):524-532. doi:10.1038/nrn.2016.57
- [3] Cross AL, Viswanath O, Sherman AL. Pregabalin. In: StatPearls. Treasure Island (FL): StatPearls Publishing; November 14, 2022.

PP220. The inflammatory ATP-enriched environment drives microglia towards an m1-like phenotype that inhibits astrogial proliferation

Quintas C^{1,2}, Fernandez-Llimos F^{1,2}, Gonçalves J^{1,2}, **Queiroz G^{1,2}**

¹Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal, ²UCIBIO—Applied Molecular Biosciences Unit - Porto, Associate Laboratory i4HB, Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal

Astrocytes and microglia coordinate the inflammatory response associated with most of CNS pathologies. In the context of inflammation, damaged cells release ATP, activating P2 receptors in both astrocytes and microglia, thereby facilitating intercellular communication.[1] Within this ATP-enriched environment, microglia have been shown to prevent astrocyte proliferation.[2] Microglia are the main source of M1 and M2 inflammatory messengers, but those induced by ATP have not yet been comprehensively described. This study aims to characterize the microglia phenotype and identify the inflammatory mediators released, when stimulated with ATPγS, that may prevent astrogial proliferation.

Two types of primary glial cultures were prepared from the cortical hemispheres of newborn Wistar rats (P0-P2): co-cultures of astrocytes and microglia (~15% of microglia), and cultures of astrocytes (<1% microglia).[2] Confluent cultures were serum-starved for 48 hours, and cellular proliferation was evaluated by stimulation with ATPγS in the presence or absence of minocycline for an additional 48 hours. Results were compared through an ANOVA (post-hoc Dunnett's test). mRNA was extracted from both types of cultures and RT-qPCR technique was used to evaluate the expression of microglia M1 phenotype markers CD16, CD86, IL-β, TNF-α, IL6 and IL-1α, as well as the M2 phenotype markers CD206 (Mrc1), TGFβ, YM1 and Arg1. To evaluate the expression of each gene, the distributions of $2^{\Delta\Delta Cq}$ were compared through an effect size analysis using Cohen's d and their 95% confidence intervals. This analysis was used to compare ATPγS-induced gene expression in astrocyte cultures and co-cultures.

In astrocyte cultures, 0.1 mM ATPγS increased astrogial proliferation to $258 \pm 7\%$ ($n=6$, $P<0.01$). This effect was attenuated in co-cultures to $137 \pm 5\%$ ($n=6$, $P<0.05$) and restored by minocycline (0.3 µM; $230 \pm 7\%$, $n=6$, $P<0.01$), which inhibits M1 polarization of microglia.

In astrocyte cultures, ATPγS increased the expression of IL-1β (Cohen's d 2.00; 95%CI 0.12:3.60), TNF-α (d 3.47; 95%CI 0.86:5.98), and IL-6 (d 6.24; 95%CI 1.81:10.68). In co-cultures, ATPγS increased the expression of CD16 (d 1.92; 95%CI 0.24:3.52), CD86 (d 6.28; 95%CI 1.82:10.73), IL-1β (d 26.23; 95%CI 9.04:43.84), TNF-α (d 16.47; 95%CI 6.58:26.49), IL-6 (d 22.13; 95%CI 7.59:37.00), IL-1α (d 8.24; 95%CI 2.58:13.95), TGF-β (d 1.65; 95%CI 0.05:3.18) and Arg1 (d 2.82; 95%CI 0.29:5.23).

In conclusion, ATPγS primarily induces the expression of M1 markers and pro-inflammatory cytokines by microglia. This M1-like phenotype may contribute to the impairment of ATPγS-induced astrogial proliferation.

[1] Front. Pharmacol. 11:1416, 2020

[2] Purinergic Signal. 7:251, 2011

PP221. Activation of microglia P2X4 and P2X7 receptors impairs purinergic-induced astroglial proliferation

Quintas C^{1,2}, Silva R¹, Glonçalves J^{1,2}, **Queiroz G^{1,2}**

¹Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal, ²UCIBIO—Applied Molecular Biosciences Unit - Porto, Associate Laboratory i4HB, Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal

During inflammation, damaged cells release ATP that acting on P2Y receptors, induces astroglial proliferation, contributing to create a buffer zone that prevents a widespread inflammation and neurodegeneration.[1] Microglia express P2X receptors that mediate the release of interleukins and prostaglandins and impair P2Y receptor-function in astrocytes, assuming pivotal roles in the coordination of astroglial responses, including proliferation. [2] The present study aimed to clarify some of the mechanisms behind microglia-astrocyte communication involved in purinergic modulation of astrogliosis.

Two types of primary glial cultures were prepared from cortical hemispheres of newborn rats (P0-P2): co-cultures of astrocytes containing approximately 12% of microglia, and cultures of astrocytes, where microglia were almost absent (~1%).[3] Cultures of astrocytes and co-cultures with 4 weeks were serum-starved for 48 h and submitted to several assays. Cellular proliferation was evaluated by stimulation with ATPγS for 48h. [3] Cellular localization of P2X4 and P2X7 receptors was identified by immunocytochemistry and COX-2 expression of was evaluated by qPCR.

In astrocyte cultures, ATPγS increased astroglial proliferation in a concentration-dependent manner, up to $227 \pm 15\%$ (0.1 mM; n=6, P<0.01), an effect that was attenuated in co-cultures to $140 \pm 7\%$ (n=6, P<0.05). In co-cultures, the proliferative effect of ATPγS 0.1 mM was restored by minocycline (0.3 μM; $225 \pm 5\%$, n=6, P<0.01), indomethacin (10 μM; $200 \pm 9\%$, n=6, P<0.01) and the COX-2 inhibitor SC58125 (0.3 μM; $185 \pm 6\%$; n=6, P<0.01). The P2X4 antagonist 5-BDBD (1 μM) and the P2X7 antagonist AZ 10606120 (0.3 μM) also restored the effect of ATPγS 0.1 mM from $143 \pm 5\%$ (n=12, P<0.05) to $180 \pm 6\%$ (n=6, P<0.01) and $229 \pm 6\%$ (n=6, P<0.01), respectively. P2X4 and P2X7 receptors are expressed in both cell types but a higher co-localization was observed with microglia. The mRNA expression of COX-2 in co-cultures, but not in astroglial cultures, was increased by ATPγS 0.1 mM ($2^{\Delta\Delta CT} = 4.43 \pm 0.89$; n=3, P<0.05), an effect attenuated to 1.61 ± 0.20 (n=3) by BDBD (1 μM) and to 0.67 ± 0.12 (n=3) by AZ 10606120 (0.3 μM).

In conclusion, microglia impairment of ATPγS-induced astroglial proliferation is mediated by eicosanoids produced by COX-2 whose expression is upregulated by activation of microglia P2X4 and P2X7 receptors. This microglia-astrocyte communication may be relevant by modulating the inflammatory response, before glial scar formation.

[1] Neuron 81:229, 2014

[2] Front. Pharmacol. 8:937, 2017

[3] Front. Pharmacol. 9:418, 2018

PP222. The pharmacological inhibition of lysosomal two-pore channel 2 (TPC2) confers neuroprotection in stroke via autophagy regulation

Tedeschi V¹, Vinciguerra A², Sisalli M¹, Pignataro G¹, Secco A¹

¹"Federico II" University of Naples, Department of Neuroscience, Reproductive and Odontostomatological Sciences, Napoli, Italy, ²University "Politecnica delle Marche", Department of Biomedical Sciences and Public Health, Ancona, Italy

Introduction: Lysosomal function and organellar Ca²⁺ homeostasis become dysfunctional in stroke causing disturbances in autophagy, the major process for the degradation of abnormal protein aggregates and dysfunctional organelles [1]. Indeed, it has been reported that excessive or prolonged autophagy activation exacerbates ischemic brain injury [2]. A key role in the regulation of basal autophagy is played by the lysosomal channel TPC2 [3], a Ca²⁺-permeable non-selective cation channel gated by NAADP (nicotinic acid adenine dinucleotide phosphate). Currently, the contribution of this channel in the regulation of autophagy during stroke is still unknown. In this study, a fine modulation of autophagy via a proper pharmacological inhibition of TPC2 has been tested in preclinical models of the disease.

Methods: Primary cortical neurons were subjected to oxygen and glucose deprivation followed by reoxygenation to reproduce *in vitro* brain ischemia. Focal brain ischemia was induced in rats by transient middle cerebral artery occlusion (tMCAO). In neurons, the TPC2 pharmacological inhibitor Ned-19 ((1R,3S)-1-[3-[[4-(2-Fluorophenyl)piperazin-1-yl]methyl]-4-methoxyphenyl]-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid) was incubated at the onset of the reoxygenation, while in ischemic rats was intracerebroventricularly infused at the onset of the reperfusion. Under these conditions, TPC2 protein expression as well as autophagy and endoplasmic reticulum (ER) stress markers were studied by Western blotting, while TPC2 localization and activity were measured by immunocytochemistry and single-cell Fura-2 video-imaging, respectively.

Results: TPC2 expression and function were highly modulated in cortical neurons exposed to hypoxia causing dysfunction in organellar Ca²⁺ homeostasis, ER stress and cell death. TPC2 knocking down and pharmacological inhibition by Ned-19 during hypoxia induced neuroprotection. The effect of Ned-19 was reversed by the permeable form of TPC2 endogenous agonist, NAADP-AM. Of note, Ned-19 prevented ER stress, as measured by GRP78/BiP and caspase-9 protein downregulation. Moreover, Ned-19 restored the proper organellar Ca²⁺ levels. Ned-19 prevented hypoxia-induced neuronal death (n=4 experiments) and reduced the infarct volume (n=8 animals) and the neurological deficits in rats subjected to tMCAO by blocking the autophagic flux.

Conclusions: Collectively, our results provide evidence that the pharmacological inhibition of the lysosomal channel TPC2 rescued cortical neurons from hypoxia-induced cell death and exerted neuroprotective effects in ischemic rats by hampering a hyperfunctional autophagic flux. These findings suggest a pivotal role for TPC2 in the pathogenesis of stroke, thus identifying this channel as a novel druggable target in stroke therapy.

- [1] Nixon R.A. Nat Med, 2013
- [2] Zhang X. et al. Autophagy, 2021
- [3] García-Rúa V. et al. J Physiol, 2016

PP223. Antisense Oligonucleotides as a novel RNA-targeted therapeutic approach against Tau-driven neuronal malfunction

Megalokonomou A^{1,2}, Campos-Marques C^{3,4}, Barros-Santos B^{3,4}, Papadimitriou G^{1,3,4}, Vamvakalakovou A^{1,5}, Godinho B^{6,7}, Watts J^{6,8}, Dalla C⁹, Kokras N^{9,10}, Skourti K¹, Katsaitis F¹, Silva J^{3,4}, Sotiropoulos I^{1,3,4}

¹Institute of Biosciences and Applications (IBA), National Center of Scientific Research "Demokritos", Athens, Greece, ²Faculty of Medicine, University of Crete, Heraklion, Greece, ³Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, ⁴ICVS/3B's-PT Government Associate Laboratory, Braga, Portugal, ⁵Department of Biological Applications & Technology, University of Ioannina, Ioannina, Greece, ⁶RNA Therapeutics Institute, Medical School, University of Massachusetts, Worcester, USA, ⁷Department of Molecular Medicine, Medical School, University of Massachusetts, Worcester, USA, ⁸Department of Biochemistry and Molecular Pharmacology, Medical School, University of Massachusetts, Worcester, USA, ⁹Department of Pharmacology, Medical School, National and Kapodistrian University of Athens, Athens, Greece, ¹⁰First Department of Psychiatry, Eginition Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Antisense Oligonucleotides (ASOs) are increasingly considered as a promising therapeutic platform for many human disorders, as they regulate the RNA levels of the protein of interest with great safety for humans and animals. In light of the emerging evidence suggesting the mediating role of Tau protein and its malfunction in neuronal deficits found in a variety of brain pathologies with diverse etiology, including Alzheimer's disease and other Tauopathies, epilepsy, stress-driven pathology, traumatic brain injury and chronic pain [1,2], this project designed and tested novel Tau-ASOs for reducing total Tau or regulating Tau splicing.

Methods: In collaboration with the RNA Therapeutics Institute (Massachusetts Medical School, USA), we designed 22 novel ASOs against total Tau (n=9) or selectively 4R-Tau (n=13), with three different chemical modifications (LNA, MOE and fully MOE-ASOs). Tau-ASOs efficiency was monitored in a pipeline of cellular and animal models of Tau pathology; Tau P301L SH-SY5Y cells, Tau P301L primary neurons and THY-Tau22 transgenic mice {5 animals/group; bilateral i.c.v. ASOs injection under anesthesia [Bupaq (1µL/mg) - medetomidine/ketamine (4µL/mg), i.p.]}, using different types of molecular, cellular and neurostructural analysis.

Results: The screening of the 22 Tau-ASOs led to the selection of four ASOs that were most efficient in reducing mRNA and protein levels of Tau in cells, as monitored by qRT-PCR and Western Blot analysis respectively. The Tau-lowering effect was verified on Tau P301L primary neurons by immunohistochemistry, while three out of the four Tau-ASOs also restored neuronal complexity, as shown by MAP2-based neurostructural analysis. In a pilot *in vivo* study, two out of the four tested ASOs were very effective in reducing Tau levels in the hippocampus of THY-Tau22 mice, and they were selected to proceed with the next *in vivo* experiment, in order to explore the effects of the selected ASOs on Tau-induced mood and cognitive deficits.

Conclusion: Providing both *in vitro* and *in vivo* evidence, these studies have generated a set of novel and highly efficient Tau-ASOs for reducing Tau or modulating the 4R/3R isoform ratio. Based on the emerging role of Tau in brain pathologies with diverse etiology, the generation of efficient Tau-ASOs may advance our therapeutic portfolio against a broad spectrum of brain disorders.

[1] Sotiropoulos I, et al. "Atypical, non-standard functions of the microtubule associated Tau protein." *Acta Neuropathol Commun.* 2017 29;5(1):91. doi: 10.1186/s40478-017-0489-6

[2] Guerreiro SR, et al. Chronic pain causes Tau-mediated hippocampal pathology and memory deficits. *Mol Psychiatry.* 2022;27(11):4385-4393. doi:10.1038/s41380-022-01707-3

PP224. A dose-response study evaluating the antidepressant-like effects and safety profile of ketamine in adolescent female rats

Jornet-Plaza J^{1,2}, Garcia-Fuster J^{1,2}

¹*University of The Balearic Islands, Palma, Spain*, ²*Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain*

Introduction: Currently, ketamine is approved for the treatment of resistant depression in adults, but its efficacy and safety profile in adolescents has not been proven. Previous studies of our laboratory focused on evaluating the antidepressant-like efficacy of a dose of 5 mg/kg in adolescent naïve rats, observing a lack of response in adolescent females. In this context, the present study aimed to evaluate different doses of ketamine in adolescent female rats, as well as its long-term safety profile.

Methods: Adolescent female Sprague-Dawley rats (n=52) were treated (i.p.) with ketamine (1, 5 or 10 mg/kg) or vehicle (n=8-18/experimental group) for 7 consecutive days (postnatal day, PND, 33-39). While acute (30 min post first injection, PND 33) effects were tested in the forced-swim test (FST), repeated antidepressant-like responses were measured both in the FST (PND 40) and novelty suppressed feeding test (NSF, PND 43). Rats were left undisturbed until adulthood when they were scored for long-term safety through the Barnes maze (i.e., cognitive performance) and the conditioned-place preference (i.e., reinforcing properties of a 10 mg/kg dose of ketamine). The results were analyzed using one-way ANOVA (treatment as the independent variable), with Dunnett's for post-hoc analysis.

Results: Ketamine induced an acute antidepressant-like effect, but only with the 10 mg/kg dose (i.e., decreased immobility (**p<0.001) paired with increased climbing (**p=0.005) in the FST). Moreover, ketamine showed an antidepressant-like effect for all doses tested after the repeated paradigm: decreased immobility (*p=0.046) (*p=0.018) and increased climbing (*p=0.031) in the FST for doses 1 and 10 mg/kg; decreased latency to center (*p=0.049) and increased feeding time in the NSF (**p=0.001) for dose 5 mg/kg. The adolescent treatment did not induce long-term changes in adulthood, both in cognitive performance and in the expected reinforcing effects induced by ketamine.

Conclusion: The observed antidepressant-like effects of ketamine in adolescent female rats were dose-dependent, since acute significance was only observed with the higher dose tested (10 mg/kg), while all three doses (1, 5 and 10 mg/kg) showed efficacy following a repeated treatment paradigm. Interestingly, drug treatment during adolescence did not induce long-term changes in adulthood in cognition or reward-mediated responses, suggesting a good safety profile. Future studies should further characterize the beneficial vs. potentially harmful effects following ketamine administration in adolescence. Funded by PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033), PDR2020-14 (CAIB and Tourist Stay Tax Law ITS2017-006) to MJG-F, and "FPI_022_2022" predoctoral scholarship (CAIB) to JJ-P.

PP225. Effects of the Recombinant Human Erythropoietin in the Entorhinal Cortex and Thalamus of Rats Exposed to Focal Cerebral Ischemia

Mršić-Pelčić J¹, Pelčić G², Vitezić D¹, Pilipovic K¹

¹*Department of Basic and Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*, ²*Clinical Hospital Centre Rijeka, Clinics for Ophthalmology, Rijeka, Croatia*

Introduction: The neuroprotective potential of recombinant human erythropoietin (rhEpo) was reported in various experimental models of brain damage [1,2] but the exact mechanism of its effect is still unclear. In the present study, the effect of rhEpo administration on the level of neuronal loss and neurodegenerative changes in the dorsolateral band of the entorhinal cortex and ventral posteromedial nucleus of the thalamus in rats following focal cerebral ischemia was examined. All procedures were performed following requirements of the EU Directive 2010/EU/63.

Methods: Focal cerebral ischemia was induced in male Hanover Wistar rats (250-350 g) by right middle cerebral artery occlusion (MCAO) model for 1 h. After 23 h of reperfusion, ischemic animals were sacrificed and the neuronal damage was detected using the Fluoro Jade B fluorescent staining to detect neurodegeneration, together with NeuN immunostaining used to detect neuronal loss. Ischemic animals received either vehicle or rhEpo (5000 IU/kg, intraperitoneally) 3 hrs after MCAO, and were sacrificed 21 hrs later. Sham-operated, vehicle treated animals served as the control group.

Results: An overall ANOVA revealed a statistically significant difference among tested groups in entorhinal cortex [$F(2,8) = 26,447; p<0,05$] but did not reveal a statistically significant effect of the treatments on the number of NeuN immunopositive cells in thalamus [$F(2,8) = 2,173; p>0,05$]. Also, rhEpo treatment caused a decrease in the Fluoro- Jade B staining intensity in entorhinal cortex [$F(2,8)=6,6075;p<0.05$]. An overall ANOVA did not reveal significant differences in the levels of Fluoro Jade B staining intensities between the investigated experimental groups in thalamus [$F(2,8)=4,1727;p<0,05$].

Conclusion: Administration of rhEpo significantly increased the NeuN immunoreactivity in the entorhinal cortex compared to the neuronal loss detected in ischemic, non-treated animals and decreased the number of Fluoro Jade B positive neurons in comparison to neuronal damage detected in ischemic, non-treated animals. The effect of rhEpo treatment in thalamus was not significant. Supported by grant uniri-biomed-18-115.

- [1] Ma Y, Zhou Z, Yang GY, Ding J, Wang X. The Effect of Erythropoietin and Its Derivatives on Ischemic Stroke Therapy: A Comprehensive Review. *Front Pharmacol.* 2022;13:743926. doi: 10.3389/fphar.2022.743926.
- [2] Juenemann M, Braun T, Schleicher N, Yeniguen M, Schramm P, Gerriets T, Ritschel N, Bachmann G, Obert M, Schoenburg M, Kaps M, Tschernatsch M. Neuroprotective mechanisms of erythropoietin in a rat stroke model. *Transl Neurosci.* 2020;11(1):48-59. Doi: 10.1515/tnsci-2020-0008.

PP226. A dose-response study evaluating the antidepressant-like effects and safety profile of cannabidiol in adolescence male and female rats

Gálvez-Melero L^{1,2}, García-Fuster J^{1,2}

¹IUNICS, University of Balearic Islands, Palma, Spain, ²Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain

In searching for novel pharmacological options to treat adolescent depression, cannabidiol emerges as a good candidate, since preclinical data published up to date seemed to corroborate its antidepressant potential, with a differential response depending on some variables (e.g., biological sex, age, dose). However, most of these studies mainly characterized its effects in adult male rodents, with scarce data available on cannabidiol's effects during early ages, such as adolescence, and/or driven by sex. In this context, our group recently demonstrated in adolescent rats that cannabidiol (10 mg/kg, 7 days, i.p.) induced both rapid and sustained antidepressant-like effects in males, while no responses were observed in females. In this follow-up study, we evaluated the potential antidepressant-like response induced by higher doses of cannabidiol in adolescent rats of both sexes, as well as its long-term safety in adulthood. To do so, adolescent Sprague-Dawley rats of both sexes (n=29 females, n=30 males) were treated (i.p.) with cannabidiol (10, 30 or 60 mg/kg, n=7-8 per group/sex) or vehicle (DMSO, 1 ml/kg, n=7 per sex) for 7 consecutive days (from postnatal day, PND, 43-49). Acute (30 min post injection, PND 43) and repeated (24 h post treatment, PND 50) antidepressant-like responses were measured in forced-swim test sessions. Later on, rats were left undisturbed until adulthood when long-term effects on cognition were measured in the Barnes maze (i.e., short- and long-term memory). Data was analysed with two-way ANOVAs in which Sex and Treatment were the independent variables. Together with our prior study proving efficacious effects with the dose of 10 mg/kg in male adolescent rats, the main results showed a lack of acute and/or repeated antidepressant-like responses of the higher doses tested (30 and 60 mg/kg) for both sexes (no significant Treatment x Sex interaction). Moreover, no changes were observed in memory tasks in the Barnes maze during adulthood after cannabidiol's exposure in adolescence (no significant Treatment x Sex interaction). In conclusion, cannabidiol is a good antidepressant candidate to be used in adolescence, with no signs of long-term damage, although exclusively for male rats. Therefore, future studies should aim at finding efficacious doses for adolescent female rats and to better characterize the molecular mechanisms behind its actions and long-term safety profile, as well as to test the possible role of sexual hormones behind the lack of response observed in females. Funded by Delegación del Gobierno para el Plan Nacional sobre Drogas (2020/001, Ministerio de Sanidad) to MJG-F.

PP227. Investigation of the anti-allodynic effects of cibinetide (ARA290) in cisplatin-induced peripheral neuropathy in rats

Ozkan B¹, Cengelli Unel C¹, Harmanci N¹, Yigitaslan S¹, Ozatik O²

¹Eskisehir Osmangazi University, Faculty of Medicine, Department of Medical Pharmacology, Eskisehir, Turkiye, ²Kutahya Health Sciences University, Faculty of Medicine, Department of Histology and Embryology, Kutahya, Turkiye

Introduction: Peripheral neuropathy (PN) is an important side effect that limits the use of cisplatin (Cis) [1]. Cis induces damage by accumulating in the dorsal root ganglia. An effective agent for PN treatment has not yet been found. Cibinetide (Cib), a nonhematopoietic erythropoietin analogue, binds selectively to the EpoR/CD131 (innate repair receptor) and has reported to have tissue-protective effects in diabetic retinopathy/neuropathy [2]. The study aims to investigate the potential activity of Cib in Cis-induced PN.

Methods: The study has been approved by the Eskişehir Osmangazi University Local Ethics Committee for Animal Experiments (approval no: 909-1/2022). 32 male Sprague Dawley rats (200-250g) were divided into 4 groups: control (saline 2 ml), Cis, Cib30 (Cis+Cib 30 mcg/kg), Cib120 (Cis+Cib 120 mcg/kg). PN was induced by administering 3 mg/kg/week Cis intraperitoneally for 5 weeks. Cib was given every two days for the first 2 weeks and then weekly. Saline was administered simultaneously to prevent kidney damage in Cis-treated rats. PN was evaluated through mechanical/cold allodynia, thermal hyperalgesia, locomotor activity, and rotarod tests. Measurements were taken at baseline and on the 6th day after drug administration. Hemoglobin levels were determined using the Sahli method. One-way or two-way analysis of variance followed by Tukey tests were applied, and p<0.05 was considered significant.

Results: Baseline measurements showed no differences among groups, indicating homogeneity. Cis did not show a significant difference in thermal hyperalgesia test. In the mechanical and cold allodynia tests, there was a significant decrease in paw withdrawal latency (PWL) in the Cis group on 35th day (p<0.01). Cib120 group showed a significant increase in PWL (p<0.05). Body weight changes in Cib groups were not different from the Cis group. Cib did not restore the locomotor activity and motor balance disrupted by Cis. Hemoglobin levels significantly decreased in the Cis group compared to control, and were not different in Cib groups from Cis group.

Conclusion: Cib was found to exert anti-allodynic effects and may be a new therapeutic candidate for Cis-induced PN. Supported by Eskişehir Osmangazi University "Scientific Research Projects" commission (TDK-2022-2572).

- [1] Ibrahim, E.Y. and B.E. Ehrlich, Prevention of chemotherapy-induced peripheral neuropathy: A review of recent findings. Crit Rev Oncol Hematol, 2020. 145: p. 102831.
- [2] O'Leary, O.E., et al., The vasoreparative potential of endothelial colony-forming cells in the ischemic retina is enhanced by cibinetide, a non-hematopoietic erythropoietin mimetic. Exp Eye Res, 2019. 182: p. 144-155.

PP228. Evaluation of the antinociceptive properties of a new N-substituted 1H-isoindole-1,3(2H)-dione derivative based on in vivo studies

Dziubina A¹, Szkatuła D², Sapa J¹

¹*Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*, ²*Department of Medicinal Chemistry, Faculty of Pharmacy, Wrocław Medical University, Wrocław, Poland*

Introduction: Phthalimide derivatives, also known as 1H-isoindole-1,3(2H)-diones, have high potential for pharmacological properties, making them candidates for new drugs. In preliminary studies, the structure of the new compound (F) was confirmed and the physicochemical properties, scavenging activities and cyclooxygenase (COX) inhibitory activity were determined [1].

Based on the above results, the present study was designed to examine the analgesic and anti-oedematous properties of the new N-substituted 1H-isoindole-1,3(2H)-dione compound (F) by in vivo models.

Methods: All procedures were conducted in accordance with the EU Directive 2010/EU/63 and were approved by the I Local Ethics Committee for Experiments on Animals of the Jagiellonian University in Krakow, Poland (701/2023, 801/2023). The study was performed in male albino Swiss mice (CD-1) and Wistar rats. Antinociceptive activity was evaluated in the neurogenic and tonic pain models (capsaicin and formalin tests) in mice. Antiallodynic and anti-hyperalgesic activity was estimated in the oxaliplatin-induced peripheral neuropathy in mice. In a rat model of carrageenan-induced inflammation, the anti-edematous and analgesic effects on thermal hyperalgesia and mechanical allodynia were evaluated. The effects of compound (F) on locomotor activity and motor coordination were also evaluated. Compound (F) (5,10,15 mg/kg) and meloxicam (5,10, 15 mg/kg) (as suspensions in 1% Tween 80 in sterile water) was administered intraperitoneally, 30 min before experiments. Oxaliplatin (10 mg/kg) was prepared in 5% aqueous solution of glucose. Capsaicin was dissolved in physiological saline solution. 0.1ml of 1% carrageenan dissolved in 0.9% saline was injected into the plantar side of the right hind paw of rat

Results: The compound (F) (5, 10,15 mg/kg) attenuated the capsaicin- induced pain and similar to meloxicam (5, 10,15 mg/kg) reduced the pain reaction in both phases of the formalin test. In the dose of 10 mg/kg decreased oedema formation and attenuated mechanical hyperalgesia in carrageenan-induced paw inflammation in rats. Moreover, compound (F) (10 mg/kg) demonstrated significant antiallodynic activity in oxaliplatin pain model. At active, analgesic doses, it did not impair motor coordination and had no sedative effect.

Conclusion: The results indicate that the derivative (F) of 1H-isoindole-1,3(2H)-dione exhibits a broad spectrum of activity in pain models, encompassing neurogenic, tonic, inflammatory, and chemotherapy-induced peripheral neuropathic pain.

This study was financially supported by the Jagiellonian University Medical College grants No.:N42/DBS/000325.

[1] Szkatuła D, et al. A new N-substituted 1H-isoindole-1,3(2H)-dione derivative -synthesis, structure and affinity for cyclooxygenase based on in vitro studies and molecular docking. Int. J. Mol. Sci. 2021, 22, 7678.

PP229. Investigating the impact of G protein-coupled estrogen receptor 1 (GPER1) and ketamine on behavioral and neurochemical changes in male and female rats: Advancing new treatments for mood disorders

Pavlidi P¹, Theodoulou E¹, Kokkinou P¹, Polissidis A^{2,3}, Sotiropoulos I⁴, Antoniou K⁵, Kokras N⁶, Dalla C¹

¹Department of Pharmacology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, ²Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation, Athens, Greece, ³Department of Science and Mathematics, Deree-American College of Greece, Athens, Greece, ⁴Institute of Biosciences & Applications NCSR "Demokritos", Athens, Greece, ⁵Department Pharmacology, Faculty of Medicine, University of Ioannina, Ioannina, Greece, ⁶First Department of Psychiatry, Eginition Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Affective and anxiety disorders significantly burden individuals and society, with a higher prevalence in women. These disorders are commonly treated with selective serotonin reuptake inhibitors, but their efficacy is not universal, leading to a subset of patients diagnosed with Treatment Resistant Depression (TRD). Recently, G protein-coupled estrogen receptor 1 (GPER1) has been implicated in depression/anxiety and it is suspected that has a role in rapid effects, similar to those of glutamatergic drugs, such as esketamine. This study investigated GPER1's function and its viability as a therapeutic target for mood disorders.

Methods: Adult male and female Wistar rats were used, to evaluate the effects of G1 (GPER1 agonist), G15 (GPER1 antagonist), ketamine, and fluoxetine. The drugs were administered at specific doses, via intraperitoneal injections. Following treatment, rats underwent behavioral tests such as the Open Field (OF), Light/Dark (LD) test, Novelty suppressed feeding test (NSFT), and Forced swim test to assess changes in activity, anxiety, and depressive-like behaviours. High-performance liquid chromatography (HPLC) with electrochemical detection was employed to analyze neurotransmitter levels in the rats' brain tissue. This allowed for the quantification of monoamines and their metabolites, providing insights into the neurochemical mechanisms underlying the behavioral effects observed. To delve deeper into GPER1's molecular pathways, a separate group of rats underwent stereotactic surgery for the implantation of cannulas in the dorsal hippocampus. This enabled intracranial co-infusion of the PI3K/Akt and ERK pathway inhibitors, along with the G1 agonist. Behavioral assessments were conducted post-infusion to evaluate the necessity of these signalling pathways in GPER1's antidepressant and anxiolytic rapid non-genomic effects.

Results: Results indicated that GPER1 activity influences ketamine- and fluoxetine-induced behaviors in a sex-dependent manner. Co-administration of G1 agonist with ketamine induces an anxiogenic effect only in females during the OF test (interaction: $F(3,64)=3.529$, $p=0.020$, post-hoc: females Vehicle vs G1+Ket: $p*=0.034$, males vs females G1+Ket: $p++=0.002$), and a anxiolytic/antidepressant effect in males during the NSFT test (interaction: $F(3,64)=2.811$, $p=0.046$, post-hoc: males vehicle vs G1+Ket: $p**=0.003$). Interestingly, combination of G15 and Fluoxetine had an acute anxiolytic effect only in males during the LD test (interaction: $F(3,64)=3.022$, $p=0.036$, post hoc: males vehicle vs G15+Flx: $p*=0.049$).

Conclusions: The study underscores the importance of considering sex in preclinical research to improve the efficacy of antidepressant/anxiolytic treatments. Overall, these results contribute to identifying GPER1 as a potential target for novel mood disorder treatments.

PP230. Molecular underpinning of the cannabidiol's therapeutic potential against stress and Alzheimer's disease brain pathology

Vamvaka-Iakovou A^{1,2,3,4}, Silva J^{3,4}, Gomes P^{3,4}, Campos-Marques C^{3,4}, Samiotaki M⁵, Panayotou G⁵, Skaltsounis L⁶, Halabalaki M⁶, Tzimas P⁶, Kokras N^{7,8}, Dalla C⁷, Megalokonomou A^{1,3,4}, Katsaitis F¹, Skourti K¹, Serfioyi E¹, Brakatselos C⁹, Antoniou K⁹, Sotiropoulos I^{1,3,4}

¹Institute of Biosciences And Applications, NCSR Demokritos, Agia Paraskevi, Greece, ²Department of Biological Applications & Technology, University of Ioannina, Ioannina, Greece, ³Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal,

⁴ICVS/3B's - PT Government Associate Laboratory, Braga/Guimaraes, Portugal, ⁵Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece, ⁶Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupolis Zografou, Athens, Greece, ⁷Department of Pharmacology, Medical School, National and Kapodistrian University of Athens, Athens, Greece, ⁸First Department of Psychiatry, Eginition Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece, ⁹Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece

Introduction: The CBD product industry has experienced tremendous growth over the last few years, with recent interest focussing on its therapeutic potential against neurological and neuropsychiatric disorders such as stress-driven depression and Alzheimer's disease (AD)[1]. However, it remains unclear whether and how CBD can affect AD-related Tau brain pathology, attenuating the precipitating role of chronic stress and related neuronal atrophy/malfunction and memory deficits.

Methods: 3-4-month-old Tau22 transgenic mice (expressing human Tau with P301S and G272V mutations; 10-12 animals/group) were exposed to chronic unpredictable stress (7 weeks) with animals i.p. receiving vehicle (5% ethanol, 5% Cremophor EL, 90% saline) or CBD (30mg/kg; 5 weeks; isolated from C. XXX; >99% purity monitored by HPLC) [2]. At the end of stress period, animals performed cognition- and mood-related behavioral analysis accompanied by molecular, cellular, neurostructural and proteomic analysis of hippocampal brain area.

Results: We found that CBD treatment ameliorated stress-induced memory impairment in Tau22 mice monitored by CFC, Y-maze and NOR behavioral tests. Furthermore, Golgi-based 3D neuronal reconstruction analysis of hippocampal neurons showed that CBD treatment blocked stress-driven reduction in dendritic length and number of branches, suggesting a neuroplastic beneficial impact of CBD on the hippocampus. Next, label-free proteomic analysis of hippocampal synapses revealed that CBD treatment reversed stress-driven changes in synaptic proteome related to the cellular response to stress and gliogenesis.

Conclusion: These results support the potential therapeutic use of CBD treatment against stress-driven Tau pathology contributing to our limited knowledge of how cannabinoid signaling can modulate brain pathology.

[1] Fonseca C., Ettcheto M., Bicker J. et al., "Under the umbrella of depression and Alzheimer's disease physiopathology: Can cannabinoids be a dual-pleiotropic therapy?" Ageing Res. Rev. 2023 90;101998. doi: 10.1016/j.arr.2023.101998

[2] Tzimas P., Petrakis E., Halabalaki M. et al., "Effective determination of the principal non-psychoactive cannabinoids in fiber-type Cannabis sativa L. by UPLC-PDA following a comprehensive design and optimization of extraction methodology" Anal. Chim. Acta Mol Psychiatry. 2021 1150:338200. doi:org/10.1016/j.aca.2021.338200

PP231. Possible dose-dependent neuroprotective effect of ketamine in the brain in a rat head trauma model

Gölcükçü Aydin E¹, Aydin O², Aydin S³, **Kılıç Tatlıcı C¹**, Yazıcı Z¹, Yıldırım C⁴, Şahintürk V⁵, Kılıç F¹
¹Eskişehir Osmangazi University, Faculty of Medicine, Eskişehir, Turkey, ²Erzurum City Hospital, Intensive Care Clinic, Erzurum, Turkey, ³İzmir Tinaztepe University Health Services Vocational School, Department of Pharmacy, Izmir, Turkey, ⁴Ankara University Faculty of Dentistry, Department of Basic Medical Sciences, Ankara, Turkey, ⁵Eskişehir Osmangazi University Faculty of Medicine, Department of Medical Histology and Embryology, Eskişehir, Turkey

Introduction: Traumatic Brain Injury (TBI) is a major health and socioeconomic problem [1]. In our study, we aimed to investigate neuroprotective and anti-inflammatory effects of ketamine in experimental head trauma model in rats.

Methods: The rats were divided into six groups(n:6) as control, trauma and ketamine-treated trauma(Trauma+10,Trauma+20,Trauma+40,Trauma+60mg/kg ketamine). Ketamine was administered intraperitoneally everyday until seventh day. Neurological examination was performed on 24th,48th hours and seventh day after trauma. Serum IL-1 β ,IL-10,TNF- α levels were measured at 24th hour and seventh day. On seventh day serum and brain levels of MDA, glutathione-peroxidase(GSH), catalase, caspase-3 and serum levels of AST,ALT,ALP,BUN,LDH,creatinine were studied. Hematoxylin-Eosin(H-E) and TUNEL staining was performed on brain tissues. One and two-way analysis of variance(ANOVA) was used for statistical evaluations.

Results: Neurological examination scores were impaired in tketamine 60mg/kg group($p<0.05$) and a decrease in IL-1 β levels was observed in 40mg/kg ketamine group ($p<0.05$) on seventh day. For IL-1 β an increase was observed at the 60mg/kg ketamine compared to 40mg/kg ketamine($p<0.05$). For IL-10 and glutathione-peroxidase, a decrease was observed in all trauma groups compared to control($p<0.05$).

An increase in catalase was observed in the trauma+ketamine40mg/kg($p<0.05$). There was an increase in lipid-peroxidase(MDA) in all trauma groups compared to control($p<0.05$). There was significant increase at Trauma+Ketamine60mg/kg($p<0.001$). In biochemical evaluations there was a decrease in LDH in trauma+20mg/kg compared to trauma($p<0.05$). Apoptosis was not observed but necrosis was observed in histopathological examinations in all trauma and ketamine groups.

Conclusions: In our study, 60mg/kg ketamine negatively affected neurological scores,IL-1 β ,brain glutathione-peroxidase,lipid-peroxidase levels. Ketamine 40mg/kg positively affected IL-1 β and catalase. At this dose, the number of necrotic cells tented to decrease. No beneficial or harmful effects of ketamine were observed at other doses. Ketamine prevented worsening of post-traumatic injuries at all doses except 60mg/kg.

This study was supported by Eskişehir Osmangazi University Scientific Research Projects Coordination Unit within the scope of project number 202011A215.

- [1] Critchley G, Rogers I, Memon A. Epidemiology of Head Injury. In: Whitfield PC, Welbourne J, Thomas E, Summers F, Whyte M, Hutchinson PJ, eds. Traumatic Brain Injury: A Multidisciplinary Approach. 2nd ed. Cambridge: Cambridge University Press; 2020:1-11. doi:10.1017/9781108355247.003

PP232. The effect and mechanism of the new antiepileptic drug Lacosamide on experimentally induced schizophrenia-like models

Kılıç Tatlıcı C¹, Erdogan Erden E¹, Sanlı Z¹, Aydin S², Kılıç F¹, Yıldırım E¹

¹Eskişehir Osmangazi University, Faculty of Medicine, Department of Medical Pharmacology, Eskişehir, Turkey, ²İzmir Tinaztepe University, Vocational School of Health Services, Pharmacy Services Program, Izmir, Turkey

Introduction: Many studies have shown that schizophrenia is a disorder of the dopamine signaling system. However, dopamine dysfunction is not sufficient to explain the psychopathology and treatment results of schizophrenia [1]. Abnormalities of GABA in specific cortical inhibitory neurons and CRMP-2 expression were shown to be related to the pathophysiology of schizophrenia [2]. In our study, we aimed to investigate the effects of different doses of the new antiepileptic drug Lacosamide, which has effects on both GABAergic system and CRMP-2, on schizophrenia models and compare it with typical and atypical antipsychotic drugs [3].

Methods: Wistar male rats (250-300 g) and cd-1 male mice (25-35 g) were used. The rats were divided into 9 groups and received drug treatment for 5 days. On Day 5, MK-801 injection was made 1 hour after the last dose of drug treatment. The rats were subjected to Prepulse Inhibition Inhibition (P.P.I) and Passive Avoidance tests, respectively. Cd1 albino mice were also given drug treatment for 5 days and climbing, catalepsy and locomotor activity tests were performed. Statistical analyzes were evaluated with one-way analysis of variance.

Results: MK-801 reduced mean %P.P.I. compared to control. The effect of MK-801 on P.P.I. was significantly reversed by MK-801+Lacosamide 30 mg/kg. The average % P.P.I. value in the MK-801+Lacosamide 30 mg/kg+Bikukulin group decreased significantly compared to the MK-801+Lacosamide 30 mg/kg group. While Lacosamide did not produce cataleptic behavior in mice, it reversed the effect of apomorphine-induced increase in climbing time. Although there is not a statistical difference in GABA levels in ELISA results, Lacosamide demonstrated GABA agonistic effect potential in behavioral tests.

Conclusions: Considering that it does not show the effects seen in typical antipsychotic drugs in terms of its side effect profile, we think that more studies can be conducted on it as an adjunctive treatment alternative to classical antipsychotic treatment.

Supported by Eskişehir Osmangazi University Scientific Research Projects Coordination Office.

Ethical Approval number:886

- [1] Berridge KC. From prediction error to incentive salience: mesolimbic computation of reward motivation. Eur J Neurosci. 2012;35(7):1124-1143. doi:10.1111/j.1460-9568.2012.07990.x
- [2] Kedracka-Krok S, Swiderska B, Jankowska U, et al. Clozapine influences cytoskeleton structure and calcium homeostasis in rat cerebral cortex and has a different proteomic profile than risperidone. J Neurochem. 2015;132(6):657-676. doi:10.1111/jnc.13007
- [3] Wilson SM, Khanna R. Specific binding of lacosamide to collapsin response mediator protein 2 (CRMP2) and direct impairment of its canonical function: implications for the therapeutic potential of lacosamide. Mol Neurobiol. 2015;51(2):599-609. doi:10.1007/s12035-014-8775-9

PP233. A virtual-aided design of small compounds to prevent BDNF receptor cleavage in Alzheimer's disease

De Oliveira S^{1,2}, Fernandes L^{1,2}, Coelho T^{1,2,3}, Manso M^{1,2,3,4}, Sebastião A^{1,2}, Enguita F², Diógenes M^{1,2}

¹Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, ²Instituto de Medicina Molecular - João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, ³Research Institute for Medicines (iMed.), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal, ⁴Department of Pharmaceutical Sciences and Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal

Brain-Derived Neurotrophic factor (BDNF) promotes neuronal protection through the activation of its full-length receptor, Tropomyosin-related Kinase B (TrkB-FL)[1]. In Alzheimer's Disease (AD), this BDNF/TrkB-FL system is compromised due to an amyloid-beta (Aβ)-mediated calpain TrkB-FL cleavage originating two fragments: a truncated receptor (TrkB-T') and an intracellular fragment (TrkB-ICD)[2]. Focusing on the BDNF/TrkB-FL signalling reestablishment as a potential therapeutic strategy for AD, the aim of this work was to search for small compounds able to prevent TrkB-FL cleavage.

A computer-aided model of TrkB-FL was created resorting to homology modelling and molecular threading. To search for potential cleavage inhibitors, a virtual screening of chemical compounds was performed. The top 8 candidates (named A to H)* were chosen according with their ability to cross blood brain barrier, solubility, gastrointestinal absorption, interference with cytochromes and patent-free.

The selected compounds were applied, in different concentrations (25, 50, 100, and 1000nM), in the presence and absence of Aβ, to primary neocortical neuronal cultures from embryonic day (E18) Sprague Dawley rats. The degree of TrkB-FL cleavage was evaluated by the ratio of TrkB-ICD/TrkB-FL levels by Western-Blot analysis. To evaluate the effect of the compounds upon hippocampal basal synaptic transmission, electrophysiological experiments were performed in 8-9 weeks old Sprague Dawley rats. Statistical analysis was performed by One-way ANOVA with Dunnett's multiple comparisons post-test and Kruskal-Wallis Dunn's multiple comparisons.

Regarding the obtained data, Compound B reduced by ~70% the TrkB-ICD/TrkB-FL levels in all studied concentrations compared to control condition ($p<0.05$, $n=5$). Compound C, at the 100 nM concentration, also decreased the TrkB-ICD/TrkB-FL levels in ~70% when compared to its control ($p<0.05$, $n=4$). Remarkably, compound H showed potential in decreasing TrkB-ICD/TrkB-FL levels (~30%) in the presence of Aβ ($p<0.05$, $n=9$). Importantly, compound H did not affect BDNF-mediated phosphorylation of TrkB-FL receptor nor hippocampal basal synaptic transmission ($p>0.05$, $n=7$).

In conclusion, the data suggest that compounds B and C present potential to prevent TrkB-FL cleavage being now important to test them in toxic conditions. Compound H co-incubated with Aβ prevented TrkB-FL cleavage without interfering with basal synaptic transmission nor TrkB-FL phosphorylation, and thus, is being considered as a promising compound which will be further explored.

[1] Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci. 2001; 24:677-736. doi:10.1146/annurev.neuro.24.1.677

[2] Jerónimo-Santos A, Vaz SH, Parreira S, et al. Dysregulation of TrkB Receptors and BDNF Function by Amyloid-β Peptide is Mediated by Calpain. Cereb Cortex. 2015;25(9):3107-3121. doi:10.1093/cercor/bhu105

PP234. Anti-glycation properties of Zinc-enriched *Arthrospira platensis* (*Spirulina*) prevent diet-induced “metaflammation” in a mouse model

Aimaretti E¹, Porchietto E², Mantegazza G³, Gargari G³, Collotta D⁴, Einaudi G², Ferreira Alves G²,

Marzani E⁴, Algeri A⁵, Dal Bello F⁶, Aragno M¹, Cifani C², Guglielmetti S³, Mastrocoda R¹, Collino M⁴

¹University of Turin, Torino, Italy, ²School of Pharmacy, University of Camerino, Camerino, Italy,

³Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Milan, Italy, ⁴Department of Neurosciences “Rita Levi Montalcini”, University of Turin, Turin, Italy, ⁵Unione Spirulina Biologica Italiana (USBI), Mantova, Italy, ⁶Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy

Introduction: Advanced glycation end products (AGEs) exert key pathogenic roles in the development of obesity [1]. Thanks to its abundance in bioactive compounds, the microalga *Arthrospira platensis* (*Spirulina*, SP) is proposed as nutritional supplement [2]. We investigated the potential anti-glycating properties of *Spirulina* enriched with zinc (Zn-SP) and the impact on diet-induced metabolic derangements.

Methods: Thirty male C57BL/6OlaHsd mice were fed a standard diet (SD, n=10) or a high-fat-high-sugar diet (HFHS, n=10) for 12 weeks; after 5 weeks of dietary manipulation, a subgroup of the HFHS-fed mice received 350 mg/kg Zn-SP orally three times a week (HFHS+Zn-SP, n=10). Blood and liver samples were collected for ex vivo analyses. Bacterial community structure was investigated using metataxonomics on fecal and ileum samples collected at the end of the protocol. The Shapiro-Wilk test was used to assess data distribution. The statistical analysis was performed by one-way ANOVA (Bonferroni's post-hoc test). The threshold for statistical significance was set to p<0.05. Statistical calculations on metataxononomic data were carried out using R programming language. Differently abundant taxa were identified with Mann-Whitney test on read abundances that underwent centered log ratio (CLR) transformation.

Results: HFHS diet-induced obesity, glucose intolerance and increased plasma levels of pro-inflammatory cytokines and transaminases. Zn-SP administration restored glucose homeostasis and reduced hepatic dysfunction and systemic inflammation (e.g., systemic levels of interleukin-6: SD=4.16±0.46 pg/mL, HFHS=9.66±0.85 pg/mL, HFHS+Zn-SP=3.86±0.62 pg/mL). In the liver of HFHS mice, a robust accumulation of AGEs was detected, in particular of carboxymethyl-lysine (CML) while Zn-SP efficiently counteracted this derangement (SD=1.49±0.17 µg/mg of proteins, HFHS=4.76±0.57 µg/mg of proteins, HFHS+Zn-SP=1.63±0.27 µg/mg of proteins); these effects were paralleled by increased expression of the main AGE-receptor (RAGE) and depletion of glyoxalase-1, whereas Zn-SP administration prevented these alterations reducing local pro-inflammatory response. 16S rRNA gene profiling of feces and ileum content revealed altered bacterial community structure in HFHS mice compared to both SD and HFHS+Zn-SP groups.

Conclusions: Our study demonstrates relevant anti-glycation properties of Zn-SP which contribute to preventing AGE production and/or stimulate AGE detoxification, improving diet-related dysbiosis and metabolic derangements.

[1] Twarda-Clapa A, Olczak A, Bialkowska AM, Koziołkiewicz M. Advanced Glycation End-Products (AGEs): Formation, Chemistry, Classification, Receptors, and Diseases Related to AGEs. Cells. 2022;11(8):1312. Published 2022 Apr 12. doi:10.3390/cells11081312

[2] Nasirian F, Dadkhah M, Moradi-Kor N, Obeidavi Z. Effects of *Spirulina platensis* microalgae on antioxidant and anti-inflammatory factors in diabetic rats. Diabetes Metab Syndr Obes. 2018;11:375-380. Published 2018 Jul 31. doi:10.2147/DMSO.S172104

PP235. Role of TGF- β signaling and sex influence in two Diabetic Peripheral Neuropathy models in mice

Silva E^{1,2}, Saguillo A^{1,2}, Marques J³, de la Fuente R⁴, Tramullas M^{1,2}

¹Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad De Cantabria, Santander, Spain, ²Instituto de Investigación Sanitaria Valdecilla (IDIVAL), Santander, Spain,

³Servicio de Rehabilitación, Hospital Universitario Marqués de Valdecilla (HUMV), Santander, Spain,

⁴Servicio de Anestesiología, Hospital Universitario Marqués de Valdecilla (HUMV), Santander, Spain

Diabetes Mellitus (DM) is one of the most serious and common chronic diseases of our times, causing disabling, costly complications, and reducing life expectancy [1]. Around 50% of diabetic patients develop Diabetic Peripheral Neuropathy (DPN). This complication can cause pain, numbness, and loss of sensation in the extremities. It is characterized by a decline in peripheral innervations, increased neuronal inflammation, demyelination, axonal atrophy, and decreased neuronal regenerative capacity. Transforming growth factors- β (TGF- β) constitute a large family of pleiotropic and multifunctional cytokines. Mice lacking the TGF- β pseudoreceptor BAMBI present an antiallodynic phenotype after sciatic nerve injury in male mice [2]. Our study aims to investigate whether BAMBI deficiency affects the establishment of DPN in two models of DM in male and female mice.

Type 1 DM was induced in C57BL6 and BAMBI-KO male and female mice by multiple low doses of streptozotocin (STZ) in citrate buffer (40mg/kg i.p, daily for 5 consecutive days). For type 2 DM induction the mice were fed "ad libitum" with a 5% fructose solution for 3 weeks, and then, treated with STZ (n=6–9 per experimental group). Blood glucose levels were measured at baseline and 4 weeks after STZ administration. Mechanical allodynia was assessed with the von Frey test. All procedures meet the following requirements of the EU Directive 2010/EU/63. Our results show that the increase in blood glucose levels was lower in female C57BL6 mice at 4 weeks post-STZ injections compared to male C57BL6 mice ($p<0.05$) in both DPN models. Weight loss, polydipsia, and polyuria were less pronounced in females than in male C57BL6 mice. Female and male BAMBI-KO mice exhibited marked symptoms of diabetes with higher levels of blood glucose. All mice developed mechanical allodynia, however, the response intensity was significantly higher in female BAMBI-KO mice ($p<0.05$).

Our findings indicate that BAMBI-KO female mice show a higher susceptibility to developing DPN. The present study suggests the role of BAMBI in the development of DPN and the existence of sex differences in glucose handling. Supported by PID2022-1364180BI00/AEI/10.13039/501100011033 / FEDER, UE and IDIVAL (INN-VAL 23/12).

[1] Sun H, Saeedi P, Karuranga S, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. 2022;183:109119. doi:10.1016/j.diabres.2021.109119

[2] Tramullas M, Lantero A, Díaz A, et al. BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) reveals the involvement of the transforming growth factor-beta family in pain modulation. *J Neurosci*. 2010;30(4):1502-1511. Doi:10.1523/JNEUROSCI.2584-09.2010

PP236. Myelin Restoration: A Promising Approach to tackle Alzheimer's Disease

Zota I^{1,2}, Chanoumidou K^{1,2}, Magoulas G³, Calogeropoulou T³, Charalampopoulos I^{1,2}, Gravanis A^{1,2}

¹*Pharmacology Dept., Medical School, University of Crete, Heraklion, Greece*, ²*Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology-Hellas (IMBB-FORTH), Heraklion, Greece*, ³*Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece*

Introduction: Recent studies highlight myelin breakdown and loss of myelin sheath as an early stage event in Alzheimer's Disease (AD). Brain-derived neurotrophic factor hold a central role in the control of neuronal development, survival, regeneration and plasticity (Chao M.V., 2003). Endogenous BDNF, acting selectively through TrkB receptor, also enhances myelination in the CNS and tends to increase the density of oligodendrocyte progenitor cells (OPCs), both in vitro and in vivo (Xiao et al., 2012; Wong et al., 2013). However, natural neurotrophins are characterized by non-preferable pharmacological properties, such as short half-life, inability to access the blood-brain barrier and low pharmacokinetic profile. Hence, targeting OPCs with novel agonists of TrkB receptor to promote re-myelination could be a complementary strategy in AD therapies.

Methods: During experimental procedure we used in vivo animal models of AD, immunofluorescence histochemical and cytochemical analysis, western blot analysis and Celltox cell survival assay.

Results: In the present study, we compared neuronal myelination levels and OPCs population in 2, 6, and 12 months old 5xFAD (animal model of AD) mice to their wild type (wt) littermates, revealing a significant decrease of myelin and defective oligodendrogenesis in the hippocampus regions of 5xFAD mice (Zota et al., 2024). We also tested 30 novel synthetic neurotrophin analogs for their ability to selectively activate the TrkB receptor and rescue cells from death under growth factor deprivation. Our results show that two of the most effective small molecules are TC508 and TC509. Human iPSC-derived neural stem cells (hiNSCs), mouse neural stem cells (NSCs) and OPCs were then treated with these novel synthetic micromolecular compounds, and our findings indicate that both compounds highly promote primary OPC differentiation into oligodendrocytes and accelerate adult NSC differentiation into PDGFR α ⁺ OPCs, under both physiological and Alzheimer's Disease-related conditions (presence of toxic Amyloid- β). Additionally, they are capable of preventing cell death upon challenge with amyloid- β toxic isoforms.

Conclusion: In summary, our study shows that myelination process constitutes an appealing therapeutic target against neuronal loss in AD and reveals two novel BDNF- micromolecular mimetics as promising lead therapeutic agents in the field of neurodegenerative and demyelinating diseases.

- [1] Zota I. et al., Dynamics of myelin deficits in the 5xFAD mouse model for Alzheimer's disease and the protective role of BDNF. In press.
- [2] Wong, A., Oligodendroglial expression of TrkB independently regulates myelination and progenitor cell proliferation. J Neurosci (2013).

PP237. Pharmacological exploitation of the p75 Neurotrophin Receptor neurogenic properties against Alzheimer's Disease

Papadopoulou M^{1,2}, Chanoumidou K^{1,2}, **Charalampopoulos I**^{1,2}

¹*University of Crete, Medical School, Heraklion, Greece*, ²*Institute of Molecular Biology & Biotechnology, Foundation for Research and Technology Hellas (FORTH), Heraklion, Greece*

Introduction: The pan-neurotrophin p75 receptor (p75NTR) is a member of the TNF-death-receptors superfamily with pleiotropic expression in neural tissue and multifaced regulatory function. p75NTR is up-regulated under brain injury and neurodegenerative conditions like Alzheimer's disease (AD). The altered expression profile combined with its controversial signaling, makes p75NTR an appealing target in neuropathology. Several studies have implicated p75NTR in AD by demonstrating its ability to serve as a receptor for amyloid- β (A β) and mediator of neurodegeneration. However, its contribution to adult hippocampal neurogenesis, which drops sharply in AD, remains poorly understood. Evidence from studies in different animal models suggests either a pro-neurogenic or an anti-neurogenic effect of p75NTR. However, to date, there is no study addressing its function in human neural stem cells (hNSCs). Here, we aim to investigate the role of p75NTR in adult neurogenesis under physiological and AD conditions. Furthermore, a neurotrophin analog, ENT-A044, activates p75NTR, Regulating Neuronal Survival in a Cell Context-Dependent Manner. Thus, ENT-A044 is proposed as a lead molecule for the development of novel pharmacological agents, providing new therapeutic approaches and research tools, by controlling p75NTR actions.

Methods: We performed BrdU injections for detection of proliferation and immunohistochemistry analyses for key neurogenic markers in p75KO, 5xFAD and p75KO_5xFAD mice. We also generated NSCs from human iPSCs and examined the activity of p75NTR signaling with co-immunoprecipitation and Western Blot analyses. Finally, with Celltox/Brdu assays we investigated p75NTR contribution to hNSCs survival under treatments with A β -peptides. Finally, we performed treatments with ENT-A044, in mouse and hNSCs.

Results: p75 KO mice exhibit decreased NSCs proliferation and attenuated neuronal differentiation in the dentate gyrus as shown with immunohistochemistry analyses for DCX and NeuN revealing key neurogenic properties. p75KO_5xFAD model, depicts a decreased number of proliferative NSCs showing the significance of p75NTR expression, even in an AD background. Furthermore, p75NTR signaling is active in hiPSCs-derived NSCs and regulates survival in the presence of A β peptides indicating its involvement in AD. Treatment of cells with ENT-A044 showed a significant increase at cell death mediated by p75NTR in hNSCs as opposed to primary cell cultures derived from mice, where ENT-A044 led to survival.

Conclusions: The present study demonstrates for the first time the expression and activity of p75NTR in hNSCs and indicates p75NTR involvement in AD neurogenic deficits. Our results suggest p75NTR requirement for intact neurogenesis. Configuration of p75NTR signaling in NSCs and its pharmacological exploitation will enable the development of new therapies.

PP238. Loss of cysteine carbon flux to H2BK46ac redirects pluripotent stem cells towards mesodermal lineage commitment

Wittig J^{1,2}, Drekolia M^{1,2}, Wang Y³, Cordero J³, Kessler L³, Karantanou C^{1,2}, Kempf S², Weigert A⁴, Wittig I⁵, Leisegang M⁶, Fuhrmann D⁴, Schlereth K⁷, Gehrs S⁷, Janel N⁸, Zimmermann W^{9,10}, Günter S^{10,11}, Klatt S², Fleming I^{2,10}, Weichenhan D¹², Plass C¹², Looso M^{10,13}, Kurian L⁶, Hu J^{2,14}, Dobreva G^{3,10}, Bibli S^{1,2,10}

¹Department of Vascular Dysfunction, European Center for Angioscience, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany, ²Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany, ³Department of Cardiovascular Genomics and Epigenetics, European Center for Angioscience, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany, ⁴Institute of Biochemistry I, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany, ⁵Functional Proteomics, SFB815 Core Unit, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany, ⁶Institute for Cardiovascular Physiology, Centre for Physiology, Goethe University, Frankfurt am Main, Germany, ⁷Division of Vascular Oncology and Metastasis, German Cancer Research Center (DKFZ-ZMBH Alliance), Heidelberg, Germany, ⁸Laboratoire Processus dégénératifs, Stress et Vieillissement, Unité de Biologie Fonctionnelle et Adaptative (BFA), UMR 8251 CNRS, Université Paris Diderot-Sorbonne Paris Cité, Paris, France, ⁹Institute of Pharmacology, Heart Research Center, University Medical Center, Georg-August-University, Göttingen, Germany, ¹⁰German Center of Cardiovascular Research (DZHK), Germany, ¹¹Deep Sequencing Platform, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany, ¹²Division of Cancer Epigenomics, German Cancer Research Center (DKFZ-ZMBH Alliance), Heidelberg, Germany, ¹³Bioinformatics Core Unit, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany, ¹⁴Department of Histology and Embryology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Introduction: Metabolic transitions accompany stem cell activation and differentiation, regulating redox balance, signaling mechanisms and epigenetic alterations. Here we report that pluripotency is characterized and maintained by an enhanced cysteinolytic flux that epigenetically regulates chromatin accessibility as such preserving cell self-renewal capacity and suppressing mesodermal lineage commitment.

Methods and Results: Cysteine metabolites are significantly enriched in pluripotent stem cells and decrease during early mesodermal fate commitment events. Metabolic flux analysis revealed that cysteine bioavailability supports glutathione generation to prevent ferroptotic events in human induced pluripotent stem cells (iPSCs). Depleting cysteine in the presence of ferroptosis inhibitors redirects iPSCs towards the mesodermal lineage, as does the knock down of the cysteine-metabolizing enzymes cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS). Metabolic flux analysis identified cysteine carbons as sources of acetyl-moieties and subsequent nuclear acetylomic analysis of core histones revealed an increased contribution of cysteine carbons to the acetylation of H2B. Specifically, cysteine-dependent acetylation of H2B on lysine 46 was enriched in genomic loci that support stemness, maintaining chromatin accessibility to the pluripotency transcriptional factors SOX2 and KLF4 and shielding regions that promote mesodermal lineage commitment. Loss of H2BK46ac closed chromatin in regions supporting stemness and opened chromatin in regions that favored mesodermal lineage commitment within 4 hours after cysteine removal. Genetic deletion of CSE and CBS inhibited murine embryonic development and increased expression of mesodermal lineage markers on E3.5 embryos. Cysteine as a sole carbon source maintained the self-renewal capacity, chromatin accessibility and transcription of cultured iPSCs.

Conclusions: Our results show that controlling the availability of cysteine carbons supports glutathione synthesis for stem cell survival and histone acetylation to preserve stem cells from pluripotency, suppressing mesodermal lineage commitment.

PP239. Aromatase inhibition and electroconvulsive seizures in adolescent rats: sex similarities in the regulation of some neuroplasticity markers

Ledesma-Corvi S^{1,2}, García-Fuster J^{1,2}

¹IUNICS, UIB, Palma de Mallorca, Spain, ²IdISBa, Palma de Mallorca, Spain

A recent study from our group demonstrated sex-specific differences in the antidepressant-like response induced by electroconvulsive seizures (ECS) in adolescent rats, since it worked when administered in male rats, while in females rendered ineffectual or even deleterious. However, when estrogen biosynthesis was inhibited with letrozole, ECS induced an antidepressant-like response in female adolescent rats, suggesting a role for the biosynthesis of estrogens and/or for the accumulation of testosterone in its therapeutical response that deserves future studies. In this context, the present study characterized the possible mechanism by which letrozole may potentiate the effects of ECS in female rats. Male and female adolescent Sprague-Dawley rats were pretreated with letrozole (1 mg/kg/day) or vehicle (1 ml/kg/day) for 8 days (i.p.), and treated during the last 5 days (3-h later) with ECS (95 mA, 0.6 s, 100 Hz) or SHAM. Rats were sacrificed 1-day post-treatment and brains were collected to evaluate by western blot assay several hippocampal neuroplasticity markers known to be commonly regulated by most antidepressants, such as mBDNF, mTOR and ERK1/2 signaling. Statistical analyses were performed through three-way ANOVAs (independent variables: sex, pre-treatment and treatment). The main results demonstrated that ECS increased hippocampal neuroplasticity markers independently of sex and/or pre-treatment (letrozole vs. vehicle). Particularly, the results showed that in vehicle pre-treated rats, repeated ECS significantly increased hippocampal mBDNF levels both in male (**p<0.001 vs. vehicle-SHAM) and female (**p<0.001 vs. vehicle-SHAM) adolescent rats. Similarly, when the biosynthesis of estrogens was inhibited with letrozole, ECS also induced an increase in mBDNF in both male (**p<0.001 vs. letrozole-SHAM) and female (**p<0.001 vs. letrozole-SHAM) adolescent rats. Finally, no changes were observed in any of the other markers analyzed, except for an overall effect of treatment in the rate of p-mTOR/mTOR. In conjunction, these results demonstrated that repeated ECS induced similar sex-related changes in neuroplasticity markers in hippocampus, which were not affected by letrozole pre-treatment. This data is interesting and align with prior results from our group proving a dysregulation between the behavioral and neurochemical responses elicited by ECS in rats (i.e., different behavioral responses in terms of efficacy in adolescent female and male rats but similar neurochemical responses). Further studies are therefore required to complement these results and deepen into the possible mechanisms by which letrozole may potentiate the effects of ECS in female rats.

Grants PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033) and PDR2020/14 to MJG-F, "Programa JUNIOR del proyecto INTRES: Invetir, Investigar e Innovar" to SL-C.

PP240. Erucin, a natural hydrogen sulfide (H₂S) donor, improves Duchenne muscular dystrophy (DMD)-induced SKM dysfunction

Casale V¹, Smimmo M¹, Bello I¹, Panza E¹, Bonomo M², Brancaleone V², Cirino G¹, Vellecco V¹, Bucci M¹

¹*University of Naples Federico II, Naples, Italy*, ²*University of Basilicata, Potenza, Italy*

Introduction: Duchenne muscular dystrophy (DMD) is a rare X chromosome-linked disease caused by mutations in the gene encoding for dystrophin, leading to progressive and unstoppable degeneration of skeletal muscle (SKM) tissues. Recently, we have demonstrated an impairment of the transsulfuration pathway, specifically of hydrogen sulfide (H₂S), in the SKM of dystrophic mice [1].

This study aims to evaluate the possible beneficial effect of Erucin, a natural slow H₂S donor, in a murine model of DMD.

Methods: In vivo studies were performed on mdx mice, a well-known genetic model of DMD, and their littermates (WT). Mice (n=10) were treated with Erucin (3mg/kg) for 2 weeks and locomotory activity was performed. At 7 weeks of age, mice were sacrificed, and quadriceps were collected for evaluation of oxidative stress markers (H₂O₂, and GSH/GSSG ratio). Statistical analysis was assessed using one-way ANOVA.

Results: mdx mice displayed a significant reduced SKM performance compared to healthy mice evaluated by rotarod and weight test (rotarod: 219 ± 25 sec vs. 297.4 ± 1.95 sec; weight test: 12 ± 0.49 sec vs. 14 ± 0.33 sec; mdx vs. WT respectively; **p<0.01). The reduced SKM function was associated with an increase of oxidative stress in the quadriceps of mdx mice compared to WT measured by both H₂O₂ and GSH/GSSG ratio levels (H₂O₂: 0.32 ± 0.06 μM/mg of protein vs. 0.11 ± 0.04 μM/mg of protein; mdx vs. WT respectively; **p<0.01; n=9; GSH/GSSG: 2.0 ± 0.25 vs. 3.06 ± 0.43; mdx vs. WT respectively; *p<0.05; n=4). Erucin treatment fully recovered the impaired SKM performance observed in mdx mice in both locomotory tests (rotarod: 293 ± 4.7 sec vs 219 ± 25 sec; weight test: 14.3 ± 0.26 sec vs. 12 ± 0.49 sec; Erucin vs. vehicle respectively; °°p<0.01). This effect was attributable to a reduced oxidative stress measured in quadriceps (H₂O₂: 0.10 ± 0.03 μM/mg of protein vs 0.32 ± 0.06 μM/mg of protein; Erucin vs. vehicle respectively; °°p<0.01; n=9; GSH/GSSG: 3.1 ± 0.17 vs. 2.0 ± 0.25 Erucin vs. vehicle respectively; °p<0.05; n=4).

Conclusions: our results suggest that Erucin exerts a protective effect in DMD, improving SKM performance by reducing oxidative stress via H₂S release.

- [1] Panza E, Vellecco V, Iannotti FA, Paris D, Manzo OL, Smimmo M, Mitilini N, Boscaino A, de Dominicis G, Bucci M, Di Lorenzo A, Cirino G. Duchenne's muscular dystrophy involves a defective transsulfuration pathway activity. Redox Biol. 2021; 45:102040

PP241. Investigating motor function in a preformed fibril model of alpha-synucleinopathy in rats in relation to time and age

Akgüner B¹, Akyel H², Çınar E³, Yalçın Çakmaklı G⁴, Elibol B⁴, Tel B¹

¹Hacettepe University, Faculty of Pharmacy, Department of Pharmacology, Ankara, Turkey, ²Baskent University, Faculty of Pharmacy, Department of Pharmacology, Ankara, Turkey, ³Istanbul University-Cerrahpasa, Faculty of Pharmacy, Istanbul, Turkey, ⁴Hacettepe University, Faculty of Medicine, Department of Neurology, Ankara, Turkey

Objective: Parkinson's disease, the second most common neurodegenerative disorder, associates closely with alpha-synuclein accumulation and age as a major risk factor. However, there is limited research on the role of ageing in exacerbating a-syn accumulation. The aim of this study was to evaluate the effect of pathological accumulation of a-syn fibrils on motor function in relation to dopaminergic loss in both young and aged rats over the course of disease progression.

Materials and Methods: 12 young (10 weeks) and 12 aged (44 weeks) female Wistar rats were used (Ethical Permission No:2023/04-01). Human-a-syn fibrils (2 µg/µl, in 16 µl PBS) bilaterally injected into dorsolateral striatum (6/6 young and aged rats). Further, 6/6 young and aged animals without any treatments used as controls. Open field and rotarod tests were performed at 45th and 90th days post-injection. After the behavioural tests, post-mortem a-syn and TH immunohistochemical staining was performed. Unpaired (between the groups) and paired (within the groups) Student's t-tests were used for comparisons. Results are expressed as mean±standard error, with p<0.05 considered statistically significant.

Results: On the 45th day, young-fibril group locomotor activity was lower than young-controls ($p=0.0052$, 4410 ± 184.8 vs 5195 ± 148.3); and slightly higher than aged-fibril groups ($p>0.05$, 4410 ± 184.8 vs 4189 ± 923); both young (92.4 ± 4.76) and aged-fibril groups (32.6 ± 12.17) had shorter latency of fall than controls on the rotarod ($p=0.0229$, young-control 170.8 ± 30.4 ; $p>0.05$, aged-control 67.6 ± 16.14). Contrary, on the 90th day, aged-fibril group's locomotor activity was increased compared to controls and young-fibril groups ($p=0.0007$, 5895 ± 219.2 vs 2949 ± 486.6 ; $p=0.0061$, 3945 ± 505.6); however, they had shorter latency of fall compared to controls ($p=0.0019$, 38.6 ± 5.163 vs 83.4 ± 9.821) on the rotarod. When compared to young-fibril group, aged-fibril group had shorter latency on both 45th ($p=0.0009$, 32.6 ± 12.17 vs 92.4 ± 4.76) and 90th ($p=0.0045$, 38.6 ± 5.163 vs 110.5 ± 21.94) days. In the immunohistochemical staining results, successful a-syn staining with fibril injection was confirmed in both young and old animals. Additionally, a noticeable decrease was observed in both young and old fibril injection groups compared to naive animals.

Conclusions: Our results indicate that fibril injection leads to mild motor dysfunction. Motor learning and coordination were impaired in the aged fibril group, whereas the young fibril group showed improved performance on day 90. In conclusion, our study emphasises the importance of age in modelling Parkinson's disease.

PP242. Dissection of anti-inflammatory and immunomodulatory activity of Mangifera indica extract: from CD4+CD45RBhigh T cells transfer model of colitis to IBD patients

Schettino A¹, Saviano A¹, Mansour A², Marigliano N¹, Raucci F¹, Rimmer P², Begum J², Zhi Z², Iqbal T², Iqbal A², Cirino G³, Bucci M³, Maione F¹

¹*ImmunoPharmaLab, Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*, ²*Institute of Cardiovascular Sciences (ICVS), College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*, ³*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*

Introduction: Inflammatory bowel diseases (IBDs) are chronic intestinal disorders mainly characterized by a dysregulation of both T regulatory (reg) and T helper (h) 1/17 cells. Increasing evidence demonstrates that dietary polyphenols from *Mangifera indica* L. (commonly known as mango) mitigate intestinal inflammation and splenic Treg/Th17 ratio [1]. Therefore, in this study, we aimed to evaluate the potential beneficial effects of this plant extract (here referred as MIE) in a CD4+CD45RBhigh T cells transfer model of colitis, also evaluating its protective effects on IBD patients.

Methods: Rag1(-/-) mice, transferred with CD4+CD45RBhigh T cells [2], were treated daily with MIE (90% in mangiferin, 10 mg kg⁻¹, p.o.) for 4 weeks. Thereafter, severity of colitis was evaluated coupled with the assessment of the cellular infiltrate's phenotype by Elisa and FACS analysis. Moreover, FITC-dextran assay was employed to determine intestinal permeability in addition to western blot analysis for tight junction proteins ZO-1, occludin and claudin-2. Finally, an analysis of main proinflammatory cytokines was performed on sera from IBD patients stimulated with LPS (0.1 µg ml⁻¹) and treated with MIE (0.03-10 µg ml⁻¹).

Results: Treatment with MIE revealed a reduction of body weight loss ($P \leq 0.0001$; $N=6$) and clinical score ($P \leq 0.001$; $N=6$) coupled to a significant modulation of both infiltrated and splenic Th1 (CD4+/IFN- γ +) ($P \leq 0.01$, $P \leq 0.05$ respectively; $N=6$), Th17 (CD4+/IL-17+) ($P \leq 0.001$, $P \leq 0.001$; $N=6$) and Treg (CD4+/CD25+/FOXP3+) ($P \leq 0.0001$, $P \leq 0.001$; $N=6$) cells. These data were consistent with the modulation of several pro/anti-inflammatory cytokines on colonic lamina propria. Moreover, MIE mitigated the gut permeability ($P \leq 0.001$; $N=6$) and tight junction functionality ($P \leq 0.01$; $N=3$). Interestingly, MIE significantly reduced TNF- α ($P \leq 0.0001$; $N=25$) and, in part, IL-17 ($P \leq 0.01$; $N=23$) levels on IBD sera.

Conclusions: Taken together, the results of this study demonstrate a beneficial activity of MIE on the immunological perturbation during the onset of colitis and on the systemic inflammatory reaction typical of IBD patients, paving the way for its rationale use as nutraceutical and/or functional food.

[1] A. Saviano, F. Raucci, G. M. Casillo, A. A. Mansour, V. Piccolo, C. Montesano et al. Anti-inflammatory and immunomodulatory activity of *Mangifera indica* L. reveals the modulation of COX-2/mPGES-1 axis and Th17/Treg ratio. *Pharmacol Res*, 2022;182:106283.

[2] D. V. Ostanin, J. Bao, I. Koboziev, L. Gray, S. A. Robinson-Jackson, M. Kosloski-Davidson et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol*, 2009;296:G135-46.

PP243. Lymphatic transport of cannabidiol after oral administration in pigs: development of a novel model for lymphatic transport studies

Ryšánek P¹, Lukáč P, Mlček M, Jelínek P, Kozlík P, Šodek P, Sklenárová M, Arora M, Nováková A, Šteigerová M, Bobek D, Das D, Křížek T, Šoós M, Šíma M, Slanař O

¹*First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic*

Introduction: Lymphatic transport is an important process involved after oral administration of highly lipophilic compounds like fat-soluble vitamins, some oral anticancer agents (e.g. venetoclax) and hormonal replacement therapy (e.g. testosterone undecanoate). Experimental measurement of lymphatic transport is challenging since it requires surgery and cannulation of lymphatic vessels. Multiple animal models have been developed in the recent decades with rat being the mostly used species. However, there are several drawbacks preventing extrapolation of lymphatic transport data from the rat onto human, namely different anatomy and physiology of the gastrointestinal tract and the need to use specifically modified dosage forms that do not correspond to full sized dosage forms used in humans. Therefore, there is a need to develop an animal model that closely resembles the situation in humans. The aim of this work is to develop a model for lymphatic transport measurement in pigs.

Methods: Adult landrace pigs have undergone surgery and thoracic duct cannulation in intubation anesthesia. The lymphatic catheter was exteriorized on the back of the animal and the lymph was collected in a modified urinary bag. After overnight recovery, the animals were dosed orally with the test formulation (cannabidiol 200 mg in 15 ml nanoemulsion) and the lymph was then continuously collected for 24 hours. At the same time, blood samples were taken. The serum and blood concentrations were measured using a validated HPLC method and pharmacokinetic parameters including parameters of lymphatic transport were assessed.

Results: First attempted thoracic duct cannulation on the neck was not successful due to a strong variation of the duct course and anatomical obstacles. Cannulation via right-sided thoracotomy was successful and brought the first preliminary cannabidiol lymphatic transport data in one animal with a full 24-hour serum and lymph profile. The total absolute cannabidiol bioavailability was low with 1.4%. The lymphatic transport played a major role with a relative bioavailability via lymph of 37.8%. The mean cannabidiol lymph concentrations were 408-fold higher than the serum concentrations. Cmax in the lymph was 898-fold higher than the Cmax in serum and was reached one hour later (Tmax 2h vs. 1h).

Conclusions: Lymphatic transport of drugs measurement in pigs using thoracic duct cannulation is a challenging but feasible method. Cannabidiol displayed a high lymphatic transport as expected based on the reports from lower species.

This work was supported by GAUK no. 102322 and AZV NU22-08-00346 grants.

PP244. Intracerebral application of chemically functionalized single-walled carbon nanotubes and its effects on neuronal damage, glial response, and synaptic changes in traumatic brain injury model in mice

Pilipović K¹, Dolenec P¹, Janković T¹, Mihalić V¹, Parpura V²

¹University of Rijeka, Faculty of Medicine, Rijeka, Croatia, ²Zhejiang Chinese Medical University, International Translational Neuroscience Research Institute, Hangzhou, China

Traumatic brain injury (TBI) stands as a leading cause of mortality and disability among young individuals, posing a substantial challenge to public health and socioeconomic well-being. Despite its prevalence, effective neuroprotective treatments remain elusive. We aimed to explore the influence of single-walled carbon nanotubes functionalized with polyethylene glycol (SWCNT-PEG) on neuronal loss, neurodegeneration, myelination, microglial and astrocytes response, and synaptic changes in the mice brain, five weeks after moderate TBI induction.

Moderate TBI was induced in adult male mice using the lateral fluid percussion model. SWCNT-PEG or a control solution (PEG) was administered at the injury site one week later. Non-treated TBI mice and control, non-injured animals were injected with a vehicle or the investigated nanomaterial. Subsequently, at five weeks post-TBI, mice were sacrificed, and their brains were subjected to histological and immunohistochemical analyses.

The findings revealed a notable loss of neurons in the parietal cortex (PC), disruptions in myelin integrity within white matter tracts, and the presence of neurodegeneration following TBI. Application of SWCNT-PEG demonstrated a potential to mitigate neuronal loss and alleviate pathological changes in white matter tracts. Although no significant effects on astrocytes and microglia cell numbers were observed in the PC and hippocampus (HC), morphological alterations in microglia were noted in the PC. Synaptic changes were absent in the PC, but a significant decrease in synaptophysin signal was evident in the HC post-TBI. However, the administration of SWCNT-PEG did not significantly impact these synaptic changes. Importantly, the results did not indicate adverse effects of SWCNT-PEG on brain tissue.

This research suggests the neuroprotective potential of a single application of SWCNT-PEG following TBI, with a possible impact on microglia cells.

This research was fully supported by the Croatian Science Foundation grant UIP-2017-05-9517 to KP.

PP245. Study of new immune players in Abdominal Aortic Aneurysm

Domingo Pérez E^{1,2}, Gómez Á^{1,2}, Collado A^{1,2}, Aparicio J², Marques P^{1,2}, Francisco V^{1,2}, Piqueras L^{1,2,3,5}, Real J^{1,2,3,4,5}, Blanes J^{1,2,5}, Sanz M^{1,2,3,5}

¹*University of Valencia, Valencia, Spain*, ²*INCLIVA, Valencia, Spain*, ³*University Clinic Hospital of Valencia, Valencia, Spain*, ⁴*CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Spain*, ⁵*University General Hospital of Valencia, Valencia, Spain*

Introduction: Abdominal aortic aneurysm (AAA) consists in a localized dilation of the abdominal aorta, resulting in an increase of its normal diameter by 50% or more [1]. It predominantly affects men over 65, and currently, the clinical therapeutic approach to AAA is limited to surgical repair. Nowadays, the development of AAA is considered to have a clear inflammatory component. Due to the high mortality rate associated with AAA, it is important identify new effective therapeutic strategies to prevent the progression of disease. Therefore, since inflammation appears to be the crucial force driving onset and progression of AAA, a better understanding of the inflammatory response that is involved in this process, as well as the role of the different immune players, is required to discover new therapeutic targets to either inhibit or prevent AAA development.

Method: Eight-week-old male apolipoprotein E-deficient (apoE-/-) mice were used in these studies. An osmotic minipump (Alzet, Model 2004, Charles River) implanted subcutaneously was used to infuse Ang-II (n=5) at a rate of 1000 ng/kg/min or saline (n=5) for 28 days. Lesion formation, macrophage, T lymphocyte, eosinophil and pSTAT-6+ cells infiltration as well as eotaxin-1/CCL11 and IL-4 content were determined within the lesion through histological and immunohistochemical techniques. Statistical significance was determined using a Student's t-test.

Results: Ang-II-challenged apoE-/- mice had a higher incidence of AAA than those infused with saline. The maximum diameter of the adrenal region in the animals infused with Ang II was 2.19 ± 0.16 mm, while control (saline) animals was 1.10 ± 0.02 mm. Increased diameter was also observed in both the aortic arch and the thoracic region of the aorta in those animals infused with Ang II compared to those in the control group. Moreover, enhanced macrophage (CD68+), CD3+ lymphocyte, eosinophil and pSTAT6+ cell infiltration and neovascularization were observed compared to unchallenged animals, which was accompanied by greater eotaxin-1/CCL11 and IL-4 content within the lesion area.

Conclusion: Additional studies must be conducted to understand the role of eotaxin-1/CCL11 in AAA development.

Fundings and grants: This work was supported by the Spanish Ministry of Science and Innovation: [grant number PID2020-120336RB-I00]; the Carlos III Health Institute (ISCIII) and the European Regional Development Fund (FEDER) [grant number PI21-00220]; the Generalitat Valenciana [grant numbers APOTIP/2020/011, CIPROM/2022/45].

- [1] Deroo, E., Stranz, A., Yang, H., Hsieh, M., Se, C., Zhou, T., 2022. Endothelial Dysfunction in the Pathogenesis of Abdominal Aortic Aneurysm. *Biomolecules* 12(4), 509-522.

PP246. Effect of pentadcapeptide BPC 157 on platelet aggregation and viscoelastic properties of blood clot in rats treated with aspirin, clopidogrel and cilostazol

Stupnisek M^{1,2}, Krtalic B³, Krtalic L⁴, Mircic M⁵, Konosic S⁵

¹Faculty of Dental Medicine and Health, J. J. Strossmayer University of Osijek, Osijek, Croatia,

²University Hospital for Infectious Diseases, Zagreb, Croatia, ³University Hospital Sveti Duh, Zagreb, Croatia, ⁴Children's Hospital Zagreb, Zagreb, Croatia, ⁵University Hospital Centre Zagreb, Croatia

Introduction: Previously, pentadcapeptide Gly-Glu-Pro-Pro- Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val, M.W. 1419, named BPC 157, as a prototype antiulcer agent with potent cytoprotective capability, thereby exerting innate endothelium protection, counteracted abdominal anastomosis-induced thrombosis and prolonged bleeding and thrombocytopenia after amputation and/or anticoagulant (heparin, warfarin) and aspirin and largely interacts with NO-system in various models and species.

As a natural extension of previous research that confirmed the role of BPC 157, there was a necessity to determine how BPC 157 influences platelet aggregation and viscoelastic properties of the blood clot. Therefore, these outcomes were carried out using ex vivo and in vitro studies, using impedance aggregometry and ROTEM studies.

Methods: Male albino Wistar rats, 200 g b.w., were randomly assigned; 6 rats per each group were used for the experiments, approved by the Local Ethics Committee at the University of Zagreb, Croatia. The medication procedure was performed on rats, which had food and water ad libitum before the procedure and until the end of the experiment, and was assessed by the observer unaware about the treatment. Rats received intragastrically for three days once daily treatment with antithrombotic agents - aspirin (10 mg/kg) or clopidogrel (10 mg/kg) or cilostazol (10 mg/kg). Medication (BPC 157 (10 µg/kg) or an equal volume of saline (5 ml/kg)) was given intragastrically, immediately after each antithrombotic agent application.

For multiple electrode aggregometry and modified rotational thromboelastometry studies, blood sampling was at 2 h after last application. Adenosine diphosphate (ADP test 6.5 µM), arachidonic acid (ASPI test 0.5 mM), a combination of arachidonic acid and prostaglandin E1 (ASPI test 0.5 mM and PGE1-test 30 nM), and collagen (COL test 3.2 µg/ml) were used as aggregation agonists.

Results: Given with aspirin, clopidogrel, or cilostazol in rats, BPC 157 counteracted their inhibitory effects on aggregation activated by arachidonic acid, ADP, collagen, and arachidonic acid/PGE1. Specifically, this includes recovery of the aggregation induced by arachidonic acid (vs. aspirin, vs. clopidogrel, and vs. cilostazol), arachidonic acid/PGE1 (vs. cilostazol), ADP (vs. clopidogrel), or collagen (vs. clopidogrel). Contrarily, there is no effect on the used tests (extrinsic/intrinsic hemostasis system, the fibrin part of the clot) EXTEM, INTEM, and FIBTEM; clotting time; clot formation time; alpha-angle; maximum clot firmness; lysis index after 30 minutes; and maximum lysis.

Conclusion: We can conclude that BPC 157 rescues inhibited platelet aggregation, but it has no effect on viscoelastic properties of the blood clot.

PP247. Cell cycle braker P16INK4a regulates spinal production of TNF alpha and chronic pain induced by nerve injury

Weng H¹, Bipin S

¹*California Northstate University, Elk Grove, United States of America*

Introduction: Neuropathic pain (NP) is a devastating symptom in patients due to limited effective treatments currently available. Spinal microglial proliferation and over-production of proinflammatory cytokines are critically implicated in the genesis of neuropathic pain. Over-production of TNF α proinflammatory cytokine in the spinal dorsal horn facilitates spinal excitatory glutamatergic synaptic activity, resulting in excessive activation in spinal neurons along the pain signaling pathway. Thus, identifying signaling molecules regulating TNF α production may shed light on the development of novel analgesics for treatment of neuropathic pain. P16INK4a is a key molecule that negatively regulates cell proliferation. The aim of this study is to determine the role of P16INK4a and its upstream signaling molecules in the spinal production of TNF α and genesis of neuropathic pain.

Methods: Partial sciatic nerve ligation was made in male Sprague-Dawley rats (weight: 200 to 250 g) to create a NP animal model. Behavioral assay, *in vivo* drug and small interfering RNA (siRNA) administration, and molecular techniques (western blotting) were used. All studies were approved by the Institutional Animal Care and Use Committee, and were fully compliant with the NIH Guidelines for the Use and Care of Laboratory Animals.

Results: 1. Animals with NP exhibited suppression of P16INK4a protein expression in the spinal dorsal horn, which was temporally associated with suppression of LANCL2 protein expression and AMPK activity, as well microglial activation, and over-production of TNF α (n=4). Activation of TLR4 in the spinal cord with topical application of lipopolysaccharide (100 ng/ml) produced molecular changes in the spinal dorsal horn similar to those in animals with NP (n=4). 2. Similarly, suppression of AMPK activity with spinal topical application of Compound C (200 μ M) (n=4) reduced AMPK activity and protein expression in LANCL2 and P16INK4a, which were temporally associated with microglial activation and over-production of TNF α . 3. Activation of LANCL2 by abscisic acid (20 μ M) ameliorated protein expression of LANCL2 and P16INK4a, as well as suppressed microglial activation and TNF alpha production in the spinal cord (n=4) treated with lipopolysaccharide. 4. Knockdown of P16INK4a with siRNA induced suppression of P16INK4a expression, microglial activation, over-production of TNF α and neuropathic pain behaviors (n=4).

Conclusions: P16INK4a is critically implicated in the regulation of microglial activation, production of TNF α , and genesis of neuropathic pain. P16INK4a protein expression is regulated by TLR4, AMPK and LANCL2 signaling molecules. Our study highlights that targeting signaling molecules regulating the P16INK4a function may be a novel approach to conquering neuropathic pain.

PP248. Role of TGF- β signalling and sex influence on neuropathic pain-evoked anxiety in mice

Marques J^{1,2}, Saguillo A^{1,3}, Tramullas M^{1,3}

¹Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Santander, Spain, ²Servicio de Rehabilitación del Hospital Universitario Marqués de Valdecilla (HUMV), Santander, Spain, ³Instituto de Investigación Sanitaria Valdecilla (IDIVAL), Santander, Spain

Neuropathic pain (NP) is a debilitating chronic syndrome that is often refractory to currently available analgesics. Furthermore, NP is often associated with comorbidities like anxiety and depression, resulting in a low health-related quality of life. Although both chronic anxiety and pain conditions are more prevalent in females, most of the studies are performed only in males, and to date, few studies have included females or even both sexes. Transforming growth factors- β (TGF- β) constitute a large family of pleiotropic and multifunctional cytokines. We have previously reported that male mice lacking the TGF- β pseudoreceptor BAMBI present an antiallodynic phenotype after peripheral nerve injury [1]. Our study aims to investigate whether BAMBI deficiency affects a) the establishment of NP in female mice and b) the impact of NP on anxiety-like behavior in female and male mice. Adult male and female C57BL6 and BAMBI-/- mice were exposed to sciatic nerve crush injury. Two weeks after surgery, mechanical allodynia was assessed with von Frey monofilaments. Anxiety-related behaviours were evaluated using open-field and light-dark box tests. All procedures meet the following requirements of the EU Directive 2010/EU/63.

Our results indicate that the lack of BAMBI does not protect females from the development of NP (N= 12 mice per experimental group). Both, male and female BAMBI-/- mice exhibit increased anxiety-like behaviors evaluated in the open field ($p<0.01$) and the light-dark box ($p<0.001$) tests. However, after nerve injury, female C57BL6 mice, but not male, show increased anxiety-like behaviours ($p<0.05$). Our findings highlight the key role of BAMBI in the development of neuropathic pain and unconditioned anxious behaviours and the importance of the gender perspective in these pathological processes. Supported by PID2022-136418OBI00/AEI/10.13039/501100011033/ FEDER, UE and IDIVAL (INN-VAL 23/12).

[1] Tramullas, M. Lantero A, Díaz A, et al. BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) reveals the involvement of the transforming growth factor- β family in pain modulation. *J Neurosci*. 2010;30(4). doi:10.1523/JNEUROSCI.2584-09.

PP249. TrkB-FL cleavage in human Alzheimer's disease and Fronto Temporal dementia in patient samples

Aleman-Serrano N^{1,2}, Costa-Coelho T^{1,2,3}, De Mendonça A¹, Diógenes M^{1,2}, Sebastião A^{1,2}

¹Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa (FMUL), Lisboa, Portugal, ²Instituto de Medicina Molecular-João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal, ³Research Institute for Medicines (iMed), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by the accumulation of amyloid-beta (Aβ) alongside an impairment of brain-derived neurotrophic factor signaling. We described that Aβ induces BDNF receptor (TrkB-FL) cleavage, generating an intracellular fragment, TrkB-ICD. Recently, we have shown that TrkB-ICD levels are increased in cerebrospinal fluid (CSF) of AD patients, when compared to a mild cognitive impairment (MCI) group (not due to AD). Curiously, the presence of this fragment and its generation has yet to be studied in other dementias, namely frontotemporal dementia (FTD).

Hence, this work aims to investigate the levels of TrkB-ICD and TrkB-FL in CSF from FTD patients, when compared to an MCI not due to AD (control group). In parallel, the predictive value of TrkB-FL, TrkB-ICD and TrkB-ICD/TrkB-FL in both AD and FTD was investigated.

FTD patients (n=7) comprised individuals with high levels of p-tau and t-tau but deprived of an Aβ-associated pathology, whereas MCI individuals (n=14) suffered from cognitive impairments without showing neurodegenerative markers. CSF samples were concentrated, and TrkB-FL and TrkB-ICD immunoreactivity quantified via western-blot.

CSF from FTD patients showed an increase in TrkB-ICD levels, when compared to controls ($p=0,0015$ n=7-14). Importantly, correlations were withdrawn when comparing both p-tau (TrkB-ICD/TrkB-FL:r=0.5828; TrkB-ICD:r=0,5832, n=7-14) and t-tau (TrkB-ICD/FL:r=-0,6160; TrkB-ICD:r=0,3165, n=7-14) with TrkB-ICD and TrkB-ICD/TrkB-FL ratio, respectively.

ROC analysis revealed TrkB-ICD/TrkB-FL as a potential disease monitoring marker in AD (AUC=0.71, n=23-46) and, TrkB-ICD in FTD (AUC=0.73, n=7-14).

The data show increased levels of TrkB-ICD but not a concomitant increase in TrkB-ICD/TrkB-FL in FTD samples. This suggests different fingerprints and pathophysiological mechanisms among FTD and AD, where both TrkB-ICD and TrkB-ICD/TrkB-FL are significantly increased.

PP250. Profiling the analgesic activity of selected, dually acting AChE/BChE inhibitors/ NMDA receptors antagonists

Kubacka M¹, Mogilski S¹, Sapa J¹, Gorecki L², Korabecny J², Sokoup O²

¹Jagiellonian University Medical College, Kraków, Poland, ²Biomedical Research Center, University Hospital, Hradec Kralove, Czech Republic

Introduction: Commonly used analgesics are characterized by a significant limitation in their use due to the side effects. Considering the complexity of the pain process and the multiplicity of the systems involved in the conduction, modulation, and perception of pain, the emphasis is now on searching for substances that are multifunctional ligands. The simultaneous, properly balanced blockade of AChE, BChE and specific NMDA receptor subtypes may be beneficial, especially in the case of chronic pain. In this study, the analgesic activity of three compounds: K1395, K1591, K1594 were evaluated. These compounds have proven inhibitory potency towards hAChE and hBChE, and a differently balanced ability to block GluN1/GluN2A and GluN1/GluN2B NMDA receptors [1,2].

Methods: The analgesic effect was assessed in the formalin and the hot plate test. In addition, the effects on spontaneous locomotor activity and motor coordination were assessed. The experiments were performed on adult male CD-1 mice, n=8-10. The study was carried out under experimental protocols approved by the Local Ethical Committee in Krakow (resolution no. 666/2022 and 738/2023), Poland.

Results: The most favourable profile was shown by K1591 - an effective AChE/BChE inhibitor with low inhibitory potency at NMDA receptors. K1591 showed high analgesic activity in the formalin and the hot plate tests. That showed that K1591 was efficient in attenuating acute and inflammatory pain. The observed effect was at least partially due to its cholinomimetic properties, as the simultaneous administration of K1591 with scopolamine reduced its analgesic activity. In addition, its analgesic effect was specific and did not result from sedative properties and no neurotoxic effects were observed. Compound K1594, a potent AChE inhibitor with moderate/low affinity for NMDA receptors with a preference of GLUN1/N2B subtype, showed analgesic activity in formalin test, however, at the highest dose tested, was sedative and impaired motor coordination. Compound K1395 - a potent and selective antagonist of GLUN1/N2B NMDA receptor and a weak inhibitor of both AChE/BChE- showed no significant analgesic effect.

Conclusions: The experiments allowed to select K1591, a potent AChE/BChE inhibitor and a weak NMDA receptor antagonist, as the most effective in the context of analgesic effect, without neurotoxic effect. These results suggest that increasing cholinergic transmission is more beneficial than decreasing NMDA activation in the context of analgesia and overall safety of the treatment.

[1] Gorecki L, Misiachna A, et al. Eur J Med Chem. 2021;5;219:113434, doi:10.1016/j.ejmech.2021.113434.

[2] Kaniakova M, Korabecny J, et al. Biochem Pharmacol. 2021;186:114460, doi:10.1016/j.bcp.2021.114460.

PP251. A formulation containing palmitoylethanolamide and phenolic compounds counteracts hepatic dysmetabolism and oxidative damage in obese mice: in vivo and in vitro evidence

Pirozzi C¹, Melini S¹, Lama A¹, Comella F¹, Opallo N¹, Mollica M², Paciello O³, Mattace Raso G¹, Meli R¹

¹*Department of Pharmacy, University of Naples Federico II, Naples, Italy*, ²*Department of Biology, University of Naples Federico II, Naples, Italy*, ³*Department of Veterinary Medicine and Animal Productions, Naples, Italy*

Introduction: Gluco- and lipotoxicity play a key role in the development of metabolic dysfunction-associated fatty liver disease (MAFLD) characterizing obesity. The N-acylethanolamine palmitoylethanolamide (PEA) and some phenolic compounds, such as rutin and hydroxytyrosol (HT), have shown different metabolic, anti-inflammatory, and antioxidant effects [1-3]. This study aimed at evaluating the beneficial activity of a formulation containing PEA co-micronized with rutin and associated with HT, named NORM3, in counteracting hepatic damage and metabolic alterations occurring during obesity in high-fat diet (HFD)-fed mice.

Methods: Male C57Bl/6J mice received a standard chow diet or HFD for 19 weeks; an HFD group administered NORM3 (PEA 10 mg/kg/die-Rutin 2 mg/kg/die, HT 0,5 mg/kg/die per os) from week 12 up to week 19. Metabolic and hepatic damage were evaluated by both biochemical, histological, and molecular analyses such as Western blotting and Real-Time PCR.

Results: First, NORM3 limited body weight gain of obese mice (n=12 each group), already after 3 weeks of treatment, and reduced fat mass. NORM3 improved HFD-altered insulin sensitivity reducing HOMA index, and normalized systemic leptin/adiponectin ratio, as predictive value of adipocyte dysfunction. In liver, the morphological analysis showed that NORM3 reduced macro- and microvacuolar steatosis caused by HFD (at least n=3-4 each group). Consistently, NORM3 counteracted lipid dysmetabolism of obese mice, activating AMPK, a key sensor of both glucose and lipid homeostasis, and normalizing the expression of carnitine palmitoyl-transferase (CPT)1, a rate-limiting enzyme of fatty acid β-oxidation, and other genes involved in fatty acid homeostasis. Contextually, we demonstrated the anti-inflammatory and antioxidant potential of NORM3 in liver of obese mice (reduced inflammatory mediators/cytokines and ROS, and increased transcription of detoxifying factors). All molecular determinations are obtained from n=5-7 animals each group. Finally, in HepG2 cells mimicking the oxidative damage as one of the main features of insulin resistance and obesity in vivo, we proved the synergistic effect of the single components (PEA-Rut and HT) of NORM3 not only in reducing cellular ROS production and associated inflammation but also increasing antioxidant defense.

Conclusions: Taken together, our findings identify NORM3 as a potential hepatoprotective approach in dampening hepatic dysmetabolism and associated oxidative stress in MAFLD and obesity.

- [1] Annunziata C, Lama A, Pirozzi C, et al. FASEB J. 2020 doi:10.1096/fj.201901510RR
- [2] Pirozzi C, Lama A, Simeoli R, et al. J Nutr Biochem. 2016; doi:10.1016/j.jnutbio.2015.12.004
- [3] Lee CC, Shen SR, Lai YJ, Wu SC. Food Funct. 2013; doi:10.1039/c3fo30389f

PP252. Anxiolytic effect of PEA-OXA through the blockade of neuroinflammation in mice with high fat diet induced obesity

Opallo N¹, Lama A^{1,2}, Pirozzi C¹, Comella F¹, Melini S¹, Meli R¹, Mattace Raso G¹

¹*Department of Pharmacy, School of Medicine, University of Naples Federico II, Naples, Italy, Naples, Italy, ²Università degli Studi di Trento, Trento, Italy*

Introduction: Anxiety is one of the most common mental illnesses whose prevalence is higher in patients with obesity than in normal weight individuals [1]. Preclinical evidence showed that following high fat diet (HFD) feeding of mice, neuroinflammation may affect several brain areas involved in mood regulation, determining blood brain barrier (BBB) disruption, mitochondrial dysfunction, and ER stress [2]. 2-Pentadecyl-2-oxazoline (PEA-OXA), the oxazoline of palmitoylethanolamide (PEA), is a multi-target compound. It can act as an α 2 antagonist, a histamine H3 partial agonist, and an inhibitor of the N-acylethanolamine-hydrolyzing acid amidase (NAAA), the enzyme responsible of the PEA metabolism [3]. The aim of this study was to evaluate the effects of PEA-OXA on anxiety-like behavior and the underlying pathogenic mechanisms associated to HFD feeding in obese mice.

Methods: 6-week-old male C57Bl/6J mice were fed with standard chow diet or HFD for 12 weeks. PEA-OXA (30 mg/kg/die per os) was administered for an additional 7 weeks, along with HFD.

Results: PEA-OXA counteracted obesity-induced anxiety-like behavior evaluated by open field and elevated plus maze tests. Molecular analyses performed in hippocampus showed a reduction in the expression of pro-inflammatory factors, namely cyclooxygenase-2, toll like receptor 4, interleukin-1 β and the inflammasome NLR family pyrin domain containing 3 in PEA-OXA treated mice, indicating its capability to limit neuroinflammation. Moreover, PEA-OXA restored tight junction mRNA levels (claudin 5, occludin), indicating the improvement of blood brain barrier integrity. Finally, PEA-OXA dampened unfolding protein response, interfering on glucose-regulated protein (GRP)78 expression and protein kinase RNA-like ER kinase (PERK)-elongation initiation factor (eIF)2 α pathway.

Conclusions: Taken together, our data suggest a potential therapeutic role of PEA-OXA in obesity-induced anxiety, limiting hippocampal neuroinflammation, BBB permeability, and ER stress.

- [1] Baker KD, Loughman A, Spencer SJ, Reichelt AC. The impact of obesity and hypercaloric diet consumption on anxiety and emotional behavior across the lifespan. *Neurosci Biobehav Rev*. 2017;83:173-182. doi:10.1016/j.neubiorev.2017.10.0142.
- [2] Nakandakari SCBR, Muñoz VR, Kuga GK, et al. Short-term high-fat diet modulates several inflammatory, ER stress, and apoptosis markers in the hippocampus of young mice. *Brain Behav Immun*. 2019;79:284-293. doi:10.1016/j.bbi.2019.02.016
- [3] Boccella S, Guida F, Iannotta M, et al. 2-Pentadecyl-2-oxazoline ameliorates memory impairment and depression-like behaviour in neuropathic mice: possible role of adrenergic alpha2- and H3 histamine autoreceptors [published correction appears in Mol Brain. 2021 Apr 1;14(1):64]. *Mol Brain*. 2021;14(1):28. Published 2021 Feb 8. doi:10.1186/s13041-020-00724-z

PP253. In silico and in vitro assessment of imperatorin-induced anticancer activity: The role of mitotoxicity

Albayrak G¹, Ergüç A², Okur H³

¹Department of Pharmaceutical Botany, Faculty of Pharmacy, Izmir Katip Celebi University, Izmir, Türkiye, ²Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Izmir Katip Celebi University, Izmir, Türkiye, ³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dicle University, Diyarbakır, Türkiye

Objective: This study aims to explore the impact of the Crabtree effect on mitochondria in HepG2 cells when exposed to imperatorin (IMP), a natural furanocoumarin obtained from *Prangos hulusii* S. G. Şenol, H. Yıldırım & Ö. Seçmen, under conditions of either glucose or galactose media. Additionally, the research aims to predict potential interactions between IMP and electron transport chain (ETC) complexes, shedding light on the underlying causes of mitochondrial dysfunction.

Materials and Methods: *P. hulusii* roots were collected from the western slopes of Mount Bozdağ, Ödemiş, Turkey, in June 2022. After plant authentication, air-dried roots (200 g) were crushed and powdered for extraction. Chloroform extraction (3 L) was performed using an orbital shaker for 12 hours. Following filtration and evaporation, IMP (100 mg) was isolated from the chloroform extract via repeated column chromatography. Compound identification was achieved using ¹H NMR, ¹³C NMR, and LC-MS by comparing data with literature (1). The compound was stored at +4°C in the dark until the experiments. Cell viability and membrane damage in HepG2 cells exposed to various concentrations of IMP (12.5, 25, 50, 100, and 250 µM) were assessed through MTT and LDH leakage assays in both glucose- and galactose-conditioned media. The affinity of IMP to ETC complexes was determined through molecular docking studies.

Results and Discussion: The MTT assay indicated cytotoxic activity at 250 µM IMP in glucose-conditioned media, while concentrations of 25 µM and higher in galactose-conditioned media decreased cell viability. In glucose-conditioned media, 250 µM IMP disrupted the cell membrane, and in galactose-conditioned media, 100 and 250 µM IMP increased membrane damage. Docking simulations revealed that IMP's binding affinities to ETC complexes follow the order: Complex IV > Complex I > Complex III > Complex II. The study suggests that IMP-induced inhibition of complex IV leads to mitochondrial dysfunction by impeding the transfer of electrons from cytochrome c to oxygen, causing the collapse of the proton gradient.

- [1] Sevin G, Alan E, Demir S, Albayrak G, Demiroz T, Yetik-Anacak G, et al. Comparative evaluation of relaxant effects of three prangos species on mouse corpus cavernosum: Chemical characterization and the relaxant mechanisms of action of *P. pabularia* and (+)-oxypeucedanin. J Ethnopharmacol. 2022;284(March 2021):114823.

PP254. The impact of anti-seizure medications on gut microbiota in a genetic animal model, the WAG/Rij rat

De Caro C¹, Roberti R², Ferlazzo E³, Russo E⁴

¹Department of Pharmacy, University "Federico II" of Naples, Naples, Italy, ²University Magna Graecia of Catanzaro, Catanzaro, Italy, ³University Magna Graecia of Catanzaro, Catanzaro, Italy,

⁴University Magna Graecia of Catanzaro, Catanzaro, Italy

Background: Many studies have highlighted the important role of the gut microbiota (GM) in the pathophysiology of neurological disorders including Alzheimer's disease, Parkinson's disease, epilepsy and depression¹, by the bidirectional communication between the gut and the brain named microbiota-gut-brain axis (MGBA). MGBA is traditionally considered to integrate immunological, neural, and hormonal signals². Indeed, the GM regulates the CNS through metabolites, neuroactive molecules, and inflammatory factors, whereas the CNS, in turn, exerts its effect on the GM through the vagus nerve and hypothalamic–pituitary–adrenal axis. Anti-seizure medications (ASMs) are the first line of treatment for seizure control in patients with epilepsy. The epileptic patients also have abnormalities in GI physiology, including increased intestinal permeability, constipation or diarrhea, overall GM alterations, and gut infections³. However, there is little information on whether and how ASMs may influence GM⁴.

Methods: We tested the effects of five ASMs: 1) Ethosuximide (ETH; 300 mg/kg/os); 2) Valproic acid (VPA; 300 mg/kg/os); 3) Lamotrigine (LTG; 10 mg/kg/os); 4) Carbamazepine (CBZ; 80 mg/kg/os); 5) Clobazam (CLB; 12 mg/kg/os) in WAG/Rij rats, an animal model of genetic generalized epilepsy characterized by the development of spontaneous seizures and neurological/psychiatric comorbidity. All ASMs were used daily, delivered in the drinking water with daily freshly prepared solutions. The treatments lasted 30 days. Before and after the treatments, we collected fecal samples for GM analysis by 16S Metagenomic Sequencing using Illumina MiSeq System. Moreover, at the end of treatments we collected brain, gut, and serum for immunological and histological analysis.

Results: Gut microbiota analysis showed differences in beta diversity and specific phylotypes with significant variances in the Bacteroidetes/ Firmicutes ratio between different treatments. Histological results indicated that treatments improved the morphology of intestinal villi and reduced inflammatory infiltrates. Moreover, treatments significantly reduced inflammatory cytokines.

Conclusion: These results will further improve our understanding of MGBA in epilepsy

- [1] Sharon, G. et al. The Central Nervous System and the Gut Microbiome. *Cell* 167, 915–932 (2016).
- [2] Russo, R. et al. Gut-brain Axis: Role of Lipids in the Regulation of Inflammation, Pain and CNS Diseases. *Curr. Med. Chem.* 25, 3930–3952 (2018).
- [3] Al-Beltagi, M. & Saeed, N. K. Epilepsy and the gut: Perpetrator or victim? *World J. Gastrointest. Pathophysiol.* 13, 143–156 (2022).
- [4] Ilhan, Z. E. et al. Exposure to anti-seizure medications impact growth of gut bacterial species and subsequent host response. *Neurobiol. Dis.* 167, 105664 (2022).

PP255. Fractalkine chemokine receptor 1 (CX3CR1) mediates chronic arthritic pain: in vivo study using CX3CR1-deficient mice

Horvath A^{1,2}, Gilinger P¹, Orosz P¹, Borbély É^{1,2}, Tékus V^{1,2}, Szentes N^{1,2,3}, Kovács-Rozmer K¹, Futácsi A⁴, Czéh B^{4,5}, Dénes Á⁶, Helyes Z^{1,2,3,7}

¹Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary, ²National Laboratory for Drug Research and Development, Budapest, Hungary, ³HUN-REN-PTE Chronic Pain Research Group, Pécs, Hungary, ⁴Structural Neurobiology Research Group, Szentágothai Research Centre, University of Pécs, Pécs, Hungary, ⁵Department of Laboratory Medicine, Medical School, University of Pécs, Pécs, Hungary, ⁶Momentum Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary, ⁷PharmInVivo Ltd., Pécs, Hungary

Introduction: The fractalkine chemokine receptor 1 (CX3CR1) is primarily expressed on monocytes/macrophages, T cells, osteoclast precursors and microglial cells. It was described to mediate inflammatory mechanisms both in the periphery and the central nervous system. Although the role of neuroinflammation has been described in some pain conditions, little is known about the role of CX3CR1 in chronic joint pain. Therefore, we investigated the involvement of CX3CR1 in chronic adjuvant-induced arthritis and monoiodoacetate (MIA)-induced osteoarthritis model of the mouse.

Methods: Chronic arthritis was induced by complete Freund's adjuvant (CFA), knee osteoarthritis by MIA in CX3CR1 gene-deficient (CX3CR1-/-) and C57BL6/J wild-type mice (n=6-13/group). Mechanonociception was determined by aesthesiometry, paw volume by plethysmometry, knee joint swelling by digital caliper, neutrophil myeloperoxidase (MPO) activity by luminescence, plasma extravasation and matrix metalloproteinase (MMP) activity by fluorescence in vivo imaging, histopathological alterations by semiquantitative scoring, and glia activation by immunohistochemistry. Statistical analysis was determined using Kruskal-Wallis test followed by a Dunn's multiple comparison test (in case of histopathological alterations) and ordinary or repeated measures 2-way ANOVA followed by a Sidak's multiple comparison test (in another cases). Effect sizes were also determined using Hedges' g in all cases.

Results: 20-40% mechanical hyperalgesia ($p<0.05$ vs. saline-injected group, $g>0.8$), 20-50% paw edema ($p<0.05$ vs. saline-injected group, $g>0.8$), neutrophil MPO activity ($p<0.0001$ vs. contralateral paw, $g>0.8$) and plasma extravasation increase ($p<0.05$ vs. contralateral paw, $g>0.8$), histopathological damage (mononuclear cell infiltration, synovial hyperplasia, cartilage destruction, $p<0.05$ vs. saline-injected group, $g>0.8$) and on day 3 astrogliosis in L4-L6 spinal dorsal horn ($p<0.05$ vs saline-injected group, $g>0.8$) were detected in CFA-injected wild-type mice. Mechanical hyperalgesia was lower in CX3CR1-/- mice between days 3 and 14 of the 21-day experimental period, but no differences were found in any inflammatory or tissue damage parameters. 30-50% mechanical hyperalgesia ($p<0.05$ vs. saline-injected group, $g>0.8$), 30% knee edema ($p<0.05$ vs. saline-injected group, $g>0.8$), reduction of dynamic weight distribution of the hind limb ($p<0.05$ vs. contralateral side, $g>0.08$), on day 1 neutrophil MPO activity ($p<0.0001$ vs. contralateral side, $g>0.8$) and on day 4 MMP activity increase ($p<0.01$ vs. contralateral side, $g>0.8$) on the affected side were observed in both wild-type and CX3CR1-/- mice. However, no differences were observed between the groups.

Conclusions: CX3CR1 activation mediates chronic arthritic, but not osteoarthritic pain, mainly independently of the peripheral inflammatory processes. Therefore, its inhibition offers promising novel analgesic perspectives.

Acknowledgement: NKFIH K 138046, RRF-2.3.1-21-2022-00015, TKP2021-EGA-16, Hungarian Research Network (Chronic Pain Research Group)

PP256. Role of TGF- β signalling and sex influence in a model of oxaliplatin-induced neuropathic pain in mice

Saguillo Velásquez A^{1,2}, Marques J^{1,3}, Silva E^{1,2}, López C⁴, Tramullas M^{1,2}

¹Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Santander, Spain, ²Instituto de Investigación Sanitaria Valdecilla (IDIVAL), Santander, Spain,

³Servicio de Rehabilitación, Hospital Universitario Marqués de Valdecilla (HUMV), Spain, ⁴Servicio de Oncología, Hospital Universitario Marqués de Valdecilla (HUMV), Spain

Chemotherapy-induced peripheral neuropathy (CIPN) is the most frequent complication of cancer treatment. CIPN is a complex pain syndrome that includes sensory symptoms, autonomic, and motor dysfunction. CIPN manifestations are often highly refractory to current analgesics, impacting the function and quality of life of patients. Transforming growth factors- β (TGF- β) constitute a large family of pleiotropic and multifunctional cytokines. Mice lacking the TGF- β pseudoreceptor BAMBI present an antiallodynic phenotype after sciatic nerve injury [1]. MicroRNAs (miRNAs) are small noncoding RNAs that modulate post-transcriptionally gene expression. Previous results of our group support a major role for miR-30c-5p in neuropathic pain development [2]. Our study aims to investigate whether BAMBI deficiency affects a) the establishment of CIPN in male and female mice and b) the impact of CIPN on the expression levels of miR-30c-5p in plasma and nervous tissue: spinal dorsal horn (SDH) and dorsal root ganglia (DRG).

CIPN was induced in C57BL6 and BAMBI-KO female and male mice by an oxaliplatin cycle administration consisting of five intraperitoneal injections (3mg/kg) on alternate days. Mechanical and thermal allodynia were assessed with von Frey and acetone tests, respectively. Plasma samples were obtained under basal conditions and on days 7 and 14 after oxaliplatin administration and, SDH and DRG on day 14, when maximal neuropathic pain-related behaviours were evident. All samples were processed for miR-30c quantification by qPCR.

Our results show that oxaliplatin-treated mice developed a sex-independent mechanical and thermal allodynia after two weeks of treatment, which was maintained for at least 4 weeks ($p<0.05$, N=8-15 per group). However, the response intensity was significantly lower in BAMBI-KO mice compared to C57BL6 mice ($p<0.05$). The expression levels of miR-30c-5p were dysregulated in plasma ($p<0.05$), SDH ($p<0.05$), and DRG ($p<0.05$) in C57BL6 mice treated with oxaliplatin, but not in BAMBI-KO mice.

Our results highlight the key role of BAMBI pseudoreceptor in oxaliplatin-induced peripheral neuropathy development and the importance of the gender perspective in these pathological processes. Furthermore, the lack of BAMBI confers protection against the alterations of miR-30c-5p expression in the model of oxaliplatin-induced peripheral neuropathy.

Supported by PID2022-1364180BI00/AEI/10.13039/501100011033/ FEDER, UE and IDIVAL (INN-VAL 23/12)

[1] Tramullas, M. Lantero A, Díaz A, et al. BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) reveals the involvement of the transforming growth factor- β family in pain modulation. *J Neurosci*. 2010;30(4). doi:10.1523/JNEUROSCI.2584-09.

[2] Tramullas, M. Francés R, de la Fuente R, et al. MicroRNA-30c-5p modulates neuropathic pain in rodents. *Sci Transl Med*. 2018;10(453). doi:10.1126/SCITRANSLMED.AA06299

PP257. ADAR1 modulates cell death and neuroinflammation in Multiple Sclerosis revealing novel therapeutic targets

Pettas S^{1,2}, Kanata E², Karagianni K¹, Kyriakidis K¹, Bekas N¹, Sargiannidou I³, Kleopa K³, Xanthopoulos K², Sklaviadis T², Dafou D¹

¹*Department of Genetics, Development, and Molecular Biology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*, ²*Laboratory of Pharmacology, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*, ³*The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

Multiple Sclerosis (MS) is an immune-mediated central nervous system disease causing demyelination. Microglia play a dual role in MS, contributing to both inflammation and re/demyelination. RNA editing is a post-transcriptional process that changes the sequence of RNAs, most commonly resulting in Adenosine-to-Inosine conversion (A-to-I editing) mediated by the ADAR family.

Using an 'in-house' developed bioinformatic pipeline we established an MS microglial-enriched editome profile and identified significant reduction ($p<0.0001$, Mann-Whitney's U-test) of global editing during human disease progression (human autopsy material from affected and non-affected CNS regions, n=68 MS, n=25 controls). Pathway analysis on differentially edited transcripts highlighted the Necroptosis and NOD-like receptor signaling. We validated editing alterations, through targeted resequencing (MiSeq, n=9 MS samples, n=3 controls), of two cell death modulators: XIAP (inhibitor of apoptosis) and P2RX7 (ATP receptor that induces inflammatory necroptosis).

We have then quantified all ADAR editors in terms of their gene expression and the expression of our targets in correlation with their RNA editing frequency. Only ADAR1 was inversely correlated with XIAP and P2RX7 gene expression, indicating ADAR1 as the main editor.

We generated ADAR1 knock-out (KO) murine (BV2) and human (HMC3) microglial cell lines, using the CrispR-Cas9 method, to decode ADAR1 functional connection. Direct targeting of the 3' UTRs of XIAP and P2RX7, revealed altered protein expression affecting death modulation and neuroinflammation. ADAR1 KO cells showed an increased pro-inflammatory phenotype compared to controls after stimulation and inability to convert into an anti-inflammatory state. Next, transcript stability assay revealed a connection between ADAR1 and XIAP/P2RX7 mRNA stability. ADAR1 RNA-immunoprecipitation confirmed XIAP and P2RX7 binding during induced inflammation.

Our findings hold promise for treating MS, through the direct targeting and inhibition of XIAP and P2RX7. Inhibiting XIAP may aid in clearing damaged cells by promoting apoptosis, while P2RX7 inhibitors could limit tissue damage and inflammation by preventing necroptosis. These targeted approaches offer potential therapeutic interventions, although further research and clinical validation is needed.

PP258. Anthocyanin-rich extracts derived from wine byproducts exert neuroprotective and anti-inflammatory properties

Christoudia N¹, **Kanata E²**, Chatziefstathiou A¹, Schmitz M³, Zerr I³, Tsamesidis I⁴, Xanthopoulos K², Dafou D¹, Sklaviadis T²

¹*Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece*, ²*Neurodegenerative Diseases Research Group, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*,

³*Department of Neurology, German Center for Neurodegenerative Diseases (DZNE), University Medicine Göttingen, Göttingen, Germany*, ⁴*Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Introduction: Anthocyanin (ACN) neuroprotection is a topic that explores the beneficial effects of ACNs, which are natural pigments found in fruits and vegetables. They are safe compounds that can be easily supplemented through dietary intake, on the brain and nervous system. ACN have been shown to have antioxidant, anti-inflammatory, and anti-apoptotic properties, which may protect neurons from oxidative stress, inflammation and death. Prion diseases are fatal neurodegenerative disorders, characterized by the conversion of the normal prion protein (PrP^c) to its pathogenic isoform (PrP^{Sc}) with no available treatment yet.

Methods: We have established a GRAS (stands for generally recognized as safe) ACN industrial scale extraction from dried grape skins (from several varieties) with optimized factors that can affect the efficiency and quality of anthocyanin extraction (temperature, time, pH). Combining HPLC AND UV-Spectroscopy fraction characterization, we identified Oenin and Myrtillin as two of the most prevalent ACNs present in fractions. To assess their neuroprotective and anti-inflammatory activity, we utilized a well-accepted prion murine infected cell line, 22LN2a58 with matching non-infected (N2a58) controls and murine microglial BV2 cells.

Results: ACN-rich fractions reduced ROS production by ~75% in accordance with pure Oenin and Myrtillin when compared to stress levels induced by an H2O₂ pre-treatment in both neuronal and microglial systems. Next, neuroprotective effects were quantified following the ability of ACNs to reduce/prevent in vitro conversion/aggregation of recombinant PrP^c in RT-QuIC assays by using Cerebrospinal Fluid (CSF) from twelve sporadic Creutzfeldt–Jakob disease (sCJD) patients. Anti-prion effects were estimated in vitro, by ~30% significant reduction in aggregation of pathologic PrP^{Sc}, in 22LN2a58 cells. ACNs were also found to modulate various signaling pathways, such as Nrf2/HO-1, NF-κB and PI3K/Akt, and which are involved in neuronal survival, plasticity, and function.

Conclusions: Vinification byproducts which are usually discarded as waste, have high abundance of ACNs and are termed as a valuable source of natural antioxidants. ACNs-fractions exert pleiotropic neuroprotective effects, potentiating their use against prion diseases and other proteinopathies.

Funding: This research was carried out as part of the project «Utilization of anthocyanin extracts from vinification waste» under the framework of the Action «Investment Plans of Innovation» of the Operational Program «Central Macedonia 2014 - 2020», that is co-funded by the European Regional Development Fund and Greece. Grant Number: KMP6-0079465.

PP259. Limonene ameliorates acute renal inflammatory injury induced by Lipopolysaccharide by targeting the TLR4/AP1/IRF3/NF-κB pathway in mice

Kathem S¹, Shareef S²

¹*University of Baghdad, Baghdad, Iraq*, ²*Al-Esraa University, Baghdad, Iraq*

Background: Acute kidney injury (AKI) is a serious disease with rapid onset and a high mortality rate. It is therefore particularly important to identify a suitable method for treating AKI. A large body of evidence supposes that the inflammatory response is a crucial characteristic of sepsis as a direct cause of AKI. Consequently, LPS-induced AKI has been broadly utilized as one of the most prevalent animal models for elucidating the processes behind AKI induced by sepsis. D-limonene is a monoterpenoid, present in citrus and numerous other plants. It has been revealed that D-limonene has wide-ranging anticancer, anti-inflammatory, and anti-oxidant properties. The present study aimed to investigate the effects of limonene in the treatment of sepsis-induced AKI and its molecular target.

Method: AKI induced in mice by LPS 10mg/kg intraperitoneally. Mice in the treatment groups received either 50, 100, or 200 mg/kg/day of oral Limonene for 5 consecutive days before LPS injection. Twenty-four hours after the LPS injection, mice were euthanized and samples were taken for measurement and analysis.

Results: Our results showed that treatment with small, medium, and high doses of Limonene rescued renal function. Treatment with Limonene (200 mg/kg) in mice revealed a significant lowering of serum urea (36.26 ± 2.90 vs. 116.35 ± 17.35 mg/dl) and creatinine (19.05 ± 1.34 vs. 80.99 ± 2.60 $\mu\text{mol/L}$) compared to non-treated mice. KIM-1 gene expression as a marker of renal function was also reduced by limonene significantly compared to non-treated mice (19.90 ± 2.28 vs. 54.95 ± 7.38 folds). To evaluate the effect of limonene on renal inflammatory injury, the levels of TNF- α , IL-1 β , and iNOS were measured and analyzed in renal tissue. Our results revealed that limonene exerted a strong anti-inflammatory effect by significantly attenuating the levels of TNF- α (244.07 ± 1.63 vs. 655.97 ± 19.13 ng/L) and IL-1 β (11.20 ± 2.29 vs. 28.64 ± 1.37 ng/L). In addition, the level of gene expression of iNOS was significantly downregulated (12.11 ± 1.68 vs. 40.87 ± 5.59 folds) compared to nontreated mice. We further investigated the inflammatory signaling pathway induced by LPS, and our results uncovered that limonene significantly downregulated gene expression of TLR4 (1.79 ± 0.24 vs. 15.74 ± 2.92), AP-1 (1.22 ± 0.25 vs. 18.16 ± 1.98), IRF3 (4.20 ± 0.94 vs. 37.14 ± 2.99), and NF-κB (0.77 ± 0.41 vs. 21.70 ± 2.50) folds compared to nontreated mice in renal tissue. The mortality rate was also measured and provided further support for the reno-protective effect of limonene.

Conclusion: Our study revealed that limonene exerted remarkable renal protective and anti-inflammatory effects against LPS-induced AKI by attenuating inflammatory signaling molecules in both TLR4/Myd88 dependent and independent pathways.

PP260. Limonene exerts anti-inflammatory effect on LPS- induced jejunal injury in mice by inhibiting NF-κB/AP-1 pathway

Kathem S¹, Nasrawi Y²

¹*University of Baghdad, Baghdad, Iraq*, ²*Alzawhrawi University, Kerbala, Iraq*

Introduction: The intricate ecosystem of the human gastrointestinal system is crucial for nutrient absorption, immune function, and overall well-being. Within this complex network, the jejunum, a vital part of the small intestine, plays a pivotal role in the digestion and absorption of nutrients. In sepsis, the injury of the gut is a common pathophysiologic condition, which is proposed to be the main cause of serious illness due to the resultant malfunction of the intestinal barrier. Limonene, a monocyclic terpene, is known for its diverse array of biological properties and has gained significant attention for its potential therapeutic applications in various fields.

Aim: The study explored the protective and antiinflammatory effects of limonene against LPS-induced jejunal injury in mice.

Method: Twenty-four albino male mice were randomly divided into four groups (6 in each group): control group, LPS model group, 100mg/kg limonene plus LPS treatment, and 200 mg/kg limonene plus LPS treatment. Limonene was orally administrated for 4 continuous days. LPS was administered interperitoneally (10 mg/kg) on day 5 with limonene 24hrs before euthanization.

Results: Pretreatment of mice with limonene (both 100 mg/kg & 200 mg/kg) resulted in significant decline in TNF-α, iNOS, IL-1β and COX-2 gene expression (6.97 ± 1.14 & 2.81 ± 0.71 vs 35.35 ± 1.47), (9.82 ± 3.48 & 9.41 ± 1.87 vs 35.12 ± 6.99), (18.81 ± 3.14 and 12.28 ± 1.09 vs. 33.39 ± 1.87) & (6.41 ± 1.14 & 3.32 ± 0.504 vs 15.75 ± 2.43) compared to the non-treated LPS-induced mice, respectively. Interestingly, mice pre-treated with both doses of limonene exhibited a significant downregulation of TLR4 (6.48 ± 1.27 & 2.90 ± 0.20 vs 25.67 ± 4.16 folds) in jejunal tissue compared to non-treated animals. Furthermore, in this aspect both Myd88-dependent and independent pathways investigated by measuring NF-κB, AP-1 and IRF3. Limonene administration (100 & 200 mg/kg) resulted in a remarkable decline in gene expressions of NF-κB compared to non-treated mice (1.98 ± 0.95 & 0.48 ± 0.17 vs 24.61 ± 4.22). In the same context, mice treated with limonene (100 & 200 mg/kg) showed a significant attenuation in jejunal AP-1 expression compared to non-treated mice (3.50 ± 1.079 & 1.89 ± 0.56 vs 25.26 ± 8.78). In contrast, IRF3 expression didn't revealed any significant change.

Conclusions: The results revealed that limonene holds promise as a potential therapeutic agent for mitigating intestinal inflammation and preserving gastrointestinal health. Limonene showed a strong anti-inflammatory and protective effects by targeting Myd88 dependent pathway through TLR4/AP-1 / NF-κB pathway in the jejunum.

PP261. Development of silicone-based transdermal patches against postoperative and inflammatory pain conditions

Pintér E¹, Hajna Z¹, László S², Göntér K¹, Pozsgai G¹, Wagner Ö²

¹Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Hungary, Pécs, Hungary, ²Department of Inorganic and Analytical Chemistry, Faculty of Chemical Technology and Biotechnology, University of Technology and Economics, Budapest, Hungary, Budapest, Hungary

Introduction: Transdermal therapeutic systems (TTS) provide a convenient and painless dosing in drug delivery. Modified silicone-polymer-based patches are well-controlled and cost-effective matrix diffusion systems. We have previously developed a capsaicin containing TTS providing sustained release of that. Moreover, the capsaicin-induced increase of local microcirculation might facilitate the absorption of non-steroidal anti-inflammatory drugs (NSAIDs). Therefore, we aimed to investigate the analgesic effect of TTS containing capsaicin in low-concentration (<1%), or diclofenac or the combination of them in rat models of postoperative and inflammatory pain. We also investigated the release and skin penetration of the active ingredients.

Methods: Release properties were measured in Franz diffusion cell and continuous flow-through cell. Compounds were detected with HPLC-UV and UV spectrophotometry. Raman spectroscopy was used to analyse the penetration of capsaicin through the layers of the human skin exposed to the TTS. Postoperative pain was elicited with plantar skin-muscle incision and thermal hyperalgesia was assessed with increasing temperature water bath 18 h after surgery, as well as 2.5 h and 6 h after the application of the TTS. Inflammatory pain was induced with carrageenan (3%, i.pl.) and mechanical hyperalgesia was determined with dynamic plantar aesthesiometer 3 h after carrageenan treatment, as well as 2.5 h and 6 h after the application of the TTS.

Results: Patches exhibited controlled, zero-order kinetic release of the active ingredients. According to the Raman mapping, capsaicin penetrated into the epidermis and dermis of human skin where the target receptors are expressed. Thermal pain threshold drop was reversed by capsaicin treatment in the operated rat paws compared to the controls. Thermal hyperalgesia was decreased 2.5 h after the application of diclofenac-containing TTS and 6 h after the application of capsaicin-containing TTS. In the case of combined capsaicin-diclofenac containing TTS, thermal hyperalgesia was reduced both 2.5 h and 6 h after TTS application. Mechanical hyperalgesia was decreased 6 h after capsaicin TTS, as well as 2.5 h and 6 h after diclofenac and combined TTS.

Conclusions: Silicone-based TTS containing low-concentration capsaicin reduces postoperative pain. Capsaicin prolongs the short-term analgesic effect of diclofenac, if used in combination. Furthermore, combo capsaicin-diclofenac TTS is also effective in the relief of inflammatory pain. Because of the different mechanism of actions and pharmacokinetic features of the ingredients our combo TTS could be effective in various pain conditions.

Funding: TKP2021-EGA-13, Proof of Concept Research Grant, University of Pécs, KA-2023-07

PP262. *Smilax domingensis* regulates carbohydrate and lipid metabolism through dual agonist action on PPARs

Ortiz Barragan J¹, Almanza Pérez J², Giacoman Martinez A², Alarcón Aguilar F², Estrada Soto S³

¹Posgrado en Biología Experimental, Universidad Autónoma Metropolitana-Iztapalapa, Vicentina, Iztapalapa., Ciudad de México, México, ²Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Vicentina, Iztapalapa, Ciudad de México, México, ³Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Chamilpa, Cuernavaca, México

Introduction: *S. domingensis* is used in Mexican traditional medicine to diabetes mellitus treatment. Some reports showed the antihyperglycemic effect, and phytochemical analysis suggested the presence of alkaloids, coumarins, tannins, saponins, triterpens and flavonoids. These could have an agonist activity at PPARs [2]. PPARs regulate lipid and carbohydrate metabolism, these participate in reestablishing metabolic homeostasis [3]. Thus, the aim of this investigation was to determine the effect of *S. domingensis* and isolated compounds in PPAR α and PPAR γ activity.

Methods: The *S. domingensis* extract was fractionated by column chromatography. Gene expression of PPARs, GLUT-4 and FATP were quantifying by RT-qPCR in C2C12 myocytes treated with the fractions. Lipid accumulation in 3T3-L1 adipocytes and the effect on translocation of GLUT-4 in C2C12 myocytes by immunofluorescence were evaluated. The most active fraction was analyzed by nuclear magnetic resonance (NMR) and the majority molecule was evaluated by molecular docking to PPAR α and PPAR γ . Luteolin, was isolated, identified and evaluated in genic expression of PPARs. The experiments were carried out in duplicate with n=5 and were analyzed by ANOVA p<0.05

Results: The fractions F3 and F7 increased the PPAR α expression, 5.5 and 4 times, while the FATP expression increased twice. In addition, the fractions F3 and F6 increase the PPAR γ expression 5 and 3.8 times respectively and increased the GLUT-4 expression in 2.8 and 3.5 times respectively. A 21% decrease in lipid accumulation was observed at 24h and a 24.5% at 72h. Also, these fractions induced GLUT-4 translocation. Luteolin was identified in the most active fraction analyzed by NMR. Likewise, increase the PPAR γ expression (5 times), and the action in PPAR α agonist (-8.27 Kcal/mol.) and PPAR γ (-6.75 Kcal/mol.) was evaluated by molecular docking.

Conclusion: The results suggest a dual agonist activity of luteolin that could be associated with the decrease in lipid storage and GLUT-4 translocation.

- [1] Boeing, T., Speca, S., Souza, P., Mena, A. M., Bertin, B., Desreumax, P., Mota da Silva, L., Faloni de Andrade, S., Dubuqoy L. The PPAR γ -dependent effect of flavonoid luteolin against damage induced by the chemotherapeutic irinotecan in human intestinal cells. Chem. Biol. Interact. 2022. 351:109712. doi: 10.1016/j.cbi.2021.109712.
- [2] Loza-Rodríguez, H., Estrada-Soto, S., Alarcón-Aguilar, F. J., Huang, F., Aquino-Jarquín, G., Fortis-Barrera, Á., Giacoman-Martínez, A., & Almanza-Pérez, J. C. Oleanolic acid induces a dual agonist action on PPAR γ/α and GLUT4 translocation: A pentacyclic triterpene for dyslipidemia and type 2 diabetes. Eur. J. Pharmacol. 2020. 883:173252. doi:10.1016/j.ejphar.2020.173252.

PP263. A bioguided study on Artemisia species extracts from Greece reveals promising antileishmanial sesquiterpene lactones

Karoutzou O¹, Kalpoutzakis E¹, Smirlis D², Skaltsounis L¹

¹*Division of Pharmacognosy and Chemistry of Natural Products, Pharmacy Department, National and Kapodistrian University of Athens, Athens, Greece*, ²*Molecular Parasitology Laboratory, Microbiology Department, Hellenic Pasteur Institute, Athens, Greece*

Introduction: According to WHO, one billion people are at risk of Leishmania infection while it is estimated that there are 12 million infected people worldwide [1]. Artemisia species have been used as traditional medicine since ancient times for various purposes [2], including parasitic diseases. In this work, we sought to investigate the antileishmanial activity of 5 Artemisia species from Greece. The most active extract was then analysed and fractionated leading to the isolation of 10 compounds from different chemical classes, which were tested against *Leishmania infantum* promastigotes in order to determine the extract's active components.

Methods: Ultrasonication with ethyl acetate was used for the preparation of the extracts. For the isolation of the secondary metabolites Countercurrent Partition Chromatography, Column Chromatography, Preparative-HPLC and Preparative-TLC were used. All the structures and configurations were elucidated by extensive 1D- and 2D-NMR spectroscopic analysis in combination with MS experiments, and comparison with literature data. *Leishmania infantum* promastigotes (strain GH14, MHOM/GR/2003/GH14) were cultured at 26°C in RPMI 1640 (RPMI) medium supplemented with 10 mM HEPES, penicillin/streptomycin (final concentration 100 U ml⁻¹) and 10% (v/v) heat-inactivated fetal bovine serum. The inhibitory activity was determined with the use of the Resazurin viability assay by measuring the optical density (wavelength: 550 nm, reference wavelength: 620 nm). The half maximal effective concentration (EC₅₀) of the isolated compounds was calculated using a nonlinear regression curve fit with increasing concentrations of the compound. Amphotericin and miltefosine were used as reference drugs.

Results: The total ethyl acetate extract of *Artemisia umberlliformis* subsp. *eriantha* showed an IC₅₀ of 12 µg/mL against *Leishmania infantum* promastigotes and a good selectivity index (THP-1 macrophages IC₅₀=80 µg/mL). After testing the isolated compounds from this extract against *Leishmania infantum* promastigotes, the sesquiterpene lactones telekin and 5-deoxy-5-hydroperoxy-telekin were the most active with an EC₅₀ of 5.1 µM and 4.5 µM respectively. 5-deoxy-5-hydroperoxy-telekin, which also presented a good selectivity index of 6.7 against THP-1 macrophages, was then tested against *Leishmania infantum* amastigotes to give an EC₅₀ of 2.5 µM.

Conclusions: 5-deoxy-5-hydroperoxy-telekin, a eudesmane type sesquiterpene lactone derived from *Artemisia umbelliformis* subsp. *eriantha*, exhibited strong antileishmanial activity against promastigote and amastigote form, along with a good selectivity index, making this compound a promising antileishmanial agent.

[1] Alvar J, Velez I, Bern C, et al. (2012). PLoS ONE, 7(5), 1-12

[2] Nigam M, Atanassova M, Mishra AP, et al. (2019). Nat Prod Commun, 14(7)

PP264. Cannabidiol's antipsychotic effects on two distinct pharmacological models of schizophrenia

Antoniou K¹, Brakatselos C¹, Asprogerakas M¹, George Ntoulas G¹, Tsarna O¹, Messinis A¹, Delis F¹, Polissidis A²

¹*Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece*, ²*Center of Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece*

Cannabidiol (CBD), a non-addictive compound of Cannabis sativa is reported to present antipsychotic properties in clinical and preclinical research, but the mechanisms involved are poorly understood. The lack of experimental schizophrenia models with construct validity dictates the use of multi-model approaches for mapping the antipsychotic properties of new compounds. Chronic d-amphetamine (AMP) treatment is used as model of the aberrant dopaminergic neurotransmission in schizophrenia. On the other hand, repeated ketamine (KET) administration has been used to simulate NMDA receptor dysfunction that is manifested in schizophrenia. This study aims to evaluate the antipsychotic effects of CBD in both models, emphasizing on behavioral phenotype and the underlying mechanisms.

Male adult Sprague-Dawley rats have been treated intraperitoneally (i.p.) with 30 mg/kg/day KET or saline (SAL) for 10 days. Another subset of rats has been treated with escalating doses of AMP (1-8 mg/kg i.p.) twice daily for 5 days. Subsequently, rats received CBD (10 mg/kg/day, i.p.), or vehicle (VEH) for 5 days. Consequently, rats underwent a battery of behavioral tests including motor activity, cognitive and social interaction tasks. The neurochemical substrate concerning dopaminergic, glutamatergic, and GABAergic activity in specific brain areas involved in the neurobiological substrate of schizophrenia was investigated using HPLC-ED. Protein expression levels of glutamatergic receptors and downstream signaling were evaluated with western blot. Additionally, the density and functional state of specific interneuron subtypes has been assessed using immunofluorescence.

KET- and AMP-treated rats displayed hyperlocomotion ($p<0.01$) and oversensitivity to d-amphetamine ($p<0.01$), while KET-treated animals displayed impaired recognition memory ($p<0.01$) and social dysfunction ($p<0.01$) that CBD was able to mitigate ($p<0.01$). Neurochemical analysis revealed differentiated dopaminergic and glutamatergic dysfunction profiles in the two models that CBD was able to modulate in a region-dependent manner ($p<0.01$). Additionally, KET model disrupted NMDA receptor protein expression, parvalbumin interneuron immunoreactivity, and CB1 receptor expression in cholecystokinin interneuron synapses ($p<0.001$). CBD was able to mitigate ($p<0.01$) the abovementioned alterations with emphasis on the PFC.

This study pinpoints the neurobiological diversity between classic preclinical approaches of schizophrenia and enhances our understanding of the neurobiological underpinnings of widely used models of schizophrenia. Importantly, it also highlights the behavioral and neurobiological basis of CBD's antipsychotic action in a detailed action with symptomatology relevance and neuroanatomical characterization, enriching our understanding of the antipsychotic potential of CBD.

Funding: The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 1203).

PP265. Modulatory Effects of Cannabidiol on the Behavioral and Neurobiological Alterations in a Rat Model of Fragile X Syndrome

Antoniou K¹, Ntoulas G¹, Nakas G¹, Messinis A¹, Brakatselos C¹, Tzimas P³, Halabalaki M³, Polissidis A²

¹*Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece*, ²*Center of Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece*, ³*Department of Pharmacognosy and Natural Product Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece*

Fragile X Syndrome (FXS) is the most common monogenic cause of autism spectrum disorder (ASD) characterized by cognitive and behavioral deficits. Preclinical and clinical data support the ameliorating effects of cannabidiol (CBD) in autism-related symptomatology, but the underlying mechanisms remain elusive. In this study we are evaluating the effects of CBD administration on a genetic rat model of FXS.

Male Long-Evans FMR1 KO or WT rats have been treated with CBD (30 mg/kg, i.p) or Vehicle (n=8 per group) and behavioral paradigms including motor activity in the Open Field test, and recognition and spatial memory using Object Recognition Task (NORT) and Novel Object Location Task (NOLT), were evaluated. In parallel, indices of glutamatergic, GABAergic and dopaminergic function were investigated in the dorsal and ventral hippocampus and prefrontal cortex.

Our results have shown that the Fmr1 KO rats exhibited a hyperactive motor profile (WT/VEH vs KO/VEH: p***= 0.001) and cognitive deficits (WT/VEH vs KO/VEH: p**=0.01). Administration of CBD induced a reduction in motor activity (KO/VEH vs KO/CBD30: p**=0.01). Specific alterations in the glutamatergic, GABAergic and dopaminergic function observed in Fmr1 KO rats were modulated by CBD administration (WT/VEH vs KO/VEH: p**=0.05, KO/VEH vs KO/CBD30: p**=0.01).

These findings suggest that CBD has an impact on the behavioral and neurobiological profile of Fmr1 KO rats. The effects of CBD on these FXS animal model might have important implications for amelioration of FXS-related cognitive and behavioral abnormalities. Present results contribute to issues related to the therapeutic interventions of phytocannabinoids on ASDs.

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE project code: T2EDK-02075

PP266. Antidiabetic and anti-inflammatory action of 4-HBA and B-sitosterol, isolated of *Cucurbita ficifolia*

Almanza-Perez J¹, Rosiles-Alanis W², Pazaran-Aguinaga I¹, Ortíz Barragán E¹, Giacoman-Martínez A¹, Alarcón-Aguilar F¹, Zamilpa A³

¹*Universidad Autónoma Metropolitana, Mexico, Mexico*, ²*FES-Iztacala UNAM, Mexico, Mexico*, ³*CIBIS-IMSS, Xochitepec, Mexico*

Introduction: *Cucurbita ficifolia* Bouché is used in traditional medicine as an alternative treatment for diabetes [1]. Its antidiabetic-related effects have been studied previously, where hypoglycemic and antihyperglycemic effect have been described [2], along with insulin secretion, glycogen storage, and antiinflammatory effect. Nevertheless, the phytochemical composition and compounds responsible for these beneficial effects have not been yet conclusive. The present study has focused on conducting a bio-guided phytochemical study to elucidate the molecules with potential hypoglycemic/antihyperglycemic effect.

Methods: Three different preparations of *C. ficifolia* were evaluated in vivo, to choose the most effective and perform a subsequent separation. Fractions and sub-fractions were administered in RINm5F pancreatic cells and C2C12 myocytes, as biological guidance models. 4-Hydroxybenzoic acid and B-sitosterol were evaluated in C2C12 and RINm5F. Additionally, cytokines expression and release were quantified on macrophages RAW 264.7 treated with both compounds. The experiments were carried out in duplicate with n=5 and were analyzed by ANOVA p<0.05

Results: Ethyl acetate fraction derived from aqueous extract showed antihyperglycemic effect in OGTT and was further separated, β-sitosterol and 4-Hydroxybenzoic acid were identified as active compounds that increased insulin secretion, GLUT4, PPAR γ and Adiponectin mRNA expression. Additionally, 4-HBA decrease the TNF- α and IL-6 expression and release. and B-sitosterol decrease IL-6 and IL-1B expression and release. Both compounds increase IL-10 expression and release. Molecular docking revealed potential as partial agonists over GPR40 and PPAR γ receptors.

Conclusion: These compounds could be responsible for antidiabetic and anti-inflammatory effect that *C. ficifolia* exerts, being good candidates as a basis for the design of new anti-diabetic drugs.

[1] Alarcon-Aguilera FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. *J. Ethnopharmacol* 1998. 61:101–110. doi:10.1016/s0378-8741(98)00020-8

[2] Díaz-Flores M, Angeles-Mejia S, Baiza-Gutman LA, Medina-Navarro R, Hernández-Saavedra D, Ortega-Camarillo C, Roman-Ramos R, Cruz M, Alarcon-Aguilar FJ. Effect of an aqueous extract of *Cucurbita ficifolia* Bouché on the glutathione redox cycle in mice with STZ-induced diabetes. *J. Ethnopharmacol* 2012. 144:101–108. doi:10.1016/j.jep.2012.08.036

PP267. Pharmacological modulation of Nrf2 pathway in α -synuclein mouse model of Parkinson's disease

Gardoni F¹, Salvadè M¹, Zianni E¹, DiLuca M¹

¹*Università degli Studi di Milano, Milano, Italy*

Parkinson's Disease (PD) is the second most common neurodegenerative disorder. Multiple mechanisms contribute to PD pathogenesis with a clear involvement of inflammatory events, oxidative stress and mitochondria dysfunctions in disease progression. In addition, accumulating evidence demonstrated that misfolded proteins and inclusions contribute to the pathology of familial and sporadic PD. Alpha-synuclein (α -syn) is the main component of these inclusions. Unmet needs in the field include the availability of accurate biomarkers and disease-modifying drugs.

Nrf2 transcription factor is a master regulator of cell protection, involved in a defensive response, which embraces key pathogenic events in PD. Preclinical studies supported the centrality of Nrf2 pathway in pathogenic cascade of PD, specifically highlighting its protective role. However, only few studies are currently available addressing cellular mechanisms by which Nrf2 regulates neuronal homeostasis and neurodegeneration caused by α -syn. Of relevance, the role of Nrf2 in modulating α -syn-driven neuroinflammation and synaptic toxicity is almost unknown. Importantly, since several small molecules modulating Nrf2 activity are available, Nrf2 pathway could represent a target for novel disease-modifying treatments: Nrf2-modulating drugs are already validated *in vivo*, at different phases of clinical development. Thus, Nrf2 represents one of the first targets fully embraced by classic and systems medicine approaches to facilitate both drug development and drug repurposing and therefore may rapidly gain a concrete transferability to patients. For instance, the repurposing of dimethyl fumarate (DMF), a potent Nrf2 activator, already marketed as an oral drug for relapsing forms of multiple sclerosis, offers a compelling rationale and unique opportunity to design novel clinical trials.

Here we used state-of-the-art α -syn mice model allowing for a careful evaluation of the toxic effects induced by α -syn preformed fibrils (α -syn-PFFs) from early to late stages of disease. A combination of confocal imaging, biochemistry, molecular biology and behavioral assays will be used for the analysis of the progression of the disease and to evaluate the efficacy of DMF to counteract α -syn-mediated toxicity.

This work has been supported by Fondazione Regionale per la Ricerca Biomedica (Regione Lombardian), project ID 3433068 - Acronym: LINKING PARK

PP268. Effects of MIA-602, GHRH receptor antagonist, on emotional disorders in mice

Libero M^{1,2,3}, Recinella L¹, Schally A⁴, Salvatori R⁵, Brunetti L¹, Leone S¹

¹"g. D'annunzio" University of Chieti-Pescara, Chieti, Italy, ²Instituto Maimónides de Investigación Biomédica de Córdoba, Córdoba, Spain, ³University of Cordoba, Córdoba, Spain, ⁴University of Miami Leonard M. Miller School of Medicine, Division of Medical/Oncology and Endocrinology, Miami, USA, ⁵The Johns Hopkins University School of Medicine, Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, Baltimore, USA

The role of growth hormone-releasing hormone (GHRH) in brain function has been suggested. Recent behavior studies by our group clearly demonstrate a powerful anxiolytic and antidepressant-like effects of a novel growth hormone releasing hormone (GHRH) antagonist of MIAMI class, MIA-690, probably related to modulatory effects on the inflammatory and oxidative status [1]. Our investigation in this work was focused on the potential beneficial effects of MIA-602, another recently developed GHRH antagonist on emotional disorders and examined how mood disorders are related to the endocrine system. In this context, the effects induced by MIA-602 were also analyzed in comparison to vehicle-treated mice with GH deficiency due to generalized ablation of the GHRH gene (GHRH knock out (GHRHKO)). The beneficial effect of MIA-602 on inflammatory and oxidative status and synaptogenesis resulting in anxiolytic and antidepressant-like effects could be related by increases of nuclear factor erythroid 2-related factor 2 (Nrf2) and of brain-derived neurotrophic factor (BDNF) signaling pathways in the hippocampus and prefrontal cortex. MIA-602 exhibited antinflammatory and antioxidant effects in ex vivo and in vivo experimental models, inducing anxiolytic and antidepressant-like behavior in mice subcutaneously treated for 4 weeks. Moreover, immunohistochemical and Western blot analyses suggested an evident activation of Nrf2, HO1, and NQO1 in the prefrontal cortex of both +/+ mice treated with MIA-602 (+/+ MIA-602) and homozygous GHRHKO (-/- control) animals. Finally, we also found significantly decreased COX-2, iNOS, NFkB, and TNF- α gene expressions, as well as increased P-AKT and AKT levels in +/+ MIA-602 and -/- control animals compared to +/+ mice treated with vehicle (+/+ control).

We hypothesize that the generalized ablation of the GHRH gene leads to a dysregulation of neural pathways, which is mimicked by GHRH antagonist treatment.

- [1] Recinella, L. et al. Antinflammatory, antioxidant, and behavioral effects induced by administration of growth hormone-releasing hormone analogs in mice. Sci Rep 10, 732 (2020).

PP269. Protective effects induced by an aqueous aged black garlic extract on prostate and bladder cancer

Libero M¹, Montero-Hidalgo A^{2,3,4,5}, Recinella L¹, Leone S¹, Luque R^{2,3,4,5}, Brunetti L¹

¹"G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy, ²Maimonides Institute of Biomedical Research of Cordoba (IMIBIC), Cordoba, Spain, ³Department of Cell Biology, Physiology and Immunology, University of Cordoba, Cordoba, Spain, ⁴Reina Sofia University Hospital (HURS), Cordoba, Spain, ⁵CIBER Physiopathology of Obesity and Nutrition (CIBERObn), Cordoba, Spain

Prostate (PCa) and bladder cancer (BCa) are two highly prevalent tumor pathologies characterized by chronic inflammation associated with tissue dysfunction that have become a global health problem due to its high mortality rate. Hence, it is crucial to identify more effective therapeutic agents for these pathologies. In this regard, the use of aged black garlic (ABG), which is obtained from fresh garlic fermented at high temperature and humidity, has significant beneficial effects on various pathologies, including cancer. In fact, some studies indicate that ABG-derived elements might exert antitumor actions on genitourinary tumors, especially PCa and BCa. Therefore, we aimed to characterize the potential therapeutic role and associated molecular mechanisms of ABG extract (ABGE) on PCa and BCa models. ABGE was analyzed by HPLC-DAD. Then, we evaluated the putative beneficial effects of ABGE (0-1 mg/mL) by carrying out different functional and molecular assays on normal-like, and PCa- and BCa-derived cells at different stages of cancer progression. Our results indicated that ABGE was rich in polyphenolic compounds, being gallic acid and catechin the most abundant compounds. The extract was able to modify different in vitro functional parameters of tumor aggressiveness in PCa- and BCa-derived cell lines in a dose-dependent manner. Furthermore, ABGE treatment altered critical signaling pathways related with tumor development/progression. Therefore, our results suggest that ABGE might be potentially used as a diet supplement for health promotion and a source of bioorganic compounds with antitumor properties in PCa and BCa patients.

PP270. Karnofsky index as a predictive factor in the pharmacotherapy of bone metastases cancer pain

Krtinic D^{1,2}, Nedin Rankovic G¹, Petkovic I^{3,2}, Cvetanovic A^{3,2}, Conic I^{3,2}, Lucic Prokin A⁴, Todorovic Mitic M², Radic M^{3,2}, Jovanovic H¹, Trajkovic H¹, Cevrljakovic M², Pejcic V⁵, Cekic M⁶

¹Department for Pharmacology with Toxicology, Faculty of Medicine, University of Nis, Nis, Serbia,

²Clinic for Oncology, University Clinical Center Nis, Nis, Serbia, ³Department for Oncology, Faculty of

Medicine, University of Nis, Nis, Serbia, ⁴Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia,

⁵Clinic for Physical Medicine and Rehabilitation, University Clinical Center Nis, Nis, Serbia, ⁶Clinic for Cardiovascular Diseases, University Clinical Center Nis, Nis, Serbia

Introduction: Tapentadol (3-dimethylamino-1-ethyl-2-methyl-propyl-phenolhydrochloride) is an opioid analgesic with a dual mechanism of action because it also acts by inhibiting the noradrenaline reuptake, which results in a reduction of incoming pain signals and an increase in descending pain inhibition. The Karnofsky index (KI) is one of the reliable indicators of survival in oncology patients with bone metastases. This index is determined by the attending physician at the time of the patient's examination. Symptomatic therapy of oncological patients with bone metastases, inevitably includes palliative radiotherapy of this changes. The aim of this study was to compare the predictive factor of survival in oncology patients with bone metastases who underwent only radiotherapy and those who, in addition to that type of treatment, also received tapentadol pharmacotherapy.

Patients and Methods: This study was conducted at the Oncology Clinic, University Clinical Center Nis, in a follow-up period of 3 months after approval by the local Ethics Committee. The patients who were included in the research were divided into two groups. The first group consisted of 30 patients with primary breast cancer and proven painful bone metastases with neuropathy, who were prescribed tapentadol orally and who were undergoing palliative radiation treatment. The second group consisted of 30 patients with the same diagnosis who were treated only with palliative radiation therapy. KI was determined for all patients in three follow-up moments - before the start of radiotherapy, when the patients of the first group were introduced to pharmacotherapy with long-acting oral tapentadol formulation; one month after the radiotherapy; two months after the radiotherapy.

Results: There was no statistically significant difference in the values of KI between the two examined groups at the beginning of the measurement ($\chi^2=5.876$; $p=0.118$) and after one month ($\chi^2=1.786$; $p=0.618$). After three months, it was determined that a greater number of patients from the Tapentadol group had KI 90 compared to patients from the control group ($\chi^2=6.092$; $p=0.046$).

Conclusion: Tapentadol represents an adequate pharmacotherapeutic solution with palliative radiation therapy for pain relief in oncologic patients with bone metastases. Palliative radiation therapy of these patients does not adequately control the neuropathic component of this pain, but it is achieved synergistically by prescribing long-acting tapentadol to these patients who are also undergoing radiotherapy treatment. KI has proven to be an excellent landmark that serves us as an orientation in supportive oncology therapy and assessment of the general condition of patients and response to prescribed therapy.

PP271. α -amyrin and lupeol, triterpenes from *Hibiscus sabdariffa* L. with anti-inflammatory effect and activity

Luna Hernández F¹, Giacoman Martínez A^{1,2,3}, Alarcón Aguilar F¹, Huang F², Almanza Pérez J¹

¹Laboratorio de Farmacología, Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Mexico City, Mexico, ²Laboratorio de investigación en Obesidad y Asma, Hospital Infantil de México "Federico Gómez", Mexico City, México, ³Sección de Estudios de Posgrado, Escuela Superior de Medicina, Instituto Politécnico Nacional, Mexico City, México

Introduction: Inflammation is a protective response of the body's tissues to irritation or injury, where macrophages play an important role, producing key cytokines. Although they are crucial for the resolution of damage, chronic inflammation favors the development of metabolic diseases such as obesity, type 2 diabetes and cardiovascular diseases [1]. Existing anti-inflammatory medications that are useful, however, their long-term use leads to unwanted side effects. This has motivated the search for new compounds with safer and more effective anti-inflammatory properties. In traditional Mexican medicine, *Hibiscus sabdariffa* L. is reported to be used to treat obesity and diabetes. Studies have identified in this plant the triterpenes α -amyrin and lupeol, dual agonists of PPAR δ/γ , which may be related to anti-inflammatory effects [2], and evaluate the possible anti-inflammatory effect and activity of these triterpenes in vivo and in vitro studies.

Methodology: Mice of the CD-1 strain were used to evaluate the anti-inflammatory effect, through topical administration of TPA in the ear, triterpenes was administrated a 10 mM. Macrophages from the RAW264.7 line were treated with LPS (1 μ g/mL) to induce an inflammatory response. Subsequently, the cells were treated with the compounds α -amyrin [10 μ M] and lupeol [10 μ M] for 24 hours. Secreted cytokines were quantified in medium culture by the ELISA method. Cytokines expression were analyzed by real-time RT-PCR.

Results: α -amyrin and lupeol reduce ear edema caused by TPA; α -amyrin generates 68% inhibition and lupeol 85% compared to the CT. Both compounds even have a greater anti-inflammatory effect than positive CT, Indomethacin. On the other hand, these triterpenes reduce the expression of TNF- α and IL-6, in addition to increasing IL-10 compared to the CT, generating an effect like positive CT, Celecoxib. Likewise, both compounds decrease the secretion of proinflammatory cytokines in cultured RAW 264.7 macrophages.

Conclusions: α -amyrin and lupeol are compounds with anti-inflammatory potential, modulate the expression and secretion of pro- and anti-inflammatory cytokines. It is important to continue with the elucidation of the mechanism of action of these triterpenes, with the aim of proposing them for the development of new drugs.

[1] Giacoman-Martínez A, Alarcón-Aguilar FJ, Zamilpa A, et al. Triterpenoids from *Hibiscus sabdariffa* L. with PPAR δ/γ Dual Agonist Action: In Vivo, in Vitro and in Silico Studies. *Planta Med.* Published online 2019. doi:10.1055/a-0824-1316

[2] Chung HY, Kim DH, Lee EK, et al. Redefining chronic inflammation in aging and age-related diseases: Proposal of the senoinflammation concept. *Aging Dis.* 2019;10(2):367-382. doi:10.14336/AD.2018.0324

PP272. Striatal astrocytes of neonatal and adult rats display distinct kinetic and molecular characteristics of dopamine uptake

Sočan V¹, Kržan M¹

¹*Institute of Pharmacology and Experimental Toxicology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

Introduction: Astrocytes play crucial roles in maintaining homeostasis, regulating synaptic transmission, and influencing neuronal development and plasticity. Despite their involvement in various functions, the specific contribution of astrocytes to dopamine homeostasis remains underexplored. Astrocytes have been shown to express dopamine receptors as well as to contribute to the clearance of dopamine by multiple transporters, such as the high-affinity, low-capacity dopamine and norepinephrine transporters (DAT and NET) and various low-affinity, high-capacity transporters such as the organic cation transporter 3 (OCT3) and plasma membrane monoamine transporter (PMAT). Our study aimed to investigate the role of adult and neonatal rat astrocytes in dopamine uptake.

Methods: Primary astrocyte cultures from neonatal and adult Wistar rats' striata were used, and kinetic and molecular characteristics of dopamine uptake were examined in confluent cell cultures in 12-well plates. The kinetic characteristics of dopamine uptake, including time-, temperature- and concentration-dependence, were investigated using radiolabelled [³H]-dopamine. qPCR was used to evaluate mRNA expression of transporters involved in astrocyte dopamine uptake. Additionally, astrocyte cultures were subjected to 24-hour treatment with 100 µM concentration of dopamine receptor agonist apomorphine, dopamine receptor antagonist haloperidol and dopamine precursor L-DOPA.

Results: [³H]-dopamine was taken up in a temperature-, time- and concentration-dependent manner and was significantly inhibited by 1mM ouabain in both neonatal and adult rat striatal astrocyte cultures. Specific [³H]-dopamine uptake displayed distinct saturation kinetics in adult and neonatal rat striatal astrocytes. qPCR analysis showed prominent mRNA expression of NET and PMAT in both neonatal and adult rat astrocytes, whereas low level mRNA expression of all OCT isoforms and DAT was detected. Dopamine uptake was significantly inhibited by GBR12909 and desipramine and only modestly impeded by decynium 22 (D22) in neonatal rat striatal astrocytes, whereas in adult rat striatal astrocytes dopamine uptake was potently inhibited by desipramine, nortriptyline and D22. mRNA expression of NET and PMAT was significantly upregulated in adult rat astrocytes in response to apomorphine, while only NET mRNA expression exhibited changes in neonatal astrocytes. Haloperidol and L-DOPA did not induce significant alterations in transporter mRNA expression.

Conclusion: Overall, our findings suggest that adult rat astrocytes possess multiple carrier-operated dopamine uptake systems involving NET and PMAT. In contrast, neonatal rat striatal astrocytes may mediate dopamine uptake through DAT, NET, and PMAT. Apomorphine induced changes in mRNA expression of various dopaminergic targets, highlighting the need for further exploration of these molecular mechanisms.

PP273. The fractalkine receptor 1 (CX3CR1) mediates hyperalgesia and neuroinflammation the passive transfer-trauma mouse model of complex regional pain syndrome

Tékus V^{1,2,3}, Szentes N^{1,3,4}, Dénes Á⁵, Sensi S^{6,7}, Neiland H^{6,7}, Goebel A^{6,7}, Helyes Z^{1,3,4,8}

¹*Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary*, ²*Faculty of Health Sciences, Department of Laboratory Diagnostics, University of Pécs, H-7624, Pécs, Hungary*, ³*Hungarian Research Network, University of Pécs, Pécs, Hungary*, ⁴*National Laboratory for Drug Research and Development, Budapest, Hungary*, ⁵*Momentum Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary*, ⁶*Pain Research Institute, University of Liverpool, Liverpool, United Kingdom*, ⁷*Department of Pain Medicine, The Walton Centre National Health Service Foundation Trust, Liverpool, Hungary*, ⁸*PharmInVivo Ltd., Pécs, Hungary*

Background and aims: Complex Regional Pain Syndrome (CRPS) is a severe, chronic pain condition, which develops after a small injury. The most common symptoms are hyperalgesia, edema and autonomic disorders. Autoimmunity, complex sensory-immune-vascular interactions and neuroinflammation are involved in its pathophysiology. Since its therapy is unsatisfactory, it is necessary to identify novel therapeutic targets. We investigated the role of the fractalkine inflammatory chemokine receptor 1 (CX3CR1) expressed on microglia and macrophages in our previously developed and characterized CRPS passive transfer-trauma mouse model.

Methods: Female C57Bl/6 mice (6-8/groups) were treated i.p. daily with purified IgG from CRPS patients or healthy volunteers. Plantar skin-muscle incision was performed to model the microinjury. The role of the CX3CR1 receptor was investigated by gene-deficient mice and the selective receptor antagonist AZD 8797 (80 µg/kg i.p./day). The paw mechanonociceptive threshold was measured by dynamic plantar aesthesiometry and volume by plethysmometry, astrocyte and microglia in pain-related central nervous system regions by glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1) immunohistochemistry.

Results: CRPS IgG significantly increased plantar incision-induced mechanical hyperalgesia by 40-50% throughout the 7-day experiment. Both CX3CR1 deficiency and antagonist treatment significantly reduced CRPS IgG-induced mechanical hyperalgesia. Genetic deletion of CX3CR1 reduced microglia activation and increased astrocyte activation in the periaqueductal gray matter and the somatosensory cortex in both treatment groups compared to wild-type mice. Antagonist treatment did not affect the levels of reactive microglia, but reduced the number of astrocyte cells in the periaqueductal gray matter.

Conclusions: CX3CR1 activation mediates CRPS-associated chronic pain presumably via neuroinflammation. Therefore, CX3CR1 inhibition may represent novel analgesic perspectives in this primary chronic pain condition.

Support: National Brain Research Program 3.0, OTKA K-138046; OTKA FK-146283, TKP2021-EGA-16, RRF-2.3.1-21-2022-00015

PP274. Evaluation of medication protocols for asthma patients admitted to a tertiary healthcare institution

Basbug Y¹, Kirmizi Sonmez N², **Vizdiklar C³**, Akici A³

¹*Department of Pulmonary Medicine, Haydarpasa Numune Research and Training Hospital, Istanbul, Turkey*, ²*Department of Pharmacology, School of Pharmacy, Bahcesehir University, Istanbul, Turkey*, ³*Department of Medical Pharmacology, School of Medicine, Marmara University, Istanbul, Turkey*

Introduction: Asthma is known to affect up to one-fifth of the population in different countries [1]. Establishing protocols in compliance with asthma guidelines is crucial for rational treatment. We aimed to evaluate the admissions to a tertiary care center for asthma, where a medication protocol was set up.

Methods: Direct or indirect admissions of ≥18-year-old patients to the pulmonary medicine department of a tertiary healthcare institution in Istanbul between 01.08.2022 and 31.08.2023, diagnosed with asthma and subsequently prescribed a medication treatment protocol, were retrospectively examined (n=1700). The patients were stratified into two age groups, non-elderly (18-64 years old) and elderly (≥ 65 years old), and their demographic characteristics were assessed. The distribution of the drugs prescribed to the patients was determined according to the Anatomical Therapeutic Chemical (ATC) classification. Additionally, the details of the medication protocol were compared by patient demographics.

Results: The average age of the patients admitted for asthma and had medication protocols created for was 54.3 ± 15.7 years, of which 72.8% were non-elderly and 81.4% were women. Most of the protocols (93.2%) included inhaler drugs (ATC code: R03A/R03B), which were more frequently prescribed to women (93.8% vs. 90.5% to men, p<0.05). A minority of the protocols (2.0%) contained drugs unrelated to the respiratory system (drugs with an ATC-1 code other than R) and 10.8% contained additional diagnoses other than asthma. Protocols with additional diagnoses were created more frequently for women (11.6% vs. 7.3% for men, p<0.05). There was no difference between non-elderly and the elderly in the comparisons (p>0.05). The most commonly encountered drug in the protocols was the combination of salmeterol/fluticasone (34.4%), which was the same in the breakdowns by gender and age groups.

Conclusions: Our study revealed that adult patients admitted to a tertiary healthcare center with asthma diagnosis were frequently prescribed combinations of inhaled long-acting beta-2 agonists and glucocorticoids in accordance with the guidelines. Moreover, it was shown that medication prescribing patterns for asthma differed by gender but not by age group.

- [1] Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2022. Available from: <https://ginasthma.org/wp-content/uploads/2022/07/GINA-Main-Report-2022-FINAL-22-07-01-WMS.pdf>

PP275. Deer antler extract as an anticancer agent and drug delivery carrier

Xie Y¹, Kondratyeva M¹, Nazarbek G¹, Mu C², Shaimoldina A¹, Toktarov N³, Amin A⁴

¹Department of Biology, School of Sciences and Humanities, Nazarbayev University, Astana, Kazakhstan, ²Zhejiang University, Hangzhou, China, ³Kazakh Scientific Research Institute of Maral Husbandry, Beekeeping and Organic Agricultural Technologies, LLP, Ust-Kamenogorsk, Kazakhstan,

⁴University of Sharjah, Sharjah, UAE

Introduction: Deer antler extract as a natural product, which contains peptides, growth factors and chemical compounds has been applied in traditional medicine in anti-fatigue, anti-ageing, bone disease, and wound-healing due to multiple pharmacological functions [1]. Deer antler extract processing in traditional medicine may generate nano and microscale particles, which may further assemble to form a nanozyme or co-nanozyme [2].

Methods: Here we tested the particle characteristics by morphology analysis by SEM, TEM, and AFM and measuring enzyme activities. Network pharmacology and molecular docking were applied for target analysis [3]. Cell viability assay by crystal violet staining with dose at 1:20 or 1:30 of commercial product studied (from Katon-Karagal Deer Park Company) and MET inhibitor Crizotinib (Sigma) at concentration of 7.5 μ M were applied upon treatment of cells.

Results: Deer antler extract acts as regulation of peroxidase, co-nanozyme of phosphatase, and nanozyme of SOD and catalase activities (pH13). The particle scatter analysis showed the predominately 150-250 nm peak with variations. Moreover, the zeta potential suggests the negative charge upon processing shows the potent antler extract compound mediated inhibition of MET kinase. Experimental investigation validated the better efficacy of the combination of the extract with MET kinase inhibitor in anticancer. In addition, network pharmacology analysis also showed immune mechanisms in anticancer. Finally, autofluorescence of antler water extract suggests the potential in imaging of drug delivery. Using the deer antler extract as drug delivery medium, we encapsulated the PARP inhibitor (10 μ M) with positive charged carbon nanoparticles and showed the enhanced efficacy of the complex in cancer cells.

Conclusions: Our data suggest the potent deer antler extract both function as an anticancer agent but also as a drug encapsulation medium when co-targeting MET kinase or PARP signaling, with great potential in clinical application.

[1] Jo K, Jang WY, Yun BS, et al. Effect of Deer Antler Extract on Muscle Differentiation and 5-Aminoimidazole-4-Carboxamide Ribonucleoside (AICAR)-Induced Muscle Atrophy in C2C12 Cells. *Food Sci Anim Resour*. 2021;41(4):623-635. doi:10.5851/kosfa.2021.e20

[2] Benassi E, Fan H, Sun Q, et al. Generation of particle assemblies mimicking enzymatic activity by processing of herbal food: the case of 292olygon 292olygonate and other natural ingredients in traditional Chinese medicine. *Nanoscale Adv.* 2021;3(8):2222-2235. Doi:10.1039/d0na00958j

PP276. Identification of promising molecules for Hereditary Spastic Paraplegia treatment using predictive Drosophila models

Guarato G¹, Vantaggiato C², Dianin F¹, Rossato R¹, Gumeni S³, Bassi M², Orso G¹

¹*University of Padova, Padova, Italy*, ²*Scientific Institute IRCCS E. Medea, Bosisio Parini, Italy*,

³*National and Kapodistrian University of Athens, Athens, Greece*

Introduction: SPG15 and SPG11 are two subtypes of Hereditary Spastic Paraplegia (HSP), a rare and incurable neurodegenerative disorder marked by progressive corticospinal tract degeneration[1]. Spastizin (SPG15) and Spatacsin (SPG11) mutations cause lysosome enlargement, autophagic lysosome reformation (ALR) defects and autophagic cargo accumulation in patient-derived cells and SPG15 Drosophila. Our *in vitro/in vivo* pharmacological screen pinpointed lysosome regeneration as a crucial target for SPG15 rescue, with SMER28 emerging as the most effective[2]. As SMER28 is not in any clinical trial, we explored three alternatives—Tideglusib, Miglustat, and Naringenin—all in trials, to facilitate potential initiation of an SPG15 trial. Tideglusib is a brain permeable GSK3 β inhibitor that can activate TFEB. Naringenin regulates lysosomal calcium channels and has preclinical success in Alzheimer's and Parkinson's models. Miglustat, a glucosylceramide synthase inhibitor, under clinical investigation for SPG11 (NCT04768166), yielded disappointing outcomes. We compared them with SMER28 as potential lysosomal function modulators and to assess the pharmacological predictiveness of Drosophila models for HSP therapy.

Methods: We employed Drosophila RNAi-Dspastizin and tubulin-Gal4 strains for loss-of-function studies, conducting eclosion rate and climbing assays to reveal developmental and locomotor deficits. Confocal microscopy assessed autophagy and lysosome defects. ALR analysis followed established methods[2]. SMER28 (20 μ M), Tideglusib (5 μ M), Miglustat (1 μ M) and Naringenin (0.5mM), complexed with hydroxypropyl- β -cyclodextrin, were administered chronically in the fly food.

Results: *In vivo* intracellular analysis of autophagosome maturation and lysosome function revealed that Tideglusib and Naringenin ameliorate this phenotype, whereas Miglustat had no effect across all evaluated parameters, mimicking the negative results seen in the clinical trial. Tideglusib and Naringenin, similarly to SMER28, promoted autophagosome maturation ($p=0.0003$, $n=15$) and enhanced autolysosome degradation ($p<0.0001$, $n=15$). Naringenin reduced lysosome size and numerosity ($p<0.0001$, $n=15$). Indeed, only Naringenin reactivated autolysosome tubulation during ALR process ($p<0.0001$, $n=15$), thus restoring to control levels the locomotor function in adult flies ($p<0.0001$, 100 flies for each condition).

Conclusions: Here we proved our SPG15/SPG11 Drosophila models are predictive for *in vivo* effects of bioactive compounds, showcasing their value for upcoming screenings. Remarkably, Naringenin and SMER28 emerged as promising candidates for the treatment HSP forms with ALR defects.

- [1] Vantaggiato C, Panzeri E, Castelli M, et al. ZFYVE26/SPASTIZIN and SPG11/SPATAC SIN mutations in hereditary spastic paraplegia types AR-SPG15 and AR-SPG11 have different effects on autophagy and endocytosis. *Autophagy*. 2019;15(1):34-57. doi:10.1080/15548627.2018.1507438
- [2] Vantaggiato C, Orso G, Guarato G, et al. Rescue of lysosomal function as therapeutic strategy for SPG15 hereditary spastic paraplegia. *Brain*. 2022;139(4):16-17. doi:10.1093/BRAIN/AWAC308

PP277. Glucoraphanin a natural hydrogen sulfide donor exerts anti-inflammatory effect in macrophagic cells

Indolfi C¹, Saviano A², Esposito E², Marigliano N², Schettino A², Iqbal A³, Maione F², Mitidieri E², Sorrentino R², d'Emmanuele di Villa Bianca R²

¹*Department of Molecular Medicine and Medical Biotechnology, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy* ²*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy* ³*Institute of Cardiovascular Sciences (ICVS), College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom*

Introduction: Hydrogen sulfide (H₂S) is a signaling molecule endogenously produced from L-cysteine by the action of three enzymes: cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and 3-mercaptopropruvate sulfotransferase (3-MST) [1]. It plays an important role in inflammation, exerting anti-inflammatory effects [1]. Therefore, there is a growing interest in identifying natural H₂S donors [2]. In this view, glucoraphanin, one of the main glucosinolates found in cruciferous vegetables, has been recognized as a slow-releasing H₂S [3]. Here, we aimed to investigate the anti-inflammatory effect of glucoraphanin by using murine and human in vitro models of inflammation.

Methods: Cell viability was assessed in murine macrophage (J774) stimulated with glucoraphanin (3, 10, 30 μM for 24h). The anti-inflammatory effect of glucoraphanin (30 μM) was tested in J774 cells stimulated with lipopolysaccharide (LPS, 10 μg/ml) or vehicle. The canonical inflammatory signaling (iNOS, COX-2, IL-6, and PGE2) coupled with H₂S and NO levels were evaluated. Finally, the effect of glucoraphanin on human M1 and M2 macrophage polarisation was investigated.

Results: Glucoraphanin did not affect cell viability up to 30 μM (n=3). The treatment with glucoraphanin (30 μM) did not modify, under physiological or inflammatory conditions, the expression of CSE, CBS, and 3-MST (n=3). Glucoraphanin significantly inhibited LPS-induced iNOS and COX-2 expression (P<0.001 and P<0.01, respectively; n=3). IL-6 and NO levels but not PGE2 were significantly reduced after the treatment in J774 cells stimulated with LPS (P<0.05, P<0.01; n=3). As expected LPS challenge caused a significant reduction in H₂S levels in J774 cells whereas glucoraphanin treatment reversed it (P<0.05; n=3). Furthermore, glucoraphanin significantly reduced TNF-α content in M1 polarised macrophages (P<0.01; n=5) while IL-10 levels were increased in M2 phenotype (P<0.05; n=5).

Conclusions: Taken together our results suggest that glucoraphanin, a natural H₂S donor, could represent a promising new pharmacological approach in the treatment of inflammatory disorders as key regulator of “trained immunity”.

- [1] G. Cirino, C. Szabo, A. Papapetropoulos. Physiological roles of hydrogen sulfide in mammalian cells, tissues, and organs. *Physiol Rev.* 2023, 1;103(1):31-276.
- [2] A. Martelli, R. d'Emmanuele di Villa Bianca, G. Cirino, R. Sorrentino, V. Calderone, M. Bucci. Hydrogen sulfide and sulfaceutic or sulfanutraceutical agents: Classification, differences and relevance in preclinical and clinical studies. *Pharmacol Res.* 2023, 196:106947.
- [3] L. Gambari, M. Barone, E. Amore, B. Grigolo, G. Filardo, R. Iori, et al. Glucoraphanin Increases Intracellular Hydrogen Sulfide(H₂S) Levels and Stimulates Osteogenic Differentiation in Human Mesenchymal Stromal Cell. *Nutrients.* 2022, 14:435.

PP278. Merits and constraints of inflammatory and neuropathic rat models of trigeminal activation

Harmat M¹, Mohos V¹, Kitka T², Pintér E^{1,3,4,5}, Farkas S², Helyes Z^{1,3,4,5}

¹*Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary*, ²*Vascular Research Group, Budapest, Hungary*, ³*Hungarian Research Network, PTE HUN-REN Chronic Research Group, National Laboratory for Drug Research and Development, Budapest, Hungary*, ⁵*PharmInVivo Ltd., Pécs, Hungary*

Introduction: Primary headache diseases such as migraine are widespread severe pain conditions affecting a large population. The pathophysiological mechanisms behind primary headache syndromes are not fully understood, but activation and sensitization of trigeminal sensory neurons and neurogenic inflammation via the release of pro-inflammatory neuropeptides in the dura mater play important roles. Given the lack of optimal translational models, it is necessary to characterize and compare different *in vivo* paradigms to identify key mediators and novel therapeutic targets. Inflammatory and neuropathic orofacial or periorbital pain models are accepted surrogate models for investigating pain mechanisms related to primary headaches. Here we performed functional studies in three rat models of different origin.

Methods: Chronic orofacial inflammation and consequent allodynia was induced by subcutaneous injection of Complete Freund's Adjuvant (CFA) into the right whisker pad of adult male Sprague-Dawley rats. Meningeal inflammation and periorbital allodynia was evoked by supradural infusions of an "inflammatory soup" containing 2 mM histamine, bradykinin, serotonin, and 0.2 mM prostaglandin E2. Neuropathic allodynia was induced by partial ligation of the infraorbital nerve (pIONL model). The mechanonociceptive threshold values were measured using von Frey filaments.

Results: In the CFA model approximately 60% of all rats showed allodynia ($n = 29$), the threshold values decreased from 18.30 g to approximately 5 g, which lasted for 9 days. In case of the inflammatory soup infusion approximately 45% of the rats exhibited periorbital allodynia ($n = 13$) shown by mechanonociceptive threshold decrease from 18.30 g to around 12 g, with small variations throughout the 27-day experiments. In the pIONL model approximately 30% of the animals developed orofacial allodynia ($n = 36$) (threshold decrease from 18.30 g to 13 g) and the degree of sensitisation varied considerably between measurements lasting for 16 days.

Conclusion: All three models are appropriate for testing trigeminovascular activation-induced allodynia, therefore, they can all be used for investigating the pathophysiological mechanisms behind primary headache diseases. Since CFA induced the most severe and stable allodynia and in the highest number of animals, this model seems to be suitable for testing the effects of potential novel antimigraine drugs.

Acknowledgements: Supported by the Gedeon Richter's Talentum Foundation; the New National Excellence Program (ÚNKP-23-4-II), the National Brain Research Program 3.0 (NAP-3; Chronic Pain Research Group); the National Research, Development, and Innovation Fund of Hungary, under the EGA 16 funding scheme (TKP 2021-EGA-16) and by the European Union (Project no. RRF-2.3.1-21-2022-00015).

PP279. Effect of cannabidiol on dynorphinergic and BDNF systems in the CNS of neuropathic pain suffering rats

Rullo L¹, Morosini C¹, Losapio L¹, Boccella S², Luongo L², Posa L³, Maione S², Gobbi G⁴, Candeletti S¹, Romualdi P¹

¹Department of Pharmacy and Biotechnology, Alma Mater Studiorum, University of Bologna, Bologna, Italy, ²Department of Experimental Medicine, Pharmacology Division, University of Campania "L. Vanvitelli", Naples, Italy, ³Department Biochemistry, Weill Cornell Medicine, New York, US, ⁴Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University Health Center, McGill University, Montreal, Canada

Introduction: Chronic neuropathic pain is a complex experience characterized by maladaptive plasticity within neural networks. This pathological condition is frequently accompanied by emotional and cognitive impairments¹ which can affect painful experience. In this regard, recent studies highlighted the potential ability of cannabidiol (CBD) to reduce mechanical allodynia and anxiety-like behaviour predominantly through TRPV1 and serotonin (5-HT)_{1A} receptor activation, in a rat model of neuropathic pain². Given the role of the dynorphinergic system in pain-induced negative affect ^{1,3} and, since an interaction between this opioid system, the brain derived neurotrophic factor (BDNF) and 5-HT has been also suggested³⁻⁴, this study aimed to investigate the effects of the repeated CBD administration on dynorphinergic and BDNFergic alterations in the CNS of neuropathic pain suffering rats.

Methods: Male Wistar rats were subjected to the spared nerve injury (SNI) model of neuropathic pain and treated with CBD (5 mg/kg, s.c.) or vehicle from day 7 to 14 after SNI. At the end of treatments, pain aversion was assessed by conditioned place aversion paradigm. Then, brain areas were collected and mRNA levels for prodynorphin (pDYN) and KOR as well as for BDNF and TrkB were assessed in the dorsal raphe nucleus (DRN), anterior cingulate cortex (ACC), nucleus accumbens (NAc), by quantitative RT-PCR.

Results: Results showed that the repeated administration of CBD counteracted the increase of pDYN gene expression caused by SNI injury in the rat ACC and DRN. Moreover, CBD significantly reversed the SNI-induced increase of KOR gene expression in the NAc of neuropathic rats. In addition, a reduction of BDNF mRNA levels was assessed in DRN and NAc of SNI rats receiving the repeated CBD administration. The prolonged CBD treatment was also able to counteract the pain aversive component as detected by the conditioned place aversion test.

Conclusions: Together with previous behavioural results², these neurochemical data underline the potential therapeutic value of CBD in neuropathic pain. Indeed, these findings indicated that the repeated CBD treatment can be useful to revert some chronic pain-induced dynorphinergic and BDNFergic selective alterations^{1,3,5} known to be associated with the development of negative affective states.

- [1] Bushnell et al., Nat Rev Neurosci, 2013. 14(7):502-11.
- [2] De Gregorio et al., Pain, 2019. 160(1): 136–150.
- [3] Palmisano et al., Genes, Brain and Behaviour, 2019. 18(6):e12467.
- [4] Pomrenze et al., Neuron, 2022. 110(24):4125-4143.
- [5] Yu and Chen, Acta Pharmacol Sin., 2011. 32(1):3-11.

PP280. Topiramate showed anti-allodynic effect on the adjuvant-induced orofacial allodynia model

Mohos V¹, Harmat M¹, Kitka T², Farkas S², Pintér E^{1,3,4,5}, Helyes Z^{1,3,4,5}

¹*Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary*, ²*Vascular Research Group, Budapest, Hungary*, ³*Hungarian Research Network, PTE HUN-REN Chronic Research Group, Budapest, Hungary*, ⁴*National Laboratory for Drug Research and Development, Budapest, Hungary*, ⁵*PharmInVivo Ltd., Pécs, Hungary*

Introduction: Orofacial pain is a frequently occurring debilitating condition, which commonly develops due to the sensitization of extra- and intracranial trigeminal primary afferents [1, 2]. Orofacial inflammation activates the peptidergic, trigeminal sensory neurons, inducing mechanical allodynia/hyperalgesia [3]. As a large portion of patients are without appropriate therapy, it is important to identify new therapeutic approaches in validated animal models. Therefore, we aimed for the pharmacological validation of the Complete Freund's Adjuvant (CFA)-induced orofacial allodynia model with reference compounds (5-HT1B/1D receptor agonist: sumatriptan, mainly voltage-gated Na⁺ channel blocker: topiramate, mainly voltage-gated Ca²⁺ channel blocker: gabapentin) to prove its relevance for testing novel drug candidates.

Methods: Orofacial allodynia was induced by s.c. injection (0.5 mg/mL, 50 µL s.c.) of CFA into the right whisker pad of adult, male Sprague-Dawley rats (n=65), after which orofacial allodynia was measured using von Frey filaments. The effects of sumatriptan (1 mg/kg s.c., 0.1 mL/100 g volume), topiramate (30 mg/kg p.o., 0.5 mL/100 g volume), and gabapentin (30 mg/kg p.o., 0.5 mL/100 g volume) were tested on the mechanonociceptive threshold values (on days 3, 5, and 7), 60, 120, and 180 minutes after the treatments. Data were analysed using the GraphPad Prism 9 software. Results are presented as the means ± standard errors of the means (SEM).

Results: Approximately 60% of all rats developed robust mechanical allodynia in response to CFA (threshold values decreased from 18.30 g to approximately 5 g). Topiramate induced statistically significant anti-allodynic effect compared to the vehicle-treated group on all experimental days. Furthermore, significant differences in the threshold values were also observed before and after topiramate treatment within the same group. In contrast, neither sumatriptan nor gabapentin altered CFA-induced allodynia in any investigated doses.

Conclusion: This model is appropriate to investigate chronic orofacial allodynia related to trigeminovascular activation and to evaluate the effect of new drug candidates in comparison with topiramate, as the reference compound.

Acknowledgment: Supported by TKP 2021-EGA-16, NAP-3, PTE-ÁOK-KA-2023-23, ÚNKP-23-4-II.

- [1] Burstein R, Yamamura H, Malick A, et al. Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons. *J. Neurophysiol.* 1998;79(2):964–982. doi:10.1152/jn.1998.79.2.964
- [2] Romero-Reyes M, Uyanik J.M. Orofacial pain management: current perspectives. *J. Pain res.* 2014;7:99–115. doi: 10.2147/JPR.S37593
- [3] Iwata K, Takeda M, Oh S.B, Shinoda M, (2017). “Neurophysiology of orofacial pain,” in Contemporary Oral Medicine, eds C. Farah, R. Balasubramaniam, and M. McCullough (Cham: Springer), 1–23.

PP281. Exploring triterpenic acid metabolism pathways through fecal metabolomics with mass spectrometry

Mikropoulou E¹, Amerikanou C², Kaliora A², Mitakou S¹, Dedoussis G², Halabalaki M¹

¹*Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece*, ²*Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University of Athens, Athens, Greece*

Introduction: Triterpenic acids are a widespread class of natural products, with many reported pharmacological activities. Nevertheless, their exact mechanism of action in several pathological conditions remains inconclusive [1]. Fecal metabolomic analysis can be a powerful tool in disease diagnosis, prognosis, and treatment because it presents several advantages. The fecal metabolome offers a window into the intestinal microbiota composition and thus presents a great metabolic diversity, including a plethora of metabolites that cannot be detected in other biological substrates [2].

Methods: The current work utilizes an untargeted metabolomics workflow and high-resolution mass spectrometry (HRMS) to investigate metabolism aspects of naturally occurring triterpenoids from Chios mastic gum (CMG) in human fecal matter. Stool samples from a double-blind, placebo controlled clinical trial involving daily CMG administration in NAFLD/NASH patients [3], were analyzed by means of LC-HRMS (Orbitrap and QTOF analyzers) and data were submitted to statistical processing with univariate and multivariate methods.

Results: Several compounds of diverse chemical classes were tentatively annotated in stool samples of volunteers. Interesting observations could be made about the role of diet and medication on the stool metabolome, while metabolic biomarkers of the disease appeared to be differentially altered among patient groups (CMG, placebo). Most importantly, a group of statistically significant features that were only detected in stool samples of individuals receiving the CMG formulation, were further investigated with advanced dereplication methods and spectral processing software to reveal aspects of triterpenic acid biotransformation within the human metabolism machinery.

Conclusion: A number of metabolic products of CMG's triterpenoids were detected and tentatively annotated in the fecal samples, with hydroxylation and/or sulfation being the most common reactions taking place. We consider that this work provides useful insight into the metabolic fate of an important group of natural products, that merit further investigation as potential therapeutic agents.

Funding: European Union (ERDF) and Greek national funds, Operational Program "Competitiveness, Entrepreneurship and Innovation", "STRENGTHENING RESEARCH AND INNOVATION INFRASTRUCTURES" (Plant-Up, project code: 5002803)

- [1] Mioc M, Milan A, Malița D, et al. Recent Advances Regarding the Molecular Mechanisms of Triterpenic Acids: A Review (Part I). *Int J Mol Sci.* 2022;23(14). doi:10.3390/ijms23147740
- [2] Zierer J, Jackson MA, Kastenmüller G, et al. The fecal metabolome as a functional readout of the gut microbiome. *Nat Genet.* 2018;50(6):790-795. doi:10.1038/s41588-018-0135-7
- [3] Amerikanou C, Kanoni S, Kaliora AC, et al. Effect of Mastiha supplementation on NAFLD: The MAST4HEALTH Randomised, Controlled Trial. *Mol Nutr Food Res.* 2021;65(10):2001178. doi:10.1002/mnfr.202001178

PP282. Preliminary exploration of the role of progesterone and related steroids on uterine slow potentials.

Deng Y¹, Liu Y¹, Hui X¹, Rudd J¹

¹The Chinese University of Hong Kong, Shatin, Hong Kong

Introduction: Progesterone and a range of steroids are involved in early pregnancy. Progestins are also used in the prevention of miscarriage, but the use of other steroids is controversial partly because their effects on uterine contraction are incompletely understood. It is known that uterine slow potentials (USP) play a significant role in the regulation of uterine contractions, but the involvement of progesterone and other steroids has not been defined. This study aims to explore the potential action of progesterone and related steroids to modulate USP in isolated uterine tissues taken from female *Suncus murinus*.

Methods: Animals were killed and the whole uterus was removed and transferred to oxygenated Kreb's medium containing nifedipine (1μM) to inhibit the smooth muscle contractions. Segments of the anterior main body were transferred to a microelectrode array (MEA) platform to record 5 min of USP before the addition of progesterone (1μM-100μM), norethindrone (100nM), levonorgestrel (100nM), medroxyprogesterone acetate (10nM-1μM), mifepristone (100nM-10μM), or hydrocortisone (100nM); thereafter, recordings continued for a further 7 min. USPs were analyzed spatiotemporally to obtain frequency, slope, and velocity data. The effect of pre- and post-drug conditions was analyzed using Student's paired t-tests on 5-12 determinations.

Results: The basal USP average frequency was 14.09 ± 1.378 cycle per minute (cpm), with a slope of 63.32 ± 19.73 V/s, and a velocity of 57.79 ± 21.75 mm/s. Progesterone increased the average frequency by 11.02% at 10μM ($P < 0.05$), and 8.95% at 100μM ($P < 0.05$); it increased the slope by 12.48% at 10μM ($P < 0.0001$), and 13.00% at 100μM ($P = 0.01$); there was a decrease of velocity by 10.12% at 1μM ($P < 0.05$) and 17.42% at 100μM ($P < 0.05$). Medroxyprogesterone acetate (1μM) showed a similar pattern to progesterone: the average frequency and the slope were increased by 13.14% ($P < 0.05$) and 16.81% ($P < 0.05$), respectively, while velocity was decreased by 22.17% ($P < 0.05$). In contrast, mifepristone (1μM) decreased the slope by 15.52% ($P < 0.01$). Hydrocortisone (100nM), norethindrone (10nM), and levonorgestrel (100nM) were inactive.

Conclusions: Progesterone and medroxyprogesterone acetate, but not norethindrone, levonorgestrel, or hydrocortisone, were active to alter USP. The action of mifepristone may suggest endogenous mechanisms are active at progesterone and/or unidentified receptors in the uterus of *Suncus murinus*. Further studies are necessary to understand if progesterone and medroxyprogesterone acetate have off-target effects since norethindrone and levonorgestrel were inactive.

PP283. Intracellular Ca²⁺ release pathways responsible from impaired contractile responses in a rat model of menopause

Cenk I¹, Denizalti M¹, Durlu-Kandilci N¹

¹Hacettepe University Faculty of Pharmacy Department of Pharmacology, Ankara, Turkey

Introduction: The sensitivity of detrusor smooth muscle to agonists, its spontaneous activity, the level of contractile proteins and the intracellular calcium movements may change due to aging or pathophysiologies. Estrogen levels can affect lower urinary tract functions and thus, during post-menopause, the frequency of symptoms such as incontinence, detrusor hyperactivity and urgency increases. Estrogen might affect the intracellular Ca²⁺ concentration via L-type Ca²⁺ channels and myosin light chain (MLC) phosphorylation, moreover activates Ca²⁺-dependent molecules such as protein kinase C [1, 2]. The aim of this study is to examine the effects of estrogen on intracellular Ca²⁺ release pathways responsible from agonist-induced contractions in permeabilized bladder detrusor smooth muscle in a rat model of menopause.

Material and Methods: 200-250g female Sprague Dawley rats underwent ovariectomy by excision of ovaries. Ovariectomized rats (Ovx) were kept for six weeks after ovariectomy. Urinary bladder was isolated and the urothelium was removed. Detrusor strips were mounted in 1ml organ baths containing modified-Krebs' solution under 100mg tension. Strips were permeabilized using 40μM β-escin (30 minutes). Contractions were expressed as a percentage of 80mM K⁺.

Results: Carbachol (50μM)-induced contractions were significantly increased from 19.29±1.47%; (n=6) to 52.13±5.21% (n=7) in Ovx group. The Rho kinase inhibitor Y-27632 (1μM, 10.31±2.56%; n=6) and the protein kinase C inhibitor GF-109203X (5μM, 14.248±5.062%; n=4) inhibited the increased contractile response in Ovx group, but not in control. IP3 (50μM) and caffeine (10μM)-induced contractile responses were significantly increased in Ovx group (14.21±4.28%; n=4 and 10.43±1.19%; n=6) compared to control (4.59±0.68%; n=6 and 4.48±1.38%; n=6), in respectively.

Discussion: Permeabilized smooth muscle is a good tool in observing intracellular Ca²⁺ movements induced by an agonist. Smooth muscle contraction can be mediated by sarcoplasmic reticulum, the main intracellular Ca²⁺ store that may be activated via inositol triphosphate (IP3) and ryanodine receptors, and moreover MLC phosphatase inhibition through Rho-kinase or protein kinase C [3]. The contractile responses to carbachol, IP3 and caffeine were all enhanced in Ovx group. The increased carbachol-induced contraction in Ovx group decreased in the presence of Rho-kinase inhibitor Y-27632 and protein kinase C inhibitor GF-109203X. Based on these functional data, together with Rho-kinase and protein kinase C pathways, Ca²⁺ release via both IP3 and ryanodine receptors on sarcoplasmic reticulum seem to play a role in contractile disorders associated with estrogen deficiency.

- [1] Valeri A et al., *Exp Physiol.*, 2009.
- [2] Kitazawa T et al., *J Physiol.*, 1997.
- [3] Somlyo AP et al., *Physiol Rev.*, 2003.

PP284. Incorporation of GFs in liposomes and in ES membranes for integration into bone regeneration-assisting implant

Mouzoura P¹, Marazioti A⁴, Blanchy M³, Klepetsanis P^{1,2}, Antimisiaris S^{1,2}

¹*Department of Pharmacy, University of Patras, Patras, Greece*, ²*Institute of Chemical Engineering & Science FORTH/ICES, Patras, Greece*, ³*APPLUS+ RESCOLL Société de Recherche, Pessac Cedex, France*, ⁴*Laboratory of Basic Sciences, Department of Physiotherapy, University of Peloponnese, Sparta, Greece*

Introduction: Bone regeneration involves signaling pathways, where growth factors (GFs) play important roles. GFs bioactivity is significantly reduced by conformational modifications by pH, temperature, enzymes, etc. [1]. GF-entrapment in drug delivery systems is a stability-increasing strategy. Liposomes (LIPs) are preferable due to biocompatibility, biodegradability and targeting potential [2]. Moreover, LIPs can control GF release, protecting from toxic doses. Extra protection and sustained release can be attained by incorporation of GF-LIPs in core-shell Electrospun membranes (ESM) [3]. Herein Platelet-Derived Growth Factor-BB (PDGF-BB) and Bone morphogenic protein 2 (BMP2) were investigated to demonstrate protective effects of LIP and/or ESM entrapment.

Methods: PDGF-BB -LIPs and BMP2-LIPs (with lipid composition: DSPC/Cholesterol/PEG-lipid; 1:1:0.08 mol/mol) were prepared by microfluidic mixing (Precision Nanosystems, Flow Rate Ratio =1:1, Total Flow Rate =12ml/min). LIPs were purified from non-entrapped GFs, by chromatography (Sepharose 4B-CL), and characterized for size, polydispersity index (PDI), ζ -potential (by dynamic laser scattering, Malvern) and encapsulation efficiency (EE%). GFs-LIPs (and free GF) were incorporated in ESM (Polyvinyl alcohol 5% as core and Polycaprolactone 10% as shell). GFs concentration was measured by Elisa, proliferative activity by MTT, osteogenic activity by alizarin-dye on L929 cells.

Results: Results demonstrate similar EE% about $44.3 \pm 5.93\%$ for both GFs in LIPs, and repeatable LIP characteristics (size 100 ± 4.4 ; PDI 0.172 ± 0.05 ; ζ -potential -4.07 ± 1.29). Integration of GFs in ESM is similar (65-75%). Bioactivity tests on free-GFs, GF-LIP and GF-LIP-in-ESM (after extraction) proved that: (i) LIP-encapsulation significantly preserved GF-bioactivity (Proliferative and Osteogenic) during electrospinning, compared to free GF (bioactivity reduced). Finally, time-frame incubation studies (at 37°C) of all GF types (Free, LIP and LIP-in-ESM), proved that the bioactivity of LIP-in-ESM-GFs was preserved for at least 2.5 months.

Conclusions: GFs LIP encapsulation preserves their bioactivity during electrospinning. Entrapment of GF-LIPs in ESMs prolongs their bioactivity (at 37°C), justifying application of the strategy for GF integration into biomaterials.

Acknowledgement: Implemented under context of EU's H2020 research and innovation program "SMART BONE REGENERATION (SBR)", under grant agreement No874896 .

- [1] Antimisiaris SG, Marazioti A, Kannavou M, et al. Overcoming barriers by local drug delivery with liposomes. *Adv Drug Deliv Rev.* 2021;174:53-86. doi:10.1016/j.addr.2021.01.019
- [2] Natsaridis E, Mouzoura P, Gkartzio F, Marazioti A, Antimisiaris SG. Development of growth factor-incorporating liposomes for integration into scaffolds as a method to improve tissue regeneration. *Int J Dev Biol.* 2022;66(1-2-3):137-154. doi:10.1387/ijdb.210108sa
- [3] Mickova A, Buzgo M, Benada O, et al. Core/shell nanofibers with embedded liposomes as a drug delivery system. *Biomacromolecules.* 2012;13(4):952-962. doi:10.1021/bm2018118

PP285. Hydrogen sulfide and inflammation: interplay with resolution pathways and pro-resolving mediators

Montanaro R¹, Carriero F¹, Mitidieri E², Vellecco V², Whiteman M³, Panza E², Terrazzano G¹, Cirino G², Bucci M², d'Emmanuele di Villa Bianca R², Dalli J⁴, Brancaleone V¹

¹*Department of Science, University of Basilicata, Potenza, Italy*, ²*Department of Pharmacy, University of Naples Federico II, Naples, Italy*, ³*University of Exeter, Exeter, United Kingdom*, ⁴*Lipidomic Unit, William Harvey Research Institute, QMUL, London, United Kingdom*

Introduction: Pro-resolving molecules, such as formyl-peptide-receptor2 (FPR2), annexinA1 (AnxA1) and specialized pro-resolving lipid mediators (SPMs), released during an inflammatory response play a critical role in the recovery from inflammation[1,2]. As the pro-resolutive pathway has mainly been addressed in immune cells, we investigated the molecular mechanisms underlying the role of AnxA1/FPR2 axis in endothelium and its possible crosstalk with H2S pathway.

Methods: Bovine aortic endothelial cells (BAEC) were cultured in 10%FBS-DMEM and treated with TNF α (6h,10ng/ml). The expression of CSE, eNOS, AnxA1 and FPR2 in presence of FPR2 agonist Ac2-26 (0.1-1 μ M) or H2S donor AP123 (1-10nM) was evaluated and H2S levels were quantified. Ex vivo experiments were performed by using male C57Bl/6 mice (8-12 weeks-old) treated with TNF α (6h, 500ng/mouse) alone or in combination with Ac2-26 (30 μ g/mouse) or AP123 (6h,10 μ g/mouse). Aorta was used to evaluate vascular reactivity in response to phenylephrine (PE,1nM-3 μ M) or acetylcholine (Ach,10nM-30 μ M). To assess the influence of H2S pathway in SPMs generation, we used M1 macrophages, stimulated with LPS (1ng/ml) and exposed to PAG (CSE inhibitor, 10mM) or AP123 (1nM-1 μ M) at two different time points (45 min-24h).

Results: TNF α reduced AnxA1 and FPR2 expression, which was reverted by the treatment with AP123. Similarly, TNF α reduced the expression of CSE and eNOS, while Ac2-26 counteracted TNF α effect ($n\geq 3$; $p<0,05$). H2S concentration were reduced by TNF α and Ac2-26 restored its basal concentration ($n\geq 3$; $p<0,05$). Similarly, NO levels were reduced by TNF α and both Ac2-26 and AP123 rescued this impairment. In ex vivo experiments, the responses to PE and Ach were reduced in TNF-treated mice compared to vehicle. Both AP123 and Ac2-26 restored the physiological response to PE or Ach, thus ameliorating the vascular function ($n\geq 3$; $p<0,001$). In the M1 experiments, AP123 (1 μ M,45min) positively modulates RvD2n3-DPA, a pro-resolutive mediator, compared to PAG treatment.

Conclusions: Therefore, FPR2 controls the levels on of H2S/NO by modulation of CSE/eNOS expression. Similarly, AnxA1/FPR2 axis is modulated by AP123. This evidence is corroborated by the beneficial effect of both AP123 and Ac2-26 on vascular function. In addition, the influence of H2S on the resolution process through the generation of RvD2n-3DPA also links the cooperation between AnxA1, FPR2 and H2S within the resolution process. Overall, our data suggest an interplay between H2S and AnxA1/FPR2 that plays a crucial role in the control of “physiological” inflammation.

- [1] Corminboeuf O et al., J Med Chem. 2015;58
- [2] Brancaleone V et al. J Immunol. 2011;186

PP286. Investigating the molecular effects of pregabalin on morphine-mediated signaling in human, rat, and mouse neuronal cells to implement a Quantitative Systems Pharmacology platform for chronic pain treatment

Cuna E¹, Baiula M¹, Gülave B², de Lange L², Bedini A¹

¹Department of Pharmacy and Biotechnology – University of Bologna, Bologna, Italy, ²Division of Systems Biomedicine and Pharmacology – Leiden Academic Centre of Drug Research – Leiden University, Leiden, Netherlands

Introduction: Quantitative Systems Pharmacology (QSP), which combines experimental and computational methods to predict systems-level effects of drugs, is a promising approach for drug discovery and development, particularly in the neuroscience therapeutic area [1]. To implement a QSP platform for predicting novel, more effective, and safer combinations of existing medications to treat chronic pain, the effects of pregabalin and morphine co-administration on opioid receptors expression and intracellular signaling have been investigated in different neuronal cell models.

Methods: Brain region-specific rat and mouse primary cultures and differentiated SH-SY5Y human neuroblastoma cells (16 nM Phorbol 12-myristate 13-acetate; 5 days) were employed. The activity of morphine (10^{-12} – 10^{-4} M), alone or combined with pregabalin (10^{-10} – 10^{-4} M) was determined by measuring the inhibition of forskolin-stimulated cAMP accumulation using a cAMP EIA kit (Cayman Chemicals). To quantify the expression of seven pain/analgesia-related targets via qPCR, cells were exposed to morphine (10-40-100-285 ng/mL), pregabalin (131-267 ng/mL), or both at different time points (12-24-48-96h). Radioligand binding assay was performed to calculate receptor density (Bmax) following morphine treatment (285 ng/mL; 48h), alone or combined with pregabalin (267 ng/mL; 48h).

Results: We found that 10^{-6} M pregabalin significantly enhanced adenylyl cyclase inhibition by morphine in rat cortical ($IC_{50}=0.052 \pm 0.6$ nM; $p<0.001$; $n=6$) and mouse cortical primary neurons ($IC_{50}=0.18 \pm 0.06$ nM; $p<0.001$; $n=6$). In neuron-like SH-SY5Y, lower concentrations of pregabalin (10^{-10} and 10^{-8} M) potentiated morphine-mediated inhibition of adenylyl cyclase ($IC_{50} = 0.18 \pm 0.06$ nM and 0.1086 ± 0.046 nM, respectively; $p<0.001$; $n=6$).

Following prolonged μ opioid receptor stimulation for 48 hours, morphine's ability to activate MOR is dampened, as expected. In the same experimental condition, adding 10^{-6} M pregabalin partially rescued morphine activity ($IC_{50}=0.034 \pm 0.071$ nM; $p<0.001$; $n=6$).

Morphine and pregabalin co-administration for 48 hours to neuron-like SH-SY5Y cells up-regulated their pharmacological target, e.g. OPRM1 and CACNA2D1 (respectively 1.71- and 9.5-fold more expressed as compared to OPRM1 in vehicle-treated SH-SY5Y; $n=6$).

Conclusions: The current findings showed that pregabalin potentiated morphine-dependent signaling events and increased the expression of analgesia-related targets, suggesting that it might be promising as an opioid-sparing agent for the treatment of chronic pain. Experiments aiming at evaluating pregabalin concentration-dependent effects on morphine signaling in rat cortex primary neurons are currently ongoing; results will be presented at the conference.

- [1] Bloomingdale P, Karelina T, Cirit M, et al. Quantitative systems pharmacology in neuroscience: Novel methodologies and technologies. CPT Pharmacometrics Syst Pharmacol. 2021;10(5):412-419.doi:10.1002/psp.4.12607

PP287. Antinflammatory and antioxidant effect of Kadsurenin F in intestinal epithelial cells

Rapa S¹, Schwaiger S², Popolo A¹, De Fabrizio V¹, Autore G¹, Stuppner H², **Marzocco S¹**

¹*University of Salerno, Fisciano -Salerno, Italy*, ²*University of Innsbruck, Innsbruck, Austria*

Inflammatory bowel diseases (IBDs), mainly represented by Crohn's Disease and Ulcerative Colitis are characterized by chronic inflammation and oxidative stress in the gastrointestinal tract. The exact cause remain unknown, but numerous factors, e.g. genetic, environmental and microbial dysbiosis, are involved and compromise integrity of intestinal barrier formed by intestinal epithelial cells and the innate immune system [1]. Currently, a growing interest is addressed to the discovery of new therapeutic drugs, also from natural sources, able to exert an anti-inflammatory activity and a reduction of degenerative oxidative damage, that could be useful in the treatment of IBDs [2]. This work aims to characterize the pharmacological potential of Kadsurenin F, a neolignan isolated from Japanese pepper plant *Piper Futokadsura*, in a model of inflammation and oxidative stress induced in intestinal epithelial cell line (IEC-6) treated with lipopolysaccharide from *E. coli* (LPS) plus interferon- γ (IFN) for 24 hours. Our results indicated that Kadsurenin F compound (10 – 2.5 μ M) reduced the inflammatory response induced by LPS plus IFN in IEC-6. In particular, a reduced tumor necrosis factor- α (TNF- α) release (by 22.19 \pm 1.8, 15.68 \pm 2.45, 8.35 \pm 2.77% vs LPS+IFN respectively for the concentrations of 10, 5 and 2.5 μ M) and a reduced cyclooxygenase-2 (COX-2; by 29.87 \pm 4.19, 15.33 \pm 0.75, 7.84 \pm 2.92% vs LPS+IFN) and inducible nitric oxide synthase expression (iNOS; by 54.71 \pm 3.02, 43.33 \pm 6.06, 22.47 \pm 9.7 vs LPS+IFN respectively for the concentrations of 10, 5 and 2.5 μ M) was assessed. In the same experimental model, results also indicated an antioxidant activity of Kadsurenin F through a significant inhibition of the intracellular release of radical oxygen species (ROS; by 32.35 \pm 3.59, 13.32 \pm 4.84, 9.89 \pm 3.28 vs LPS+IFN respectively for the concentrations of 10, 5 and 2.5 μ M) and nitrotyrosine formation (64.3 \pm 1.27, 49.91 \pm 10.51, 36.10 \pm 1.12% vs LPS+IFN respectively for the concentrations of 10, 5 and 2.5 μ M in IEC-6). The antioxidant potential of Kadsurenin F was also indicated by a significant increase of enzymes with cytoprotective and antioxidant activity, such as heme oxygenase-1 (HO-1; 41.23 \pm 3.91, 33.75 \pm 1.43, 31.09 \pm 6.03 mean fluorescence intensity, respectively for the concentrations of 10, 5 and 2.5 μ M; LPS+IFN 15.77 \pm 1.78,) evaluated by cytofluorimetric techniques were also exerted. The obtained results highlighted the potential use of this compound as an anti-inflammatory and antioxidant remedy for intestinal inflammatory-based diseases.

[1] Ramos, G.P.; Papadakis, K.A. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. (2019) 94, 155-165.

[2] Duan, L.; Cheng, S.; Long, L. Natural Anti-Inflammatory Compounds as Drug Candidates for Inflammatory Bowel Disease. Front. Pharmacol. 2021, 12, 684486.

PP288. Analysis of antibiotic prescribing at the University Clinical Dentistry Centre of Kosovo during 2019-2021 in the Periodontology and Oral Medicine Clinic

Hoti A¹, Šutej I¹, Jakupi A²

¹*University of Zagreb, School of Dental Medicine, Zabreb, Croatia,* ²*University for Business and Technology-UBT, Prishtina, Kosovo*

Research objectives: The Ministry of Health in Kosovo, by national decision (2020), has suspended dental services from March 20, 2020, to June 18, 2020. The suspension has complicated the situation of patients due to the lack of provision of these health services. Based on this, this research aimed to analyze the results of the administration of different classes of antibiotics to patients at the University Dental Clinical Center of Kosovo for the treatment of dental patients, compared to the time before the imposition of suspension measures.

Materials and Methods: Antibiotic prescribing was monitored for three years (2019- 2021) in the clinic of oral surgery of the University Clinical Dentistry Centre of Kosovo. The analysis included the number of prescriptions and the type of antibiotic prescribed. The World Health Organization's INN and ATC codes are used for the classification of antibiotics. Data were processed using MS Office Excel.

Results: During the study period, 578 patients received antibiotic therapy, 39% were male and 61% female. In addition, for 578 patients there are a total of 686 antibiotics. The most prominent antibiotic was Metronidazole (J01XD01), with 364 prescriptions or 53% of all prescriptions. This study noted a decrease in 2020 by 14% and 2021 by 16% of Metronidazole (J01XD01) compared to 2019 by 23% of all prescriptions. On the other hand, there is an increase in amoxicillin with enzyme inhibitors (J01CR02) 10-12%, amoxicillin + clavulanic acid 7-9% of all prescriptions.

Conclusion: The results of the study provide a clear situation on antibiotic prescribing during health emergencies such as epidemics/pandemics and provide an opportunity to establish clear models of approach to epidemic/pandemic problems for patients and dental practitioners, especially in the design and approval of therapeutic protocols for specific indications in dentistry.

PP289. Exposure to Cerliponase Alfa and fA β 1-42 induced the autophagy-regulatory/related pathways in HT-22 cells

Carikci F¹, Köse S¹, Tel B¹, Kelicen Uğur P¹, Cinar E², Akyel H³, Cakir-Aktas C⁴, Karatas H⁴

¹Hacettepe University, Faculty of Pharmacy, Department of Pharmacology, Ankara, Turkey, ²Istanbul University-Cerrahpasa, Faculty of Pharmacy, Department of Pharmacology, Istanbul, Turkey,

³Baskent University, Faculty of Pharmacy, Department of Pharmacology, Ankara, Turkey, ⁴Hacettepe University, Institute of Neurological Sciences and Psychiatry, Ankara, Turkey

Introduction: Extracellular aggregation of amyloid-beta (A β) in the brain plays a central role in the onset and progression of Alzheimer's disease (AD) [1]. Deficient autophagy, which is a lysosomal degradation process, occurs during the early stage of AD [2-4].

This study aimed to determine whether autophagy pathways play a role in intraneuronal A β accumulation decrease by a recombinant analog of the TPP1 enzyme, cerliponase alfa (CER) (Brineura®).

Methods: Soluble A β , TPP1, and the proteins involved in autophagy, including mammalian target of rapamycin (p-mTOR/mTOR), p62/sequestosome-1 (p62/SQSTM1), and microtubule-associated protein 1A/1B-light chain 3 (LC3), were evaluated using western blotting in this study.

Results: CER reduced the A β load in HT-22 cells by inducing TPP1 expression and converting pro-TPP1 into the mature form. (n=3-6, One-way ANOVA followed by Tukey's post hoc test was applied.)

Conclusion: The present findings indicate that CER could be considered a promising novel therapeutic for neurodegenerative diseases in which autophagy pathways are impaired.

- [1] Ma, L.Y.; Lv, Y.L.; Huo, K.; Liu, J.; Shang, S.H.; Fei, Y.L.; Li, Y.B.; Zhao, B.Y.; Wei, M.; Deng, Y.N.; et al. Autophagy-lysosome dysfunction is involved in Abeta deposition in STZ-induced diabetic rats. Behav Brain Res 2017, 320, 484-493.
- [2] Eshraghi, M.; Ahmadi, M.; Afshar, S.; Lorzadeh, S.; Adlimoghaddam, A.; Rezvani Jalal, N.; West, R.; Dastghaib, S.; Igder, S.; Torshizi, S.R.N.; et al. Enhancing autophagy in Alzheimer's disease through drug repositioning. Pharmacol Ther 2022, 237, 108171.
- [3] Li, Q.; Liu, Y.; Sun, M. Autophagy and Alzheimer's Disease. Cell Mol Neurobiol 2017, 37, 377-388.
- [4] Uddin, M.S.; Stachowiak, A.; Mamun, A.A.; Tzvetkov, N.T.; Takeda, S.; Atanasov, A.G.; Bergantini, L.B.; Abdel-Daim, M.M.; Stankiewicz, A.M. Autophagy and Alzheimer's Disease: From Molecular Mechanisms to Therapeutic Implications. Front Aging Neurosci 2018, 10, 04.

PP290. Effect of Cerliponase Alfa on autophagy-related pathways in mouse hippocampal neurons exposed to A β 1-42

Köse S¹, Kelicen-Ugur P¹, Tel B¹, Cinar E², Akyel H³, Karatas H⁴, Cakir-Aktas C⁴

¹Hacettepe University, Faculty of Pharmacy, Sıhhiye, Ankara, Turkey, ²Istanbul University-Cerrahpasa, Faculty of Pharmacy, Istanbul, Turkey, Turkey, ³Baskent University, Faculty of Pharmacy, Department of Pharmacology, Ankara, Turkey, ⁴Hacettepe University, Institute of Neurological Sciences and Psychiatry, Ankara, Turkey

Introduction: Intraneuronal accumulation of A β via oligomer internalization might play an important role in the progression of AD [1-3]. It was recently shown that Tripeptidyl peptidase-1 (TPP1) as a lysosomal enzyme may effectively destabilize the fibrillar β -sheet, enhance A β proteolysis, and degrade fibrillar A β (fA β). [4]

This present study aimed to measure the decrease in intraneuronal A β accumulation by a recombinant analog of the TPP1 enzyme, cerliponase alfa (CER) (Brineura®).

Methods: In this study, endogenous A β accumulation was induced by fA β 1-42 exposure, and mouse hippocampal neuronal cells (HT-22) were treated with CER (human recombinant rhTPP1 1 mg mL⁻¹). The sirtuin-1, beclin-1, and Atg5 genes were also studied using RT-PCR. A β and TPP1 localizations were observed via immunocytochemistry. (n=4-8, One-way ANOVA followed by Tukey's post hoc test was applied.)

Results: Exposure to CER and fA β 1-42 induced the autophagy-regulatory/related pathways in HT-22 cells and exposure to CER alone increased sirtuin-1 activity.

Conclusion: Based on the present findings, we consider that augmentation of TPP1 with enzyme replacement therapy may be a potential therapeutic option for the treatment of AD.

- [1] Lai, A.Y.; McLaurin, J. Mechanisms of amyloid-Beta Peptide uptake by neurons: the role of lipid rafts and lipid raft- associated proteins. International journal of Alzheimer's disease 2011, 2011.
- [2] Mohamed, A.; Posse de Chaves, E. A β internalization by neurons and glia. International journal of Alzheimer's disease 2011, 2011.
- [3] Nazere, K.; Takahashi, T.; Hara, N.; Muguruma, K.; Nakamori, M.; Yamazaki, Y.; Morino, H.; Maruyama, H. Amyloid Beta Is Internalized via Macropinocytosis, an HSPG- and Lipid Raft-Dependent and Rac1-Mediated Process. Front Mol Neurosci 2022, 15, 804702.
- [4] Sole-Domenech, S.; Rojas, A.V.; Maisuradze, G.G.; Scheraga, H.A.; Lobel, P.; Maxfield, F.R. Lysosomal enzyme tripeptidyl peptidase 1 destabilizes fibrillar Abeta by multiple endoproteolytic cleavages within the beta-sheet domain. Proc Natl Acad Sci U S A 2018, 115, 1493-1498.

PP291. Erucin, an H₂S-releasing isothiocyanate, exert anticancer effect on human triple negative breast cancer cells

Esposito C¹, Bello I¹, Smimmo M¹, Barile M¹, Bucci M¹, Cirino G¹, Panza E¹

¹*University of Naples Federico II, Department of Pharmacy, Napoli, Italy*

Introduction: Triple negative breast cancer (TNBC) subtype accounts the 20 % of all breast cancer (BC) cases and is correlated with a poor prognosis and a higher death rate. Due to the lack of effective therapies, the research for novel therapies is urgently needed [1]. Epidemiological studies indicate that the consumption of Brassicaceae, a rich source of biologically active isothiocyanates, may effectively reduce cancer risk [2]. In this study, we explored the potential anti-cancer effects of erucin, the most abundant H₂S-releasing isothiocyanate present in arugula (*Eruca sativa*) in MDA-MB-231 cells, a validated in vitro model of TNBC.

Methods: The antiproliferative effect of erucin on MDA-MB-231 cells was evaluated using MTT assay. The potential effect of erucin on necrosis and apoptosis was investigated using flow cytometry. Changes in the expression of genes and proteins were evaluated by RT-PCR and western blot analysis. Changes in the intracellular reactive oxygen species (ROS) levels were estimated using the fluorescence probe H2DCF-DA. Wound healing, clonogenic and invasion assays were used for the measurement of cellular migration, colony formation and invasion.

Results: Our results revealed that erucin inhibits the proliferation of MDA-MB-231 cells in a time (24-72h) and concentration (1–100 µM) -dependent manner by promoting: 1) apoptosis, through activation of caspase-3 and PARP; 2) autophagy, as reflected by the increased expression of key regulatory genes, including ULK1, ATG13, BECN1, and BNIP3. Additionally, erucin prevented intracellular ROS generation promoting the expression of key antioxidant genes and halted MDA-MB-231 cell migration, invasion, and colony formation.

Conclusion: Our results show that the consumption of erucin, which is a natural H₂S donor contained in Brassicaceae, could be a useful dietary support intervention to prevent and treat TNBC.

[1] Li, Y.; Zhang, H.; Merkher, Y.; Chen, L.; Liu, N.; Leonov, S.; Chen, Y. Recent Advances in Therapeutic Strategies for Triple-Negative Breast Cancer. *J. Hematol. Oncol.* 2022, 15, 121.

[2] Melchini, A.; Traka, M.H. Biological Profile of Erucin: A New Promising Anticancer Agent from Cruciferous Vegetables. *Toxins* 2010, 2, 593–612.

PP292. Genistein and curcumin inhibit proliferation and invasiveness in BRAFV600E mutant and wild-type melanoma cells: insights of the anticancer effects

Mannino F¹, Bitto A¹, Pallio G², Squadrito F¹, Vaccaro F², Irrera N¹, **Cullotta C¹**

¹*Department of Clinical and Experimental Medicine, University of Messina, Messina, Italia,*

²*Department of Biomedical and Dental Sciences and Morphological and Functional Imaging, University of Messina, Messina, Italy*

Introduction: Melanoma is one of the most deadly form of malignant cancers; ultraviolet radiation exposure together with genetic mutations, such as the BRAF ones, represent important risk factors and are involved in melanoma onset as well as in metastatic dissemination. The treatment of melanoma improved with monoclonal antibodies, such as BRAF inhibitors, that fail to increase patient survival and cause adverse effects appearance in addition to being expensive. Therefore, the discovery of innovative therapies would guarantee better results and would increase survival rate: a growing interest was addressed to nutraceuticals thanks to their anti-emetic, anti-oxidant and anti-proliferative effects, such as genistein and curcumin [1, 2]. For this reason, this study aimed at investigating the possible anticancer effects of curcumin, genistein and their association in melanoma cells.

Methods: Human A375 (BRAF-mut) and CHL-1 (BRAF wild-type) cell lines were cultured and then treated with curcumin (25 μ M; purity \geq 80%, Sigma-Aldrich, USA) dissolved in DMSO or genistein (100 μ M; purity \geq 98%, Primus Pharmaceuticals, USA) dissolved in DMSO or curcumin+genistein (25+100 μ M) for 24 hours: concentrations were chosen in accordance with cell viability assays for IC50 calculation.

Results: Genistein and curcumin induced cell death in BRAF- mut and wild-type cell lines, as demonstrated by MTT assay and FDA/PI staining. The anti-apoptotic protein Bcl-2 expression was significantly reduced after curcumin and curcumin+genistein incubation ($p<0.0001$), but unexpectedly not with genistein alone. FAK protein expression, studied for its invasion potential, was significantly reduced following treatments in CHL-1 cells and after the treatment with genistein+curcumin in the most aggressive A375 cell line ($p<0.0001$). Also phospho-p38 expression was significantly reduced ($p<0.0001$) and these anti-proliferative effects were confirmed by scratch assay used to evaluate cell migration. Moreover, adhesion assay showed that both curcumin and genistein alone and in association inhibited cell adhesion, thus indicating that these nutraceuticals could reduce invasion and metastasis.

Conclusions: The results obtained so far are promising and provide new insights for the anticancer effects of genistein and curcumin, which could be used to improve therapeutic adherence and traditional drug response. However, further studies could clarify their effects in order to set up clinical trials for a future use in the clinical practice.

[1] Kaps L, Klefenz A, Traenckner H, et al. A New Synthetic Curcuminoid Displays Antitumor Activities in Metastasized Melanoma Cells 2023;12:2619.

[2] Peterle L, Sanfilippo S, Borgia F, et al. The Role of Nutraceuticals and Functional Foods in Skin Cancer: Mechanisms and Therapeutic Potential. Foods 2023;12:2629.

PP293. siRNA-loaded nanobubbles to overcome melanoma resistance to targeted therapy

Collotta D¹, Pettineo E¹, Cucci M¹, Argenziano M², Dianzani C², Cavalli R², Pizzimenti S¹

¹Department of Clinical and Biological Sciences, University of Turin, Turin, Italy, ²Department of Scienza e Tecnologia del Farmaco, University of Torino, Turin, Italy

Introduction: Delivery of small interfering RNA (siRNA) by nanocarriers in cancer treatment have been identified as a promising strategy able to target overexpressed proteins involved in tumor progression. Notably, nuclear factor E2-related factor 2 (Nrf2) has been identified as a key player in chemoresistance across various malignant tumors [1], including melanoma's resistance to targeted therapy [2]. Our previous results revealed upregulation of Nrf2 in melanoma cells resistant to dabrafenib (DAB) and/or trametinib (TRA) [2]. DAB and TRA, BRAF and MEK inhibitors respectively, are two well-established drugs for advanced melanoma [2]. Moreover, we have demonstrated that Nrf2 inhibition can overcome resistance to DAB and/or TRA [2]. This study aims to assess the biological effects of chitosan-shelled nanobubbles (NBs) loaded with siRNA against Nrf2 (siNrf2) in melanoma cells resistant to targeted therapy.

Methods: Formulations of NBs loaded with siNrf2, fluorescent 6-coumarin or negative control siRNA were prepared by tuning a method previously described [3]. Melanoma cells (D4M) sensitive and with dual resistance to 1.5 µM DAB and 36 nM TRA [D4M_(D+T)res] were obtained and characterized [2]. After treatments, viability (MTT assay), proliferation (Colony Forming Assay, CFA), NB cellular internalization (uptake of 6-coumarin-NB observed under fluorescent microscopy), protein expressions (western blot analysis; antibodies targeting Nrf2 sc-365949, β-actin sc-47778 from Santa Cruz Biotechnology) were analyzed as previous described [2, 3]. We considered p values ≤ 0.05 statistically significant (evaluated using one-way ANOVA followed by the Bonferroni multiple comparison post-test).

Results: Empty NBs demonstrated no toxicity for both D4M_sens and D4M_(D+T)res. To assess the ability of NB to enter cells, we treated both cell lines with 6-coumarin-NB, demonstrating a rapid internalization within 15 min into both cell types. After confirming higher Nrf2 protein content in resistant cells, we demonstrated that siNrf2-NB treatments successfully down-regulate Nrf2 expression. Interestingly, siNrf2-NB treatment sensitized D4M_(D+T)res cells to DAB+TRA treatments.

Conclusions: siNrf2-loaded NBs are a promising nanomedicine for tackling melanoma resistance to targeted therapy.

Funder: Project funded under the National Recovery and Resilience Plan (NRRP), CN3 Centre, National Centre for the development of gene therapy and drugs with RNA technology.

- [1] Barrera G et al. Control of Oxidative Stress in Cancer Chemoresistance: Spotlight on Nrf2 Role. *Antioxidants* 2021;10(4):510.
- [2] Cucci MA et al. Nrf2 as a Therapeutic Target in the Resistance to Targeted Therapies in Melanoma. *Antioxidants* 2023;12(6):1313.
- [3] Argenziano M et al. Ultrasound-Responsive Nrf2-Targeting siRNA-Loaded Nanobubbles for Enhancing the Treatment of Melanoma. *Pharmaceutics* 2022;14:341.

PP294. Regulatory role of hemokinin-1 in age-related memory loss, motor coordination and muscle strength changes

Kepe E¹, Simon V¹, Tékus V¹, Helyes Z^{1,2,3}, Borbély É^{1,2}

¹*Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary*, ²*National Laboratory for Drug Research and Development, Budapest, Hungary*, ³*HUN-REN-PTE, Chronic Pain Research Group, Pécs, Hungary*

Hemokinin-1 (HK-1) is present in high concentrations in the cerebellum, reproductive organs, bones and muscles. However, little is known about its involvement in age-related alterations, learning, memory or motor functions. Therefore, we investigated learning ability as well as motor coordination and muscle function of 3-4- and 18-month-old C57BL/6 wild type (WT) and HK-1 deficient (KO) male and female mice.

Memory function was assessed with Y and radial arm mazes (YM and RAM) as well as novel object recognition test (NOR). We determined spontaneous alternations and arm entries in YM, reference and working memory errors in RAM, and discrimination and recognition indices in NOR. We assess locomotor coordination with the static rod test, and muscle strength with the grid and the horizontal bar tests. We used one-way and two-way ANOVA with Bonferroni post hoc test (n=12-16/group) for the evaluation.

In the YM test, alternation decreased in aged female WT and KO animals. Young KO animals had significantly fewer arm entries compared to WTs ($p=0,004$), which did not change with age in KOs, but reduced in WTs. In RAM, female WT mice showed worse memory with more errors than their KO counterparts, but this difference was not detected in males. Interestingly, old male KO mice have found fewer rewards in this test compared to the WTs ($p=0,0006$). In the NOR test, no difference was found between the young groups, while minimal memory loss was observed in old females but not in males compared to their young counterparts. In both male and female older animals, locomotor coordination was significantly deteriorated in the static bar test ($p=0,03;0,0001$), which was significantly more severe in male ($p=0,0009$) but not in female KO animals compared to the WTs. A significant decline in muscle strength was also detected in older WT animals in both tests ($p=0,0001$). In the grid test, the loss of muscle strength was smaller in females compared to the males, which phenomenon was also observed in the horizontal bar test. The muscle strength of 18-month-old male KO animals was found to be significantly better compared to their WT counterparts ($p=0,01$).

Our results suggest that HK-1 may play a role in age-related memory impairment, motor coordination and muscle strength deterioration. Significant sex differences can also be observed, so elucidating the mechanism of action of HK-1 and its interactions with sex hormones may help to understand its modulatory role and significance for drug development.

PP295. Unveiling the Intricacies of Simvastatin Bioaccumulation and Biotransformation in Probiotic Bacteria Model for Insightful Revelations on Drug-Microbiota Interplay

Đanić M¹, **Pavlović N²**, Lazarević S¹, Stanimirov B³, Zaklan D², Tatalović V⁴, Starčević I⁴, Vukmirović S¹, Mikov M¹

¹Department of Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia,

²Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia, ³Department of Biochemistry, Faculty of Medicine, University of Novi Sad, Serbia, ⁴Faculty of Medicine, University of Novi Sad, Serbia

Background: Addressing the considerable challenge encountered in clinical practice, the variability in drug response among individuals poses a significant hurdle. These differences, often challenging to attribute solely to genetic factors, are believed to be influenced, at least partially, by the effects of the intestinal environment. The presence of microbiota within the intestinal lumen has been recognized for its potential to modify the absorption and pharmacokinetic profile of numerous drugs [1]. Despite the significant interindividual variations observed in the response to simvastatin, there has been inadequate attention to the potential involvement of gut microbiota. To gain a more profound understanding of the underlying mechanisms and their impact on clinical outcomes in patients undergoing simvastatin therapy, our study aimed to explore the bioaccumulation and biotransformation of simvastatin in probiotic bacteria under in vitro conditions.

Methods: Simvastatin samples were subjected to anaerobic incubation with probiotic bacteria at 37°C for 24 hours. Extracellular and intracellular samples were systematically collected at predetermined time intervals and meticulously prepared for subsequent analysis through LC-MS. The concentrations were quantified utilizing LC-MS/MS. To elucidate potential biotransformation pathways, a comprehensive bioinformatics approach was employed, complemented by experimental assays.

Results: Throughout the incubation period, simvastatin was internalized into bacterial cells, leading to a progressive increase in drug bioaccumulation. The observed decline in total drug levels during incubation suggests the occurrence of partial biotransformation facilitated by bacterial enzymes. Bioinformatics analysis highlighted the lactone ring as particularly susceptible to metabolic alterations, with ester hydrolysis followed by hydroxylation identified as the most probable reactions.

Conclusion: Our findings point to the significance of bioaccumulation and biotransformation processes of simvastatin by intestinal bacteria, potentially influencing the drug's altered bioavailability and therapeutic effects. Given the in vitro focus on specific bacterial strains in our study, further comprehensive research is imperative to fully grasp the intricate interactions between drugs and the microbiota, ultimately influencing the overall clinical response to simvastatin. These insights may pave the way for innovative approaches in tailoring lipid-lowering therapy on an individualized basis.

Acknowledgements: This work was supported by the Project of Ministry of Education, Science and Technological Development, Republic of Serbia No451-03-68/2022 14/200114 and the Project for Scientific and Technological Development of Vojvodina (142-451-3522/2023-01)

- [1] Đanić, M., Pavlović, N., Lazarević, S., Stanimirov, B., Vukmirović, S., Al-Salami, H. & Mikov, M. (2023). Bioaccumulation and biotransformation of simvastatin in probiotic bacteria: A step towards better understanding of drug-bile acids-microbiome interactions. *Frontiers in Pharmacology*, 14, 1111115.

PP296. Design, synthesis and pharmacological bioactivities of thiol-derived flavan-3-ols prepared using procyanidins from avocado peels

Pastene E¹, Garcia A², Alarcón J¹, Zuñiga F², Reyes M³, Venegas M², Fiallos N¹, Berrios B², Ormazabal Y², Bustamante L²

¹Universidad Del Bío-Bío, Chillan, Chile, ²Universidad de Concepción, Concepcion, Chile, ³Universidad de Chile, Santiago, Chile

Epicatechins (EC) and its derivates are the main polyphenol families present in green tea. A wide range of pharmacological effects of green tea are associated to these compounds, among them the antimicrobial and cytotoxic effect. Nevertheless, the studies show a high variation between in vitro and in vivo assays, this is caused by EC low bioavailability. Different strategies had been considered to enhance EC bioavailability, among them, the use of nucleophilic thiols to attack epichatequin carbocations derived from natural procyanidins extracted from avocado peels. After synthesis, six new compounds were purified by chromatography techniques (CPC, HPLC) and fully characterized by spectroscopic methods (HR-MS/MS and NMR). In this work, thiol modifications were focused on enhancing natural epicatechins lipophilicity. The anti-cancer potential properties of this new set of molecules were assayed using sulphorodamine B, and apoptosis assays on Caco-2 and HDF cell lines. On the other hand, biofilm inhibitory properties were tested using pathogen and non-pathogen bacteria with crystal violet and resazurin assays. Thiol-derived adducts displayed antimicrobial properties and inhibit biofilm formation in a concentration-dependent manner, particularly in *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 9144), *Escherichia coli* (ATCC 11775) and *Salmonella enterica* (ATCC 13076). Interestingly, biofilm formation was promoted in *Escherichia coli* 25922, *Limosilactobacillus fermentum* UCO-979C and *Lacticaseibacillus rhamnosus* UCO-25A. ADME properties and molecular docking upon diadenylate cyclase and diguanylate cyclase enzymes were also evaluated. At the assayed concentrations in cell-based assays, the cytotoxic effect changed depending on the molecule polarity. In particular, the adducts formed with derivates of benzene-thiol showed a high cytotoxic potential, and the adduct formed with ethanedithiol showed a selective toxicity against cancer cells, which makes it necessary to deepen the studies on this compound behavior in vivo, and its mechanism of action. Therefore, avocado peels can be used as cheap starting material to produce new drug candidate and materials to be used as adjuvant for modulate biofilm production.

PP297. Analgesic effect of cyclodextrin derivatives through inhibition of Transient Receptor Potential Ankyrin 1 ion channel activation

Szőke É¹, Nehr-Majoros A¹, Bencze N¹, Payrits M¹, Kemény Á¹, Helyes Z¹

¹*University of Pécs, Pécs, Hungary*

Transient Receptor Potential Ankyrin 1 (TRPA1) is a non-selective cation channel expressed on nociceptive sensory nerve endings and primary sensory neurons, which play an important role in the integration of pain. Its activation is facilitated by cholesterol- and sphingolipid-rich lipid microdomains (lipid rafts) located in the plasma membrane. Previously, we proved that cyclodextrin (CD) derivatives forming an inclusion complex with cholesterol found in the raft regions are able to inhibit receptor activation, thereby exerting an analgesic effect. Our aim is to further investigate these lipid-protein hydrophobic interactions and to identify CD derivatives as analgesic and anti-inflammatory compounds with a new mechanism of action. In our experiment, we compared three different CD derivatives selected based on our previous **Results:** random methylated β -cyclodextrin (RAMEB), (2-hydroxypropyl)- β -cyclodextrin (HPBCD), sulfobutyl- β -cyclodextrin (SBECD). The *in vitro* cholesterol depletion of CD derivatives was demonstrated by fluorescence microscopy after Filipin III staining on the CHO (Chinese Hamster Ovary) cell line. We investigated the analgesic effect of CD pretreatment in a mouse model of formalin-induced acute inflammatory pain. The time spent with pain-relieving behavior was measured in two phases: in the first phase (0-5 minutes) the direct activation of free sensory nerve endings, while in the second phase (20-45 minutes) the pain caused by the release of inflammatory mediators can be observed. The cholesterol-depleting effect of intraplantar CD treatment was measured by colorimetry from mouse skin using the Abcam Cholesterol Assay kit. Using Filipin III fluorescent staining, we showed that treatment with 3 mM RAMEB and 10 mM HPBCD or 10 mM SBECD significantly reduced the cholesterol content of the membrane of CHO cells. CD pretreatment with all three derivatives (3 mM RAMEB, 10 mM HPBCD and 10 mM SBECD) reduced the duration of nocifensive behavior in the second phase of formalin-induced acute inflammatory pain. As a result of treatment with intraplantarly applied CD derivatives, the total cholesterol content in the skin of mice decreased compared to the amount of cholesterol measured in control individuals treated with physiological saline. We proved that the CD derivatives were able to deplete the cholesterol content of the plasma membrane of CHO cells and the plantar skin in mice. The cholesterol depletion could lead to analgesic effect in the formalin-induce acute inflammatory pain model in mice.

Support: TKP2021-EGA-16; TKP2021-EGA-13; RRF-2.3.1-21-2022-00015; ELKH-PTE Chronic pain research group; NKFIH K 138936; ÚNKP-22-3-I-PTE-1616

PP298. Therapeutic Drug Monitoring of Antiseizure Medications through quantitative DBS

Cancellerini C¹, Caravelli A², Esposito E², Soldà M², Vignatelli L², Bisulli F^{1,2}, Licchetta L², Fiori J³

¹Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy, ²IRCCS, Istituto delle Scienze Neurologiche di Bologna, Full Member of the European Reference Network for Rare and Complex Epilepsies (EpiCARE), Bologna, Italy, ³Department of Chemistry "G. Ciamician", University of Bologna, Bologna, Italy

Introduction: Therapeutic Drug Monitoring (TDM) of Antiseizure Medications (ASMs) is an essential tool for persons with epilepsy (PWE) follow-up, aimed to optimize individual drug therapy. Traditional venipuncture for TDM requires high-volume blood and can be challenging for uncooperative PWE [1]. Microsampling requires a lower blood volume through a less painful and invasive fingerprick and offers a reliable sampling methodology for TDM. Quantitative Dried Blood Spot (qDBS, Capitainer®-Sweden) are proposed as free to over-sampling and HCT-induced inaccuracies[2]. This study focuses on validating the extraction method of ASMs from qDBS.

Methods: Five ASMs were included in the analysis: carbamazepine (CBZ), lacosamide (LCS), levetiracetam (LEV), lamotrigine (LTG) and valproic acid (VPA). The quantification of ASMs from 10µl qDBS-device by Ultra-High Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) was performed through an extraction technical validation, according to EMA guidelines [3]. Bland-Altman analysis and Passing-Bablok regression were performed to compare ASMS concentrations in qDBS with leftover venous blood and plasma samples.

Results: The method was proven accurate and precise using chosen extraction procedure. Indeed, intra and inter-assay reproducibility analyses showed accuracy and precision ≤15% across calibration range (0,4-15µg/ml CBZ; 0,5-10µg/ml LCS; 0,5-20µg/ml LTG; 1-80µg/ml LEV; 10-120 µg/ml VPA). Recovery was found >85 % and matrix effect <10% for most of the ASMs considered. Stability was tested at 7, 15, 30 days of storage, showing robustness for all ASMs-qDBS at 7-days room temperature. Blood-to-plasma ratio was calculated and used to convert qDBS results. A preliminary statistical via Bland-Altman analysis and Passing-Bablok regression indicated a linear correlation for most of the ASMs.

Conclusions: To our knowledge, this is the first study that introduces qDBS for quantifying ASMs. A UHPLC-MS/MS method was developed and validated according to EMA guidelines demonstrating precision and accuracy[3]. Further studies are needed to validate this fingerprick device across a broader number of ASMs and evaluate its clinical applicability in real-life scenarios.

- [1] Licchetta L et al. TELEmedicine for EPilepsy Care (TELE-EPIC): protocol of a randomised, open controlled non-inferiority clinical trial. BMJ Open. 2021 Dec 3;11(12):e053980.
- [2] Carling RS et al. Evaluation of volumetric blood collection devices for the measurement of phenylalanine and tyrosine to monitor patients with phenylketonuria. Clin Chim Acta. 2022 Aug 20;535:157-166. doi: 10.1016/j.cca.2022.08.005. Epub ahead of print. PMID: 35995273.
- [3] Agency EM. European medicines agency guideline on bioanalytical method validation. Bioanalysis. 2012;4(8):865–8.

PP299. Perception, barriers, and willingness to implement telepharmacy among pharmacists in Palestine

Khdour M¹, Hilqawi Y¹

¹*Al-Quds University, Jerusalem, Palestine*

Background: One form of telemedicine is telepharmacy, which offers remote pharmaceutical services such as drug counseling, self-medication, drug monitoring, and assessment by a licensed pharmacist. It is unclear whether Palestinian pharmacists possess the necessary knowledge, attitudes, and willingness to practice telepharmacy.

Aims: The purpose of this study was to evaluate the present level of use and the factors that influence pharmacists' readiness to use telepharmacy in Palestine.

Methods: A cross-sectional descriptive study was carried out using an online validated questionnaire and targeting community pharmacists in West Bank, Palestine. The final questionnaire consisted of four sections; demographics, perception, willingness, and barrier section.

Results: A total of 375 pharmacists were enrolled in the current study. The median age was 30 (29–33). The majority of pharmacists (67.5%) were female, held a bachelor's in pharmacy (78.1%), and had less than ten years of experience (38.2%). Only 36.5% of the participants were aware of telepharmacy and (39.7%) had poor knowledge about Telepharmacy. The pharmacists showed high willingness and a positive attitude toward implementation in the pharmacy setting. The participants were most willing to utilize telepharmacy in Patient counseling and education 82.5%, communicating with care providers from home (83.2%), and identifying drug-related problems (81.5%). Health Literacy in the general population (78.9%), requiring uninterrupted Internet (74.5%), and high cost of establishment were the most often cited obstacles to implementing Telepharmacy. Regression analysis indicated that participants with higher education 2.1; CI: 1.6-3.1, p<0.001) and women (OR= 1.68, CI: 0.91-2.3, p<0.001) were more willing to use this service compared to men, and people with lower knowledge and education.

Conclusion: Despite the pharmacists' positive attitudes and willingness toward telepharmacy, several barriers were found, emphasizing the need for educational and training programs to increase pharmacists' knowledge of telepharmacy as well as for adequate funding support to address the issue of high operating costs.

PP300. The neuroprotective effects of Olea Europaea, Crocus sativus, Vaccinium myrtillus and Salvia spp in experimental models of Alzheimer's Disease

Kofinas A¹, Pavlidis N¹, Andriopoulou C¹, Paliaga A¹, Epeslidou E¹, Leondaritis G^{1,2}, Antoniou K¹, Gravanis A³, Charalampopoulos I³, Angelis A⁴, Antoniadi L⁴, Skaltsounis L⁴, Konstandi M¹, **Konstandi M¹**

¹*Department of Pharmacology, Faculty of Medicine, University of Ioannina, Ioannina, Greece,*

²*Institute of Biosciences (I.B.S.), University Research Center of Ioannina (U.R.C.I.), Ioannina, Greece,*

³*Department of Pharmacology, School of Medicine, University of Crete, Heraklion, Greece & Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology–Hellas, Heraklion, Greece,*

⁴*Department of Pharmacy, Division of Pharmacognosy and Natural Products Chemistry, National and Kapodistrian University of Athens, Athens, Greece*

Introduction: Neurotoxicity is a leading causative factor in neurodegenerative disorders. In particular, accumulation of β -amyloid proteins in the brain, a hallmark of Alzheimer's disease (AD), induces oxidative stress, which leads to neurodegeneration [1]. Antioxidants though are not sufficiently effective in the treatment of AD and several other pharmacological approaches failed to cure, prevent or retard the progression of the disease. Clinical studies indicated that several medicinal plants display beneficial effects by improving the memory and other cognitive functions of patients with mild and moderate AD [2].

Methods: In this study, the potential antioxidant and neuroprotective properties of Olea europaea, Crocus sativus, Vaccinium myrtillus and Salvia spp extracts, were assessed employing differentiated human neuroblastoma SH-SY5Y cells to cholinergic neurons. The effects of these herbs were assessed against the H2O2- and β -amyloid- induced cell toxicity using the MTS assay [3]. Furthermore, a mixture of herbal extracts was orally administrated to 5xFAD mouse model of AD and their effects on the memory of mice were assessed using various behavioural tests.

Results: Current findings indicated that S. fruticosa, S. officinalis, S. argentea leaf extracts and the active compounds, oleuropein, trans-crocin-3 and -4, display significant and dose-dependent antioxidant and neuroprotective effects on differentiated SH-SY5Y cells ($P<0.001$, $n=12$). Furthermore, the mixture of herbal extracts improved the spatial and recognition memory of 5xFAD mice ($P<0.05$ ($n=26$)).

Conclusion: The above mentioned medicinal plants mitigate the H2O2- and β -amyloid-induced neurotoxicity, an effect that might contribute to the protection against the development and progression of Alzheimer's disease.

PP301. μ -opioid receptor oligomers as targets for safer pain management

Casadó-Anguera V¹, Llopart N¹, Alonso-Carrasco A¹, Moreno E¹, Casadó V¹

¹Laboratory of Molecular Neuropharmacology, Department of Biochemistry and Molecular Biomedicine, Universitat de Barcelona, Barcelona, Barcelona, Spain

One in five Europeans suffer from moderate to severe chronic pain, impacting one in three individuals' ability to maintain independence, as reported by the International Association for the Study of Pain. μ -opioid receptor (MOR) agonists have long been recognized as the most effective treatment for chronic pain. While MORs play a pivotal role in opioid-induced analgesia, they are also responsible for the adverse side effects associated with opioid use, which can lead to addiction. Moreover, opioids can also induce locomotor activation, which depends on the presence of dopamine D1 receptor (D1R). It is noteworthy that the addictive properties of opioids have contributed significantly to the well-known "opioid epidemic," which represents a significant public health and safety crisis. Like other G protein-coupled receptors (GPCRs), MORs have the ability to form oligomeric complexes (heteromers) both in vitro and in vivo, acquiring distinct pharmacological properties. Therefore, the objective of this project was to identify non-addictive opioid drugs that could effectively treat chronic pain taking advantage of the unique properties acquired by MORs when forming complexes with other GPCRs, as well as to identify novel therapeutic approaches considering the allosteric modulations that occur within these complexes. Our research has uncovered that MORs interact physically and functionally with Gal1R and D1R, forming heteromers within crucial brain regions implicated in pain management, addiction, tolerance, and withdrawal symptoms. These regions include the ventral tegmental area (VTA) and the basal ganglia, where D1R has an important role in opioid-induced locomotor activation. Employing a diverse array of biochemical, pharmacological, and functional techniques, we have also characterized these oligomers, revealing a notable negative allosteric modulation of Gal1R and D1R on MOR function. Furthermore, we have identified several ligands with distinct affinities for MORs when forming specific oligomeric complexes. These differences in ligand binding affinity provide valuable insights into variations in abuse liability. For instance, our research underscores disparities observed with methadone, especially S-methadone, and buprenorphine when binding to MOR when forming heteromers with Gal1R, an heteromer present in the VTA, and involved in the activation of the dopaminergic system. Hence, our findings predict a dissociation between the analgesic and euphoric effects of these compounds, as they preferentially bind to peripheral MORs that do not form heteromers with Gal1R. Consequently, our research contributes to understanding the complexities of opioid pharmacology and offers potential strategies to optimize therapeutic outcomes while minimizing adverse effects.

PP302. Hydrangea paniculata coumarins alleviate adriamycin-induced renal lipotoxicity through activating AMPK and inhibiting C/EBP β

Zhang S¹, Ji M¹, Du T¹

¹*Institute of Materia Medica, CMAS, Beijing, China*

Introduction: As a traditional medical herb, *Hydrangea paniculata* Siebold has been used to treat various inflammation related diseases throughout Chinese history. Total coumarins isolated from *Hydrangea paniculata* (HP) has shown renal protective effect in several immune mediated kidney diseases 1,2. To investigate renal beneficial effect of HP on experimental Adriamycin nephropathy (AN), and further clarify whether reversing lipid metabolism abnormalities by HP contributes to its renoprotective effect and find out the underlying critical pathways.

Methods: After establishment of rat AN model, HP was orally administrated for 6 weeks. Biochemical indicators related to kidney injury were determined. mRNAs sequencing using kidney tissues were performed to clarify the underlying mechanism. KEGG pathways analysis, western blot, molecular docking, and drug affinity responsive target stability (DARTS) assay was carried out to further explore and confirm pivotal molecular pathways and possible target by which HP and 7-hydroxylcoumarin (7-HC) played their renal protection effect via modulating lipid metabolism.

Results: HP could significantly improve renal function, and restore renal tubular abnormal lipid metabolism and interstitial fibrosis in AN. In vitro study demonstrated that HP and its main metabolite 7-HC could reduce ADR-induced intracellular lipid deposition and fibrosis characteristics in renal tubular cells. Mechanically, HP and 7-HC can activate AMPK via direct interaction, which contributes to its lipid metabolism modulation effect. Moreover, HP and 7-HC can inhibit fibrosis by inhibiting C/EBP β expression in renal tubular cells. Normalization of lipid metabolism by HP and 7-HC further provided protection of mitochondrial structure integrity and inhibited the NF- κ B pathway. Long-term toxicity using beagle dogs proved the safety of HP after one-month administration.

Conclusion: Coumarin derivates from *Hydrangea paniculata* alleviate adriamycin-induced lipotoxicity and fibrosis in kidney through activating AMPK and inhibiting C/EBP β .

- [1] Li Z, Zhang X, Wu H, et al. *Hydrangea paniculata* coumarins attenuate experimental membranous nephritis by bidirectional interactions with the gut microbiota. *Communications Biology*. 2023/11/22 2023;6(1):1189. doi:10.1038/s42003-023-05581-9
- [2] Wang W, Sheng L, Chen Y, et al. Total coumarin derivates from *Hydrangea paniculata* attenuate renal injuries in cationized-BSA induced membranous nephropathy by inhibiting complement activation and interleukin 10-mediated interstitial fibrosis. *Phytomedicine*. 2022/02/01/ 2022;96:153886. doi:<https://doi.org/10.1016/j.phymed.2021.153886>

PP303. Low THC Cannabis extracts for psoriasis. A study in an in vitro model

Rizzi L¹, Gregoli G¹, Bresciani E¹, Meanti R¹, Pellati F², Corsi L², Mauri M¹, Torsello A¹

¹*Università Milano Bicocca, Monza, Italy*, ²*Università degli studi di Modena e Reggio Emilia, Modena, Italy*

Psoriasis is a chronic disease of the skin. Nowadays it is clear that psoriasis is a systemic disease, with a complex etiopathogenesis and genetic basis. Therefore, psoriasis is an inflammatory and autoimmune disease, characterized by skin hyperplasia caused by the imbalance of proliferation and differentiation of keratinocytes.

The therapeutic strategies in the treatment of psoriasis are based on the severity of injuries, and encompass topical or systemic therapies.

However, all of these pharmacological therapies have several relevant side effects. Novel therapeutic approaches are needed to control psoriasis in the long term. Cannabinoids, constituents of Cannabis sativa (CS), have been studied for several years with the aim of obtaining more effective and less invasive therapies. We have used an in vitro model mimicking the inflammatory component of psoriatic skin, to study the antiinflammatory effects of three cannabis extracts.

This model is based on the use of HaCaTs, an immortalized human keratinocyte cell line, which are treated with TNF α to induce the inflammatory state. At this point, the cells are treated with pure cannabinoids (cannabidiol and cannabigerol) or extracts of CS Carmagnola, CS Santhica and CS Bernabeo. In order to test the effects of these extracts, Real Time PCR was used to assess the changes in specific mRNA levels of cytokines and factors involved in the psoriasis pathway. The effects of the CS extracts on cell morphology was visualized using a confocal microscope.

From the results of this study indicate that cannabis extracts and cannabinoids could have antiproliferative and proapoptotic effects, promoting the activation of Bax pathway and inhibiting Bcl2. CS extract can also stimulate the synthesis of cytokines and factors such as STAT3, IL1 β , IL6 and IL36rn.

CBD and CBGA are most effective both from an anti-inflammatory and proapoptotic view. CS Santhica seems to have a markedly more anti-inflammatory action while CS Carmagnola and CS Bernabeo have proapoptotic effects probably through different mechanisms.

In summary, to the results of this research suggest cannabinoid and CS extract can be developed as effective therapies to treat psoriasis. However, further studies are needed to ensure the efficacy and safety of such compounds in the treatment of this disease.

PP304. A survey about knowledge and perception towards pharmacovigilance among nursing students at Medical Faculty, ss. Cyril and Methodius University, Skopje, North Macedonia

Gjorgjevska K¹

¹*Medical Faculty, University "Ss. Cyril & Methodius", Skopje, North Macedonia*

Introduction: Before being involved in clinical practice, healthcare students need to obtain sufficient knowledge on pharmacovigilance and adverse drug reactions (ADRs) reporting [1]. Bearing in mind limited data on this subject from North Macedonia, present survey was designed with purpose to evaluate knowledge and perception of regular pre-final year students enrolled in the Three-Year Professional Studies for Graduate Medical Nurses/Technicians at Medical Faculty, Ss. Cyril and Methodius University, Skopje.

Methods: This was cross-sectional questionnaire-based study. Survey was carried out using pre-validated questionnaire [2, 3] that included demographic data and two sections including 15 knowledge related questions and 5 perception related questions. Collected data were analyzed using Microsoft Excel and were presented as frequencies and percentages of variables from total number of participants. Survey was approved by the Ethical Committee of the Medical Faculty, University of "SS Cyril and Methodius"- Skopje, Republic of North Macedonia (number 03-1860/7).

Results: A total of 51 questionnaires were distributed with a response rate of 82% (n=42). Most of responders were females aged 20-21 years old (52.4%). The majority of participants (71.4%) had poor knowledge. The maximum knowledge score was 10, minimum score of 1 (mean knowledge score was 5.83 ± 2.26). More than half of participants (69.04%) had moderate perception towards pharmacovigilance and ADRs reporting. The maximum score for perception in students was 21 and minimum score was 11 (mean knowledge score was 16.14 ± 2.9).

Conclusions: Pharmacovigilance topic and manner of ADR reporting is not adequately covered in present curriculum of Three-Year Professional Studies for Graduate Medical Nurses/Technicians at Medical Faculty, Ss. Cyril and Methodius University, Skopje.

PP305. Drug prescription pattern in bird practice: a retrospective study in a Spanish veterinary teaching hospital

Romero-Gómez B¹, Susperregui-Lesaca J², Vazquez-Acero M¹, Lopez-Cadenas C¹, de la Puente-Garcia R¹, Rodriguez-Lago J¹, **Sahagun-Prieto A¹**, Diez-Laiz R¹

¹*Pharmacology, Department of Biomedical Sciences, Veterinary Faculty, Institute of Biomedicine (IBIOMED), University of Leon, Leon, Spain*, ²*Applied Mathematics, University of Leon, Leon, Spain*

Introduction: One Health concept has gained more and more importance because of emerging and re-emerging of zoonotic diseases. Therefore, the health of people, animals and environment are linked and should be treated as a whole¹. The aim of the study was to describe the prescription patterns of medicines in birds treated at the Veterinary Teaching Hospital of the University of León (HVULE) in Spain.

Methods: An observational, retrospective, and descriptive study was carried out among birds attending at the HVULE between 2018-22. Data collection, processing and storage was carried out in accordance with Spanish regulations. Data obtained were processed and analysed using Microsoft Excel (2019) and SPSS Statistics 26.

Results: A total of 427 birds were treated (57.8% raptors and 42.2% synanthropic birds). The visit of the birds to the hospital took place due to an emergency (89.0%). Most of them were treated for musculoskeletal processes (66.0%), followed by digestive ones (22.5%). For those hospitalised (50.3%), the length of stay was 4.6 ± 7.1 days (range 1-71, median 2.0). The mean of administered treatments was 2.1 ± 0.9 (range 1-7, median 2.0), and more than a third (38.9%) were euthanised. Regarding the treatments followed (n=934), 902 (96.6%) were medications included in the ATCvet classification². According to the first level of this classification, half of them (n=455; 50.4%) belonged to QN (Nervous system) group. When the fourth level was considered, the most frequent chemical/therapeutic subgroup was QB05BB (solutions affecting the electrolyte balance), followed by halogenated hydrocarbons (QN01AB), barbiturates (QN51AA) and oxicams (QM01AC). According to the EMA Categorization of antibiotics for prudent and responsible use in animals³, 77 (84.6%) of the active ingredients administered were classified as Restrict; 2 (2.2%) as Caution; and 12 (13.2%) as Prudence. Marbofloxacin (Category B-Restrict) was the most prescribed one.

Conclusions: Our study provides an insight into the prescription patterns in birds. The findings of the study provide sufficient data to veterinary policymakers and education aimed at improving drug use practices in general and antimicrobial use, in particular in the veterinary profession.

- [1] Souza MJ. One Health: Zoonoses in the Exotic Animal Practice. *Vet Clin North Am Exot Anim Pract.* 2011;14(3):421-426. doi:10.1016/j.cvex.2011.05.007.
- [2] World Health Organization Collaborating Centre for Drug Statistics Methodology. Guidelines for ATCvet classification 2023. Published 2023. Accessed November 27, 2023. https://www.whocc.no/filearchive/publications/2023_atcvet_guidelines_web.pdf.
- [3] European Medicines Agency. Categorisation of antibiotics in the European Union. Published 2019. Accessed July 6, 2023.

PP306. The Effect of Cross-Linked and Linear Intercalated Hyaluronic Acid on Primary Human Osteoarthritic Chondrocytes in the Presence of Inflammatory Extracellular Vesicles Derived from Human Monocytes

Carrabs V^{1,2}, Guillén I^{1,3}, Ferrández M³, Alcaraz M³, Ferrini F², Guescini M², Zazzetta M⁴, Capparucci I², Barbieri E², Sestili P²

¹Departamento de Farmacia, Facultad de Ciencias de la Salud, Universidad CEU Cardenal Herrera, Alfara del Patriarca-Valencia, Spain, ²Dipartimento di Scienze Biomolecolari, University of Urbino Carlo Bo, Urbino, Italy, ³Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat de València-Universidad Politécnica, Valencia, Spain,

⁴Regenyal Laboratories Srl, San Benedetto del Tronto, Italy

Introduction: Osteoarthritis (OA) is a degenerative joint condition primarily affecting adults, presenting a complex treatment challenge due to its multifactorial nature. Hyaluronic acid (HA) is a polysaccharide naturally found in joints. Intra-articular administration of HA is a common therapeutic approach for OA treatment. Extracellular vesicles (EVs) released by cells constitute an intercellular signaling system that regulates the progression of OA. This study explores the potential impact of a novel formulation of a patented blend of cross-linked and linear hyaluronic acid (CLHA) (Regenflex Bio-Plus ®) on inflammatory EVs in the context of OA.

Materials and Methods: Primary cultures of osteoarthritis chondrocytes (COA) were obtained from patients undergoing total joint replacement, following approval by the Institutional Ethical Committee. EVs derived from human monocytic cells were collected from both IL-1 β -stimulated and unstimulated THP-1 cells. COA were incubated with 0.3 mg/ml of CLHA at 48h and 7 days. The production of MMP-13 (collagenase 3), HNE-adduct formation (peroxidation lipidic marker), and collagen II (main marker for chondrocyte viability) were analyzed with ELISA assay and immunohistology. To understand the mechanism responsible for the CLHA-EVs interactions, biotinylated CLHA complexes were made with streptavidin-labelled magnetic microbeads. Subsequently, the degree of uptake of EVs by the CLHA complex was assessed.

Results: IL-1 β -stimulated inflammatory EVs, when incubated with COAs significantly increase MMP-13 production at both timepoints tested and induce HNE adduct formation only at 48h. In addition, they significantly diminish collagen II synthesis at 7 days. However, when these inflammatory EVs are incubated in the presence of CLHA, MMP-13 production and oxidative stress are significantly decreased at 48h in COA. Interestingly, a significant increase in collagen II production is observed at 7 days. These results suggest that CLHA may modulate the adverse impact of inflammatory EVs at earlier times and maintain COA viability in the longer term. Experiments exposing EVs to CLHA complexes showed that CLHA binds to EVs, preventing their interaction with target cells. Co-incubation of EVs with CLHA inhibited cellular uptake of EVs by the COAs, and subsequent cargo delivery to the COAs.

Conclusion: These results shows that CLHA potentially reduces the impact of inflammatory EVs in the joint. It is possible that this modulation depended on the binding of CLHA to EVs, thus hindering their ability to interact. The effects of CLHA on EVs unraveled herein constitute an additive therapeutic mechanism whose implementation may improve the control of underlying inflammation in OA.

PP307. The effect of pretreatment with garlic oil extracts alleviates the NaT-induced damage in a human gastric cell model of peptic ulcer disease

Kuna Roguljic L¹, Kizivat T², Roguljic H³, Omanovic Kolaric T⁴, **Petrovic A⁵**, Raguz-Lucic N⁶, Vcev A⁷, Smolic M⁸, Wu C⁹, Smolic R¹⁰

¹*Faculty of Dental Medicine and Health Osijek, Osijek, Croatia*, ²*Faculty of Medicine Osijek, Osijek, Croatia*, ³*Faculty of Medicine Osijek, Osijek, Croatia*, ⁴*Faculty of Dental Medicine and Health Osijek, Osijek, Croatia*, ⁵*Faculty of Dental Medicine and Health Osijek, Osijek, Croatia*, ⁶*Faculty of Dental Medicine and Health Osijek, Osijek, Croatia*, ⁷*Faculty of Dental Medicine and Health Osijek, Osijek, Croatia*, ⁸*University of Connecticut Health Center, Farmington, USA*, ¹⁰*Faculty of Dental Medicine and Health Osijek, Osijek, Croatia*

Introduction: The lifetime prevalence of peptic ulcer disease (PUD) is found in 5-10% of the world's population (1). Proton pump inhibitors, like lansoprazole (LPZ), are first used to treat stomach ulcer. On the other hand, garlic oil extracts (GOE), have been shown to improve PUD (2). A cellular model of peptic ulcer disease (PUD) was established in the human gastric cell line (AGS) by sodium taurocholate (NaT). The aim of this research was to examine the effects of pretreatment of GOE and the addition of LPZ on oxidative stress, morphological changes in cell membrane structure, and F-actin distribution in the AGS model of PUD.

Methods: 4 mM NaT, 10 µM LPZ, 100, 150, 250 and 350 µg/ml concentrations of GOE were used in the experiments. The gastroprotective effect of GOE was assessed by the expression of thioredoxin 1 (TRX1) and ATP-binding cassette, sub-family G, member 2 (ABCG2); The mitotic potential of the cells treated with increasing concentrations of GOE was determined by staining against proliferating cell nuclear antigen (PCNA); Characterization of the effect of LPZ, NaT, and GOE on F-actin distribution in AGS cells was done by F-actin cytoskeleton visualization by semi-quantification of the Rhodamine Phalloidin stain and visualization of the cell membrane structure by semi-quantification using cholesterol (CL) and phospholipid (PH) specific stains.

Results: In our research, a positive correlation of TRX 1 ($p<0.001$) with LPZ and GOE pretreatment was seen, while ABCG2 expression was not changed. Cells stained with PCNA showed that cells treated with GOE and then exposed to NaT had increased mitotic potential ($p<0.001$). The highest concentration of GOE caused significant increase in total F-actin ($p<0.001$). However, NaT had less impact on F-actin structure due to oxidative stress. Cells damaged with NaT showed significant decreases in integrated density values of CL and PL ($p<0.001$). GOE treatments exposed to NaT did not save PL and CH completely, but the membrane preserved them despite NaT washing away.

Conclusion: This research suggests that pretreatment with GOE has a significant gastroprotective role as a preventive agent in PUD, however further pharmacological experiments are needed to confirm the protective role of GOE in ulcer disease.

- [1] Serafim C, Araruna ME, Júnior EA, Diniz M, Hiruma-Lima C, Batista L. A Review of the Role of Flavonoids in Peptic Ulcer (2010-2020). *Molecules*. 2020;25(22).
- [2] Park JJ. The Garlic Preparation as an Alternative Way for Gastropotection: From Bench to Clinic. *Gut Liver*. 2016;10(3):321-2.

PP308. Apolipoprotein E4: A possible pharmacological target for the management of diet induced obesity and related lipid disorders

Giannatou C¹, Mparnia V², Kakafoni G³, **Zvintzou E⁴**, Kypreos K⁵

¹*Pharmacology Laboratory, Department of Medicine, School of Health Sciences, University of Patras, Patras, Greece*, ²*Pharmacology Laboratory, Department of Medicine, School of Health Sciences, University of Patras, Patras, Greece*, ³*Pharmacology Laboratory, Department of Medicine, School of Health Sciences, University of Patras, Patras, Greece*, ⁴*Pharmacology Laboratory, Department of Medicine, School of Health Sciences, University of Patras, Patras, Greece*, ⁵*Pharmacology Laboratory, Department of Medicine, School of Health Sciences, University of Patras, Patras, Greece*

Morbid obesity, one of the main disorders of the metabolic syndrome, shows an alarmingly increased prevalence which is no longer limited to the countries of the western world. One of the key components of obesity is the metabolic activation of adipose tissue. Recent data show that apolipoprotein E (APOE) is directly related to diet-induced obesity. Data from our laboratory support that APOE expression in the brain is a potent mediator of the processes leading to diet-induced obesity, while hepatic APOE expression contributes to resistance to obesity [1]. Based on these observations we studied the effect of a genetically modified form of the human APOE4 isoform, APOE4mut1, on plasma lipid levels and adipose tissue metabolic activation. For this purpose, wild-type (C57BL/6) mice were fed a high-fat diet for eight or twenty four weeks. Subsequently, they were infected with an adenovirus expressing either APOE4 or APOE4mut1 [2], while the control group was infected with a control adenovirus expressing only green fluorescent protein (GFP). Our results show that both APOE4 expression and APOE4mut1 expression led to a reduction in blood glucose levels. In contrast, APOE4 expression led to an increase in plasma cholesterol and triglyceride levels, while APOE4mut1 expression did not appear to significantly affect blood lipid levels. These observed differences in lipid levels also reflect differences in metabolic activation of adipose tissue, with APOE4mut1 expression leading to an improved metabolic profile of brown and white adipose tissue. Thus far, our results support the hypothesis that the modified APOE4mut1 form of APOE4 has beneficial effects on lipid metabolism and adipose tissue metabolic activation, while wild type APOE4 is directly related to the development of diet induced obesity [3]. APOE4 could be a possible pharmacological target for the management of obesity and related lipid disorders.

- [1] Hatziri A, Kalogeropoulou C, Xepapadaki E, et al. Site-specific effects of apolipoprotein E expression on diet-induced obesity and white adipose tissue metabolic activation. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2018;1864(2):471-480. doi:10.1016/j.bbadi.2017.11.007
- [2] Kypreos KE, van Dijk KW, Havekes LM, Zannis VI. Generation of a Recombinant Apolipoprotein E Variant with Improved Biological Functions. *Journal of Biological Chemistry*. 2005;280(8):6276-6284. doi:10.1074/jbc.M413458200
- [3] Kalogeropoulou C, Hatziri A, Xepapadaki E, et al. Isoform and tissue dependent impact of apolipoprotein E on adipose tissue metabolic activation: The role of apolipoprotein A1. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*. 2020;1865(2):158551. doi:10.1016/j.bbalip.2019.158551

PP309. Development and in vitro evaluation of genistein-loaded exosome-inspired nanoparticles

Tsakiri M^{1,2}, Naziris N^{1,3}, Zivko C^{2,4}, Ahmed W^{2,4}, Sagar R^{2,4}, Ionov M³, Bryszewska M³, Mahairaki V^{2,4}, Demetzos C¹

¹Section of Pharmaceutical Technology, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece, Athens, Greece, ²Department of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD, United States, ³Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland,

⁴The Richman Family Precision Medicine Center of Excellence in Alzheimer's Disease, Johns Hopkins Medicine and Johns Hopkins Bayview Medical Center, Baltimore, MD, United States

Exosomes are extracellular vesicles that present pivotal role for intra- and inter-cellular communication. Their potential as natural information vesicles is inspiring in the field of drug delivery platforms. However, their low isolation yield and complex cargo limit their therapeutic applications. Thus, the development of synthetic nanoparticles with similar characteristics to that of exosomes could up-grade the properties of drug delivery nanoplatforms, especially in alleviating neurodegenerative diseases, for which the limitations of brain-targeting therapies hamper successful treatment options. The aim of this study was to develop and evaluate exosome-inspired lipidic nanoparticles loaded with the phytoestrogen molecule, genistein and evaluate their toxicity after administration to neurons derived from human induced pluripotent stem cells (iPSCs).

Methods: A variety of lipids and lipid ratios were employed to develop the nanoparticles. Their physicochemical stability was assessed through dynamic and electrophoretic light scattering, thermal analysis, and nano-flow cytometry. Membrane fluidity was examined at different temperatures using fluorescence spectroscopy, while interactions between nanoparticles and albumin were evaluated via circular dichroism. Viability tests were conducted on neurons derived from human IPS cells from both control and Alzheimer's disease cell lines (day 21 of differentiation). Finally, the internalization of fluorescent genistein-loaded exosome-inspired nanoparticles within neurons stained with DAPI (nucleus dye) and MAP2 (cellular membrane dye) was confirmed using confocal microscopy.

Results: Preservation of the hydrodynamic diameter, polydispersity index and drug incorporation efficiency of the nanoparticles was achieved through lyophilization and storage at 4°C. Incorporation efficiency of genistein in a molar ratio higher than 10% caused heterogeneity and instability of the platforms, while the concentration of cholesterol is the main parameter affecting the microfluidity of the membranes. Circular dichroism experiments revealed negligible alterations in the secondary structure of albumin upon interaction with nanoparticles. Furthermore, genistein-loaded nanoparticles demonstrated no toxicity on human IPSC-derived neurons from control and Alzheimer's disease cell lines at two different drug concentrations (0.05 and 0.1 µM). In vitro ROS on pre-treated iPSC-derived neurons measurements showed a moderate effect of genistein in reducing oxidative stress. Following a 2-hour incubation, evidence indicated internalization of nanoparticles with neuronal cell membranes.

Conclusion: Our study underscores the promise of exosome-inspired nanoparticles as effective drug delivery vehicles. Successful loading of the isoflavone phytoestrogen genistein was achieved without significantly affecting the stability of the platforms at genistein molar ratio below 10% or neuronal viability.

PP310. Investigation of the Antibacterial and Antibiofilm Activities of Cinnamaldehyde and Berberine Extracts on Methicillin-Resistant *Staphylococcus epidermidis* Clinical Strains

Eken B¹, **Kantarci M**², Abdalhalim K³, Arayici M⁴, Ozturk D⁵, Ellidokuz H⁴, Gumustekin M², Yilmaz O¹

¹Department of Medical Microbiology, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey,

²Department of Medical Pharmacology, Faculty of Medicine, Dokuz Eylul University, Izmir, Türkiye,

³Department of Nephrology and Hypertension, Mayo Clinic, Rochester, USA, ⁴Basic Medical Sciences

Department of Biostatistics and Informatics, Faculty of Medicine, Dokuz Eylul University, Izmir,

Türkiye, ⁵Izmir Biomedicine and Genome Center, Electron Microscopy Unit, Izmir, Türkiye

Introduction: *Staphylococcus epidermidis*, a member of the commensal skin flora of bacterial opportunistic pathogens, is a major cause of community and hospital acquired infections. Often associated with implanted medical devices and capable of forming biofilms on their surfaces and having high morbidity and mortality.

Objective: In this study, we aimed to investigate the antibacterial and antibiofilm activities of cinnamaldehyde and berberine extracts on the same methicillin-resistant *Staphylococcus epidermidis* (MRSE) clinical strains.

Methods: Fourty MRSE strains isolated from 117 inpatients or outpatients were randomly selected and included in the study. Broth microdilution and tissue culture plate (TCP) methods were used to determine the minimum inhibitory concentrations (MICs) and antibiofilm activities of cinnamaldehyde and berberine extracts, respectively. Antibacterial effects were compared with vancomycin and linezolid. Standard strains of *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 35984 were used as positive controls. Biofilm-related target genes gseA, icaA, icaB, icaC, icaD, atlE, fruA, seg, meca were examined by PCR.

Results: Cinnamaldehyde MIC value was 64 to 256 µg/mL (median 128) and berberine was 32 to 512 µg/mL (median 256). No resistance to vancomycin and linezolid was detected in the MRSE strains studied. The MIC values of cinnamaldehyde and berberine were consistent with positive controls. Strong biofilm formation was detected in 31 and moderate biofilm formation in 9 of the clinical strains (OD 575), which were found to form stronger biofilms than the standard strains. The biofilm inhibition rates were 37.56% for cinnamaldehyde and 25.44% for berberine. At high concentrations (1024-512-256 µg/mL), cinnamaldehyde provided more than 50% biofilm inhibition in all strains compared to berberine. Both extracts had efficacy below the MIC values on each MRSE strains individually also both showing effects in 64 µg/mL concentration, confirmed by SEM analysis. *Staphylococcus epidermidis* specific gseA gene was detected in all strains, the atlE gene was the most detected among all biofilm-related genes (97.5%). All study data will be compared with the clinical data of the patients and evaluated in relation to treatment.

Conclusion: In this study, both extracts showed antibacterial and antibiofilm activity on the same MRSE strains, but further studies are needed to elucidate their mechanisms of action.

[1] Albano M, Crulhas BP, Alves FCB, et al. Antibacterial and anti-biofilm activities of cinnamaldehyde against *S. epidermidis*. *Microb Pathog*. 2019;126:231-238.

[2] Wang X, Yao X, Zhu Z, et al. Effect of berberine on *Staphylococcus epidermidis* biofilm formation. *Int J Antimicrob Agents*. 2009;34(1):60-66.

PP311. Antifungal activity of Pterostilbene incorporated in the tissue conditioner on in vitro growth of Candida Albicans

Komoni T^{1,2}, Sutej I²

¹*Faculty of Dentistry, University for Business and Technology-UBT, Prishtina, Kosovo,* ²*School of Dental Medicine, University of Zagreb, Zagreb, Croatia*

Background: One of the primary causes of fungal infections in the mouth is *Candida albicans*. One of the ethiological causes for denture-related stomatitis, particularly from prosthodontic materials like tissue conditioners, which are materials for treating dental defects, is based on its ability to attach colonisation in the mouth. Such material itself has a soft surface that allows *Candida albicans* to attach to and colonise, and it lacks antifungal properties. Since fungal infections are becoming more common and resistant to treatment, novel medication delivery methods utilising dental materials as well as bioactive chemicals are thought to be crucial tools for meeting this problem. Pterostilbene (PTE), a naturally occurring analogue of resveratrol, possesses antifungal, anticancer, antidiabetic, cardioprotective, anti-allergic, anti-androgenic, and anti-atherosclerotic properties. Minor reports show that PTE has antifungal properties against *Candida albicans*. The impact of PTE, especially its incorporation in tissue conditioner material, in preventing the formation of biofilms, however, is not well understood. The purpose of this work is to evaluate PTE integrated with tissue conditioner (PTE-TC) against *Candida albicans* in vitro for antifungal efficacy.

Methods: Using the Broth Dilution Antifungal Susceptibility Testing Yeasts method, we first examined the in vitro antifungal activity of PTE in preventing the formation of biofilms in *C. albicans* standard strains 10231. We then investigated the in vitro contribution of PTE-TC, which was produced as discs 1% (v/v), to the development of *Candida albicans*. PTE vehicle absorption into the tissue conditioner served as the negative control, while 1% (v/v) ketoconazole added to the tissue conditioner served as the appropriate positive control.

Results: PTE ≤ 8 µg/ml significantly inhibits the production of biofilms ($P<0.001$). The growth of *C. albicans* was significantly inhibited by PTE-TC, 1% ($E_{max}=60.5\%$, $P<0.001$). This inhibition improved throughout time, indicating that the PTE was released continuously.

In conclusion, PTE-TC has shown antifungal properties in *C. albicans* biofilm development. As a result, TC modification with PTE may be an effective medication delivery method for TC colonisation and adherence prevention. To further elucidate this use, however, more concentration/time dependent and biocompatibility studies may be required.

PP312. Selected Growth Hormone Secretagogues (GHS) as disease modifiers in an in vitro model of Amyotrophic Lateral Sclerosis: a proof-of-concept study

Meanti R¹, Rizzi L¹, Bresciani E¹, Licata M¹, Molteni L¹, Omeljaniuk R², Fehrentz J³, Denoyelle S³, Locatelli V¹, Torsello A¹

¹*University of Milano-Bicocca, Monza, Italy*, ²*Lakehead University, Thunder Bay, Canada*, ³*Institut des Biomolécules Max Mousseron (IBMM), Montpellier, France*

Introduction: ALS is a disease characterized by rapid deterioration of both upper and lower motor neurons in the brainstem, motor cortex and spinal cord. The mutation in SOD1 with the replacement of glycine 93 by alanine is responsible for a conformational change that leads to a gain of function resulting in oxidative stress, mitochondrial alterations, and apoptosis [1].

GHS are a family of synthetic compounds capable to stimulate the release of GH, but they also exert neuroprotective effects and participate in the regulation of skeletal muscle mass, in animals and humans. Their pleiotropic roles on neurons and muscle cells suggest that GHS could be developed for the treatment of ALS. Among them, hexarelin has important neuroprotective and cytoprotective activities, both in vitro and in vivo; and JMV2894 stimulates Ca²⁺ mobilization in vitro and GH release in vivo, and modulates mitochondria functioning and ROS production [2].

Methods: human neuroblastoma cells overexpressing the SOD1G93A mutated protein (SH-SY5Y SOD1G93A cells) were treated with H₂O₂ and GHS for 24h to study the protective effect of GHS against increased oxidative stress. The mRNA expression levels were quantified by real-time PCR, whereas protein levels were measured by western blot.

Results: Morphometric evaluation showed that H₂O₂-treatment induced an apoptotic phenotype that was rescued by both GHS. The quantification of mRNA levels of the BCL-2 family and those of the effector caspase proteins suggest that GHS have anti-apoptotic effects: both GHS significantly decreased Bax/Bcl-2 ratio and hexarelin also inhibited the activation of caspase-3.

The molecular pathways involved in GHS neuroprotection include the modulation of MAPKs and PI3K/Akt phosphorylation, probably through epigenetic mediation. Immunofluorescence visualization of γH2AX nuclear foci showed that hexarelin and JMV2894 significantly decreased the percentage of γH2AX-positive cells compared to the H₂O₂-treated group.

Conclusions: Hexarelin and JMV2894 are capable of protecting cells from oxidative stress-caused cytotoxicity, suggesting the possibility of developing new anti-oxidant and neuroprotective drugs with improved therapeutic potential.

- [1] S.T. Ngo, H. Wang, R.D. Henderson, C. Bowers, F.J. Steyn, Ghrelin as a treatment for amyotrophic lateral sclerosis, *J Neuroendocrinol.* (2021). <https://doi.org/10.1111/jne.12938>.
- [2] Meanti R, Bresciani E, Rizzi L, Coco S, Zambelli V, Dimitroulas A, Molteni L, Omeljaniuk RJ, Locatelli V, Torsello A. Potential Applications for Growth Hormone Secretagogues Treatment of Amyotrophic Lateral Sclerosis. *Curr Neuropharmacol.* 2023;21(12):2376-2394. doi: 10.2174/1570159X20666220915103613.

PP313. Optimal Timing for Fluoride Sample Analysis: Mitigating Percentage Deviation Risks

Smajli Vokshi K¹, Kullashi Spahija F¹, Šutej I², Bašić K², Peroš K², Spahija K³

¹School of Dental Medicine, Zagreb, Croatia, ²School of Dental Medicine, Department of Pharmacology, Zagreb, Croatia, ³Dental Polyclinic Center, Peja, Kosovo

Introduction: Timely and accurate analysis of fluoride samples is crucial in dental research and clinical practice to ensure reliable results and appropriate patient care, particularly regarding fluoride concentration. This study aims to investigate the impact of delayed fluoride sample analysis on percentage deviation (PD%) from baseline values and to determine the optimal timeframe for fluoride sample analysis to maintain accuracy and efficiency of fluoride concentration assessments [1].

Methods: All experimental procedures were conducted in compliance with the ethical standards outlined in the Declaration of Helsinki for biomedical research. This blinded and randomized in vitro study received approval from The Ethics Committee of the School of Dental Medicine at the University of Zagreb, with protocol number (05-PA-30-17-4/2023). Fluoride was extracted from 30 enamel slabs using 1M KOH solution for 24h and under agitation of the shaker at the room temperature, by method of Caslavská. The extracts were analyzed using fluoride ion-specific electrode (Orion Research EA 940) by ISO 19448:2018 standard method, in triplicates, at the same day of extraction (n=30), one day later (n=10), 14 days later (n=30) and one year later (n=20) after storage in firmly closed polystyrene cups on room temperature. PD% was calculated for each delay period. Statistical analysis was conducted using the Friedman analysis of variance (ANOVA) test to assess overall significance and the nonparametric Wilcoxon matched-pairs test to identify specific time points with significant differences.

Results: PD% increased with longer delays: 12% for one day, 50.6% for two weeks, and 197% for one year. Friedman ANOVA revealed significant differences ($p<0.05$) across time points. The Wilcoxon matched-pairs test identified significant deviations at two weeks ($p<0.05$) and one year ($p<0.05$) time points, while the one-day delay was not statistically different from baseline.

Conclusion: Results suggest that fluoride samples should be analyzed promptly, preferably within two days, to ensure reliable outcomes. This study underscores the importance of timely fluoride sample analysis in research and clinical settings to mitigate potential inaccuracies associated with delayed analysis.

- [1] Gómez-Rioja R, Segovia Amaro M, Diaz-Garzón J, et al. A protocol for testing the stability of biochemical analytes. Technical document. Clin Chem Lab Med. 2019;57(12): 1829-1836.