XL NATIONAL MEETING OF THE SPANISH SOCIETY OF PHARMACOLOGY

6-8 September 2023

Toledo

BOOK OF ABSTRACTS

Castilla-La Mancha

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WELCOME

On behalf of the Spanish Society of Pharmacology, it is my pleasure to welcome you to the XL National Meeting of the Spanish Society of Pharmacology that will be held in Toledo in the Campus de la Fábrica de Armas belonging to the Universidad de Castilla-La Mancha from 6 to 8 of September.

The meeting is expected to gather a large number of pharmacologists and scientists from other academic disciplines such as chemists, pharmacists, and physicians. We have organized a very attractive scientific program covering frontline areas of pharmacology and therapeutics. Moreover, there will be three Invited Lectures to be delivered by highly recognized scientists such as Virgil Percec, Graeme Milligan, and Michael Spedding. We think that the future of the society in general and of scientific societies in particular relays on the young generations that will be able to surpass the achievements of their predecessors. For this reason, the meeting will put the focus in facilitating the participation of young scientists at early stages of their scientific careers through short presentations of their scientific results, as well as facilitating the assistance to the Meeting through grants to cover their expenses.

Besides the scientific part, Toledo is a marvelous city located on the riverbanks of Tajo river that has an intense history which is reflected in the large number of monuments telling the history of the city known as the city of three cultures: Muslim, Christian and Jew that lived together for centuries in the city. Toledo is also a modern city with a very attractive artistic, gastronomic, and ludic offer which makes it worth to visit.

We look forward to meet you in Toledo at the XL National Meeting of the Spanish Society of Pharmacology.

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CONGRESS TIME TABLE

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13:45-15:30	Lunch	
15:30-17:30	Round Table 1	Session 3
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12:30-14:30	Round Table 2	
14:30-16:00	Lunch	
16:00-17:00	IUPHAR Lecture: Graeme Milligan	
17:00-17:30	Coffee-break and Posters	
17:30- 18:30	Session 7	
18:30-20:00	Assembly of the SEF. Young Researchers award ceremony	
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9:30-11:30	Session 8	Session 9
11:30-12:00	Coffee and posters	
12:00-13:00	Closing Lecture: Michael Spedding	
13:00-13:45	Closing	

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ABSTRACTS

PLENARY LECTURES

From Helical Chirality to Targeted Delivery of mRNA by Reprogramming the Factory of Life

Virgil Percec

Roy & Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, United States

E-mail: percec@sas.upenn.edu

Homochiral helical self-organizations are known in nature, art, architecture and science for thousands of years [1]. However, they were discovered in the biological macromolecules proteins and DNA [2] only in the early 1950th and in molecular, macromolecular and supramolecular complex nanosystems soon after [1]. Our ability to program functions at the molecular level based on helical chirality is less advanced than the same process at the macroscopic level during Archimedes and Leonardo da Vinci times. The principles *"discover-elucidate mechanism-predict programmed primary structure"* elaborated in our laboratory and aided by synthetic methodologies developed also in our laboratory for accelerated modular-orthogonal synthesis of programmed structures including selected examples of homochiral helical self-organizations and functions together with the molecular factory of life will be briefly explained. The origins of helical homochirality is equivalent to the origins of life although the origins and rational of biological membranes homochirality continues to be debated [3]. Inspiration from amphiphilic Janus dendrimers [4a] and Janus glycodendrimers [4b] discovered in our laboratory allowed us to transit from the commercial viral and four-component lipid nanoparticle synthetic vectors for delivery of mRNA to the *one-component ionizable multifunctional sequence-defined amphiphilic Janus dendrimers (IAJDs)* delivery vectors [5]. The current status of the molecular design principles providing the least expensive and the simplest access to targeted delivery of mRNA with programmed IAJDs and the role of helical chirality to activity will be discussed in details. Targeted delivery of mRNA to all organs is expected to change the field of nanomedicine at the most fundamental level by providing unprecedented avenues to new vaccines and therapeutics.

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Acknowledgement

Financial Support by the National Science Foundation, Grants DMR-2104554, DMR-1720530, DMR-1807127, Wellcome Foundation and by P. Roy Vagelos Chair at the University of Pennsylvania is gratefully acknowledged.

Defining roles and the regulation of GPCRs for short- and medium-chain fatty acids

Graeme Milligan[1], Sara Marsango[1], Laura Jenkins[1], Cheng Zhang[2], Andrew B. Tobin[1], Natasja Barki[1]

[1]Centre for Translational Pharmacology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland UK, [2]Department of Pharmacology and Chemical Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA15261, USA

E-mail: Graeme.Milligan@glasgow.ac.uk

Free fatty acids of varying chain length mediate many of their functions via the activation of one or more members of the G protein-coupled receptor superfamily. These include the short chain fatty acid receptor FFA2 and the medium chain fatty acid receptor GPR84. I will report discuss the first cryoEM structures of receptor-G protein complexes for both FFA2 and GPR84 with bound orthosteric agonists, the generation and characterisation of phosphorylation site-specific antisera that can be used to identify these receptors post-activation in both heterologous cell lines and in native tissues and the development of novel antagonist ligand classes for each of FFA2 and GPR84. Finally, I will present the production and characterisation of unique knock-in transgenic mouse lines that in the first case replaces mouse FFA2 with a Designer Receptor Exclusively Activated by Designer Drugs (DREADD) variant of human FFA2, whilst in the second mouse GPR84 is replaced by a human-mouse chimera of GPR84 that displays human orthologue pharmacology with mouse GPR84 signal regulation.

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Acknowledgements

These studies were supported in part by grants from the Biotechnology and Biosciences Research Council (UK), grant numbers BB/X001814/1, BB/T000562/1 and BB/S000453/1

Glucocerebrosidases: Critical roles in ALS, and viral infections: links to neurodegeneration

Spedding M[1], Perera R[2], StClair L.[2], Platt F[3], Priestman D[3], Teixeira M, Henriques A[1,4,5], Loeffler J-P[4], Bouscary A[4], Turner BJ[6]

[1]Spedding Research Solutions SAS, 6 rue Ampere, 78110 Le Vesinet, France, [2]Center for Vector-borne and Infectious Diseases (CVID), Colorado State University, Fort Collins, CO 80523-1685, USA, [3]University of Oxford, Department of Pharmacology, Oxford OX1 3QT, UK, [4]University of Strasbourg, UMRS 1118, CRBS, 67000 Strasbourg, France, [5]Neuro-sys SAS, 13120 Gardanne, France, [6]Florey Institute, University of Melbourne, Melbourne, Australia.

michael@speddingresearchsolutions.fr

The diversity of glycosphingolipids (GSLs) is immense, yet tightly controlled biologically, awry in lysosomal diseases and neurodegeneration, and also recognized by viruses for entry and exit from infected cells. Glucosylceramide hydrolysis to ceramide is controlled by lysosomal (GBA1) and non-lysosomal (GBA2) glucosylceramidases and appear to be major causes of neurodegeneration. GBA2 activity is increased 8-fold in spinal cord at the very beginning of symptoms in the superoxide-dismutase1 ($SOD1^{G86R}$) model of amyotrophic lateral sclerosis (ALS), and a neurotrophic five-sugar (GM1) is lost from neuromuscular junctions (NMJs) resulting in denervation [1]. In contrast, GBA1 mutations are the main cause of hereditary Parkinson's disease, hence it is critical not to inhibit GBA1 and disturb lysosomal function.

We and others have shown that envelope viruses such as dengue and SARS-CoV-2 target GM1, and need GBA1 and GBA2 activity for replication in host cells. The generic mucolytic drug, ambroxol, inhibits GBA2 (30nM), while acting as a chaperone for lysosomal GBA1 without inhibiting synthase, and protects NMJs by preserving GM1and may be perhaps the only drug with the required profile to be active in both diseases. Ambroxol inhibits SARS-Cov-2 replication *in vitro*, at the same concentrations (10µM) as it increases NMJ formation *in vitro*. Ambroxol is now in phase II for ALS and phase III for Parkinson's disease. We have used lipid metabolomics to show remarkable similarities between neurodegeneration in ALS and the metabolic responses of host cells to viral infection.

We have obtained a JPND grant, uniting investigators in the field, to define the risks of neurodegenerative disease following long COVID, and the results (and mechanisms) will be communicated at the meeting.

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ROUND TABLES

V

Round Table 1: Perspectivas relativas a la “Ley de Garantías” y evolución reciente de su contenido. Repercusiones socio-sanitarias.

Chairman: Francisco Zaragozá. Universidad de Alcalá de Henares.

César Hernández. Director. General de Cartera Común de Servicios del Sistema Nacional de Salud y Farmacia.

Antonio Blanes. Director de Servicios Farmacéuticos. Consejo General de Farmacéuticos.

Isabel Pineros. Farmaindustria.

Carina Escobar. Presidente. Plataforma de Pacientes.

Round Table 2: Papel de los diversos agentes financiadores en la financiación de la investigación biomédica.

Chairman: Valentín Ceña. Universidad de Castilla-La Mancha.

Cristóbal Belda. Director General. Instituto de Salud Carlos III.

Javier Ponce. Director General. Centro para el Desarrollo Tecnológico y la Innovación.

Ricardo Cuevas. Director General de Investigación. Junta de Comunidades de Castilla-La Mancha.

Isabel Amat. Laboratorios Reig-Jofré.

SESSIONS

Session 1: Advances in cardiovascular therapeutics

Chairman: Juan Tamargo. Universidad Complutense de Madrid.

6/9/2023 Paraninfo Envases de Cartón

9:30-10:00 Juan Tamargo. Universidad Complutense de Madrid. **Diabetes and Heart Failure: Therapeutic role of glifozines.**

10:00-10:30 Luis Rodríguez Padial. Complejo Hospitalario de Toledo**. New approaches to the treatment of thrombosis.**

10:30-11:00 Ricardo Caballero. Universidad Complutense de Madrid**. Treatment of dyslipidemias.**

11:00-11:15 Sofia Miñano. Universidad de Granada. **Role of immune cells mitochondrial metabolism in the development of cardiovascular complications in a murine model of lupus induced by TLR7 activation.**

Therapeutic role of SGLT2 inhibitors in patients with type 2 diabetes, chronic kidney disease and heart failure

Juan Tamargo

Department of Pharmacology and Toxicology, School of Medicine, Universidad Complutense, 28040 Madrid

E-mail: jtamargo@med.ucm.es

Heart failure (HF), chronic kidney disease (CKD), and type 2 diabetes (T2D) frequently co-exist, aggravate each other, and exert synergistic effects to increase the risk of cardiac and renal events. In the last 8 years, several clinical trials have confirmed the key role of sodium-glucose cotransporter 2 inhibitors (SGLT2Is) in the treatment of patients with T2D, HF and CKD, expanding the expectations of these drugs that were originally designed to inhibit the glucose reabsorption in the proximal tubule of the kidney and, thus, increase urinary glucose excretion and improve glycemic control in patients with T2D. Unexpected, SGLT2Is were found to significantly reduce HF hospitalization and cardiovascular death in patients with HF irrespective of baseline left ventricular ejection fraction (being the first drugs effective in patients with HF with preserved ejection fraction), symptomatic impairment or diabetic status. These effects were also observed in patients with T2D but without HF at baseline, so the benefit of SGLT2Is largely reflected the prevention of incident HF in this population. In patients with CKD or HF (in whom CKD is common), SGLT2Is safely reduced the risk of kidney disease progression (renal composite comprising end-stage kidney disease, doubling of serum creatinine level, or renal or cardiovascular death) and of acute kidney injury compared with placebo. The renal cardioprotective effects were observed across the range of studied kidney function (estimated glomerular filtration rate of at least 20 mL/min/1.72 m^2), irrespective of diabetes status, or primary kidney disease diagnosis. Of note, the benefits of SGLT2Is were obtained on top of conventional therapy.

Thus, SGLT2Is represent a "conceptual revolution" in the management of T2D patients, but beyond their glucose-lowering effect, they are able to reduce the cardiovascular risk and progression of kidney disease in individuals with or without diabetes. Therefore, at the present time they are recommended in clinical guidelines for the treatment of patients with T2D, HF, and CKD patients and they should be prescribed in all patients with HF or CKD lacking contraindications. They are easy to use (no up-titration is needed), present minimal risk of hypoglycemia (its glucosuric effect disappears at blood glucose levels <70 mg/dL) or effects on heart rate or blood pressure and few contraindications. Unfortunately, the mechanisms through which these inhibitors act to provide their benefits (cardio-renal protection) are incompletely understood although multiple mechanisms (hemodynamic, metabolic, hormonal and direct cardiac/renal effects), possibly unrelated to SGLT2 inhibition, and with different roles over time and in different populations might be involved. A better understanding of the mechanisms of action, which will be discussed in the presentation, is the first step to identify the patients who could benefit most from the use of SGLT2Is.

Effects of empagliflozin on human cardiac sodium and Kir2.1 channels

Rapún J, Cámara-Checa A, Crespo-García T, Gil-Cabezudo C, Tamargo J, Gómez R, Caballero R, Delpón E.

Dpt. Pharmacology & Toxicology. School of Medicine. Universidad Complutense de Madrid 28040-Madrid. CIBERCV
E-mail: rcaballero@med.ucm.es

Empagliflozin (Empa) is a sodium-glucose cotransporter 2 inhibitor (SGLT2i) used for the treatment of type 2 Diabetes Mellitus (T2DM). Empa reduces morbidity and mortality in heartfailure (HF) patients with reduced or preserved ejection fraction, regardless of the presence or absence of T2DM. Nav1.5 channels encoded by the *SCN5A* gene carry the Na^+ current (I_{Na}) which is responsible for cardiac action potential (AP) depolarization and, thus, is a critical determinant of cardiac excitability and conduction velocity. Kir2.1 channels encoded by the *KCNJ2* gene carry the inward rectifier K^+ current (I_{K1}) that controls resting membrane potentialand the duration of the final repolarization. Our group has demonstrated that Empa, incubated for 24 hours at therapeutically relevant concentrations, increased I_{Na} and I_{K1} in human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CMs),[1] effects that could participate in the beneficial actions of the drug on HF patients, where the expression of Nav1.5and Kir2.1 channels is reduced. The increase did not involve Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) or the Na^+/H^+ exchanger. Moreover, we demonstrated that Empa directly augmented currents generated by Nav1.5 ($I_{Nav1.5}$) and Kir2.1 ($I_{Kir2.1}$) channels in heterologous systems. Our aim was to elucidate the molecular mechanisms underlying the Empa-induced increase on cardiac I_{Na} and I_{K1}. We recorded macroscopic and unitary currents in CHO cells incubated or not with Empa expressing Nav1.5 or Kir2.1 channels by using the whole-cell and cell-attached configurations of the patch-clamp technique, respectively. Furthermore, luciferase assays were conducted to determine putative effects of the drug at the transcriptional level and flow cytometry experiments to measure putative effects on themembrane expression of the channels. In all cases, Empa (1 µM) was added to the culture mediaand incubated for 24 h. Whole-cell patch-clamp recordings demonstrated that Empa produced a concentration-dependent increase of $I_{Nav1.5}$ (EC_{50}=0.8±0.01 µM) and $I_{Kir2.1}$ (EC_{50}=0.9±0.01 µM). Single channel recordings demonstrated that Empa augmented mean open time (MOT, from 0.96±0.04 to 1.42±0.13 ms, $P<0.05$) and open probability (*Po*, from 0.03±0.001 to 0.065±0.009) of Nav1.5 channels, without modifying mean current amplitude (-2.9±0.1 pA at-20 mV, $P>0.05$). On the other hand, Empa increased MOT (from 98.8±19.1 to 279±33 ms, $P<0.05$) and *Po* (from 0.56±0.06 to 0.78±0.02, $P<0.05$) of Kir2.1 channels, without modifyingthe current amplitude (-2.7±0.3 pA at -120 mV) or slope conductance (γ=28±0.06 pS) ($P>0.05$). Luciferase assays carried out in CHO cells transfected with vectors encoding the minimal promoters of the *SCN5A* and *KCNJ2* genes demonstrated that incubation with Empa does not modify their transcriptional activity. Flow cytometry experiments in HEK-293 cells, showed that Empa treatment slightly but significantly increased the expression of Kir2.1 channels at the membrane. In conclusion, our results demonstrated that Empa increased cardiacI_{Na} and I_{K1} by producing remarkable effects on single Nav1.5 and Kir2.1 channel properties andsuggested that Empa directly interacts with the respective channel proteins.

References
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Acknowledgements
This work was funded by Ministerio de Ciencia e Innovación (PID2020-118694RB-I00), Comunidad Autónoma de Madrid (P2022/BMD-7229), and Instituto de Salud Carlos III (CIBERCV; CB16/11/00303).

Role of immune cells mitochondrial metabolism in the development of cardiovascular complications in a murine model of lupus induced by TLR7 activation

Miñano, S[1]; Moleón, J[1,2]; González-Correa, C[1,2]; Robles-Vera, I[3]; de la Visitación, N[4]; Toral, M[1,2,5]; Palmiter, R[6]; Sancho, D[3]; Gómez-Guzmán, M[1,2]; Romero, M[1,2]; Sánchez, M[1,2]; Jiménez, R[1,2,5]; Duarte, J[1,2,5].

[1] *University of Granada, Granada, Spain.*
[2] *Instituto de Investigación Biosanitaria de Granada, ibs.GRANADA, Granada, Spain.*
[3] *Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.*
[4] *Vanderbilt University Medical Center, Nashville, Tennessee, USA.*
[5] *Ciber de Enfermedades Cardiovasculares (CIBERCV), Spain.*
[6] *University of Washington, Seattle, Washington, USA.*
E-mail: sofiaminano@correo.ugr.es

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease associated with a high risk of cardiovascular disease. Activation of toll-like receptors (TLR)7 leads to phenotypic and functional changes characteristic of human SLE. Enhanced lactate production and mitochondrial oxygen consumption in the CD4+ T cells from lupus-prone mice and SLE patients have been reported.[1] Moreover, the combination of the mitochondrial chain complex I inhibitor, metformin, with the glucose metabolism inhibitor, 2-deoxy-D-glucose (2DG) normalized T-cell metabolism and SLE disease activity[1], although their impact on the development of vascular dysfunction and hypertension has not been explored. The aim is to analyze the role of immune cell mitochondrial chain complex I (Ndufs4 subunit) on endothelial dysfunction and the development of hypertension in mice with lupus induced by TLR7 activation with imiquimod (IMQ).[2] Experiment 1: Irradiated female C57BL/6NRj mice received bone marrow cells extracted from the medulla of wild type (WT) or Ndufs4-/- knock out (KO) mice. After 60 days the mice were divided into 4 groups (n=10): 1) WT, 2) WT-IMQ, 3) KO, and 4) KO-IMQ. IMQ treatment was maintained for 8 weeks. Experiment 2: Female C57Bl/6NRj mice were used and randomly divided into 4 groups (n=10): 1) Control, 2) Control-treated (metformin, 3 mg/mL + 2DG 5 mg/mL in drinking water), 3) IMQ, and 4) IMQ-treated (metformin, 3 mg/mL + 2DG 5 mg/mL in drinking water). The treatment was maintained for 8 weeks. Knock-out of the Ndufs4 subunit in immune cells prevented the development of hypertension, reduced anti-ds-DNA levels, improved vascular oxidative stress and endothelial function in SLE mice induced by IMQ. Similarly, treatment with metformin+2DG prevented the development of hypertension, reduced disease progression (plasma levels of anti-dsDNA autoantibodies and splenomegaly), T lymphocyte polarization toward Th17/Th1 in secondary lymphoid organs, and improved endothelial function in mice with IMQ-induced lupus. The vascular protective effects caused appear to be associated with reduced vascular oxidative stress generated by increased NADPH oxidase activity, derived in part from reduced vascular infiltration of Th17 lymphocytes. In conclusion, our findings identify mitochondrial Ndufs4 subunit as a key regulator of the immune system polarization involved critically in the development of autoimmunity and endothelial dysfunction in SLE mice. Furthermore, mitochondrial chain complex I could be a novel therapeutic target for the prevention of cardiovascular complications in SLE.

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Supported by Grants from MINECO (PID2020-116347RB-I00), Junta de Andalucía (P20_00193, A-CTS-318-UGR20) with funds from the Fondo Europeo de Desarrollo Regional, (FEDER, "FEDER una manera de hacer Europa").

Session 2. Neuropharmacology

Chairman: José Manuel Brea. Universidad de Santiago de Compostela.

6/9/2023 Sala de Grados Sabatini

9:30-10:00 Asia Fernández Carvajal. Universidad Miguel Hernández de Elche. **New pharmacological approaches to treat alterations in the sensory nervous system.**

10:00-10:30 Olga Valverde Granados. Universidad Pompeu Fabra. **Modulatory effects of endocannabinoids on cocaine seeking behavior in mice.**

10:30-11:00 Rebeca Diez-Alarcia. Universidad del País Vasco: **Inverse agonism and functional selectivity on serotonin 5-HT2A receptors. Relevance for the design of new antipsychotic drugs**.

11:00-11:30 Mabel Loza. Universidad de Santiago de Compostela. **Novel in vitro phenotypic models in neuropharmacology**.

New pharmacological approaches to treat alterations in the sensory nervous system

Asia Fernández-Carvajal, Simona Giorgi , Angela Lamberti , Laura Butrón , Olivia Gross-Amat, Enrique Rodríguez-Cañas, Antonio Ferrer-Montiel

Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universitas Miguel Hernández, Elche, Spain

E-mail: asia.fernandez@umh.es

There are numerous alterations at the level of the sensory nervous system, such as pain and pruritus, which constitute a major health problem that greatly affects quality of life and often increases the risk of mortality[1]. Unfortunately, there is a lack of preclinical translational models to investigate alterations in sensory neurons mainly because they do not mimic the compartmentalized anatomy of nociceptors, which a central compartment containing the soma and a peripheral one housing axon endings with a diferent molecular and cellular composition. Therefore, there is a need to validate preclinical compartmentalized neurosensory models to investigate the pathophysiology of peripheral sensory disorders and to test drug candidates that result in a higher probability of success.
In our group, we have developed a microfluidic-based compartmentalized nociceptor cultures are suitable models for investigating the pro-algesic and neuropathic sensitization and resolution phases upon their exposure to pro-algesic compounds and if these cultures are appropriate models to study the interaction and crosstalk of axonal endings with keratinocytes[2]. For this purpose, we exposed axonal endings to an inflammatory soup that increased the activity of Transient Receptor Potential (TRPs) by promoting their surface expression and/or facilitating their gating or to the chemotherapeutic drug paclitaxel that directly affects nociceptor function by altering the expression and function of Nav, Kv and thermoTRP channels[3], as representative models of two peripheral disorders and measure the neural activity in both, the soma and the axonal compartments.
We demonstrate that this model reproduces the peripheral sensitization and resolution produced by an inflammatory soup and the chemotherapeutic drug paclitaxel. In addition, primary cultures of compartmentalized nociceptors were amenable to co-culture with keratinocytes in the axonal compartment. The interaction of axonal endings with keratinocytes modulated neuronal responses, consistent with a crosstalk between the two cell types. These findings pave the way toward translational preclinical sensory models for skin pathophysiological research and drug development.

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Modulatory effects of endocannabinoid system on cocaine seeking behavior in mice

Olga Valverde

Neurobiology of Behaviour Research Group (GReNeC-NeuroBio), Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain, Neuroscience Research Program, IMIM-Hospital Del Mar Research Institute, Barcelona, Spain.

E-mail: olga.valverde@upf.edu

Cocaine is the second most used illicit drug in the EU and its abuse causes a high co-morbidity among users. Despite significant progress in clinical and preclinical research, our understanding about the neurobiological bases of cocaine addiction remains limited. Several studies have demonstrated that different components of the endocannabinoid system have a modulatory role in reward and motivational processes and participate in the neuroplastic changes that lead to cocaine addiction. The inhibition of the enzyme fatty acid amide hydrolase (FAAH), and the resulting increase in anandamide (AEA) and other N-acylethanolamines, represents a promising strategy for reducing drug seeking. Interestingly, cannabidiol (CBD), the most abundant non-psychotropic constituent of the Cannabis sativa plant, is thought to reduce AEA metabolism by inhibiting FAAH enzymatic activity. Previous results showed that CBD might exert beneficial effects attenuating the acquisition of cocaine self-administration. However, the effect of URB597 in the acquisition phase, as well as the potential role of CBD and URB597 after the consolidation of drug use and the onset of associated negative consequences remains unknown.

In this study, we aimed to assess the effect of the CBD and the FAAH inhibitor URB597 on cocaine consumption. To this end, mice underwent a 10-day acquisition of cocaine self-administration under fixed ratio 1 (FR1), receiving either vehicle or CBD or URB597 30 minutes before every session. The results showed that whereas CBD attenuates cocaine-taking behavior, URB597 did not modulate cocaine seeking or taking. Moreover, we aimed to evaluate the effects of CBD and URB597 on cocaine consumption under the risk of punishment. Therefore, mice underwent acquisition of cocaine self-administration under FR1. Three punishment sessions, in which one third of the infusions were punished with a foot shock delivery of moderate intensity, followed the acquisition phase. Additionally, a predictive light stimulus was coupled to the punished infusion. Subsequently, mice were divided in 2 groups that received either vehicle or URB597 or vehicle or CBD during three regular self-administration days and one conditioned punishment session, where the predictive light was presented in the same way as in the punishment sessions, but no foot shock was delivered.

While URB957 fails to affect cocaine self-administration after punishment, it does decrease cocaine consumption upon presentation of the punishment-predictive stimulus, suggesting that URB597 treatment enhances sensitivity to punishment. Conversely, CBD fails to affect neither cocaine self-administration after punishment or cocaine consumption during the punishment-predictive session. Interestingly, the effect of URB597 was associated to increased cannabinoid receptor 1 (gene expression in the ventral striatum and medium spiny neurons activation in the nucleus accumbens shell. Our data show a key role of endocannabinoids in the development of cocaine addictive-like behavior and allow us to propose the FAAH inhibition as a therapeutic target for the management of cocaine addiction.

Inverse agonism and functional selectivity on serotonin 5-HT_{2A} receptors. Relevance for the design of new antipsychotic drugs.

Rebeca Diez-Alarcia

Department of Pharmacology, University of the Basque Country UPV/EHU. Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM. Instituto de Investigación Sanitaria Biocruces Bizkaia.

E-mail: r.diezalarcia@ehu.eus

Nowadays is well established that GPCR can work as molecular micro-switches as different ligands can stabilize them at different molecular conformations able to modulate different intracellular signalling pathways, a phenomenon known as functional selectivity. This process has already been proposed for serotonin 2A receptor (5-HT_{2A}R). This receptor is highly expressed in brain cortical regions, is the target of different psychedelic drugs, and has been proposed to play a fundamental role in the mechanism of action of drugs used to treat schizophrenia, as second generation antipsychotics, and other psychiatric disorders. Current antipsychotic treatment usually targets positive symptoms but has poor or not effect over negative and cognitive symptoms. Even more, around 30% of the patients are resistant to current antipsychotic drugs, thus new treatment options are urgently needed.

Our studies in postmortem human dorsolateral prefrontal cortex have demonstrated the functional selectivity of 5-HT_{2A}R, both for agonist and inverse agonist drugs. Moreover, the presence of constitutive activity of this receptor was also demonstrated in the same samples. In this context, two structurally similar orthosteric ligands that bind with high affinity to the 5-HT_{2A}R and that have previously been described as neutral antagonists, altanserin and ketanserin, exhibit biased agonism/inverse agonism depending on the interrogated pathway in postmortem brain tissue preparations. Moreover, hallucinogenic drugs, as DOI and LSD, are able to activate both Gαq/11 and inhibitory Gαi1 proteins through the 5-HT_{2A}R, whereas the non-hallucinogenic drug lisuride is only active on Gαq/11 proteins. These and previous data suggest that the activation of Gαi proteins could be considered a fingerprint of hallucinogenic properties.

On the other hand, 5-HT_{2A}R activation by the hallucinogenic agonist DOI displays a selective functional hyperactivity specifically involving Gi-proteins signalling pathway in postmortem schizophrenia samples. Moreover, through the use if inverse agonists, an enhanced constitutive activity of 5-HT_{2A}R receptors over the Gi-protein-mediated signalling pathway, has also been described in schizophrenia vs control samples. These findings shed light into the molecular basis of the signalling of this central target in biological psychiatry, leading to future studies of the mechanism of psychosis and antipsychotic treatment.

Furthermore, the use of new biased pharmacological probes in a present collaborative work has highlighted the complexity of GPCR signalling and the relevance of functional selectivity for the therapeutic response. A complexity that has to be considered in future drug development efforts of more efficient and safer drugs in the treatment of psychiatric disorders.

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Acknowledgements
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Drug screening in neuropathic pain employing phenotypic neuronal-like models

Loza M.I., Martínez A.L., López D., Brea J.

Universidad de Santiago de Compostela, Santiago de Compostela, Spain.

E-mail: mabel.loza@usc.es

Despite its high prevalence, neuropathic pain is still an unmet clinical need because new analgesics identified in preclinical studies failed in clinical trials due to the lack of translationality of the HTS assays, where recombinant models are routinely used, which is a clear inconvenience for drug discovery. To overcome this drawback, we developed a sensorial neuron-like proliferative model for analgesics HTS employing an immortalized cell line (1).

We set up a differentiation protocol for F11 cells (a hybrid of neuroblastoma murine cells and rat DRG neurons) to obtain cells with a phenotype of sensory neurons. We focused our study in two neuronal phenotypic features, the electrical excitability and the outgrowth of neurites. We confirmed the involvement of calcium and sodium transients in the differentiation of F11 cells because pharmacologic blocking of calcium and sodium transients hindered the acquisition of DRG neuron phenotypic features.

We observed that the excitability of F11 cells (30 mM KCl) was increased after the exposition to a combination of inflammatory mediators (histamine, serotonin, bradykinin and prostaglandin E2). After an HTS trial employing the Prestwick Chemical Library, we found that an antidepressant (protriptyline) and four calcium channel inhibitors (felodipine, nimodipine, nicardipine and nitrendipine) relieved this increase in excitability with IC_{50} values between 1 µM and 7 µM. Also, as a secondary screening, we noticed that 10 µM felodipine and 10 µM nitrendipine protected differentiated F11 cells against neurite shortening induced by the antitumor drug vincristine and the antiviral drug rilpivirine.

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Session 3. Cardiovascular Pharmacology

Chairman: Carlos Sánchez- Ferrer. Universidad Autónoma de Madrid.

6/9/2023 Sala de Grados Sabatini

15:30-16:00 Concha Peiró. Universidad Autónoma de Madrid. **Angiotensin-(1-7), a novel anti-aging drug for the vasculature?**

16:00-16:30 Susana Novella. Universidad de Valencia. **MicroRNA as potential modulators of cardiovascular function of estradiol.**

16:30-17:00 Ana Paula Dantas. Universidad de Barcelona. **Estrogen receptor in cardiovascular protection: the good, the bad and the ugly.**

17:00-17:15 Patrice Marques. Universidad de Valencia. **Platelet hyperactivity as trigger of platelet-leukocyte-endothelium interactions and subsequent cardiovascular complications in early-stage COPD.**

17:15-17:30 Miguel Angel Olivencia. Universidad Complutense de Madrid. **Novel role of hydrogen sulfide in erectile dysfunction: Regulation of guanylyl cyclase – redox state.**

Angiotensin-(1-7), a novel anti-aging drug for the vasculature?

Concha Peiró

Department of Pharmacology, Faculty of Medicine, Universidad Autónoma de Madrid

E-mail: concha.peiro@uam.es

Vascular aging is a complex and multifaceted process that provokes profound molecular, structural, and functional changes in the vasculature, which make arteries more prone to vascular disease and accelerate frailty. Thus, preventing or delaying the hallmarks of vascular aging is nowadays a major health goal, especially in our aged societies. Angiotensin (Ang)-(1-7), a main component of the protective branch of the renin-angiotensin system (RNA), has gained relevance over recent years as a potential anti-aging peptide. Indeed, Ang-(1-7) has revealed itself as a senostatic agent, capable of preventing human endothelial cell senescence induced not only by Ang II but also by other RAS-independent stimuli like the pro-inflammatory cytokine, interleukin (IL)-1β. The mechanism underlying such antisenescence actions of Ang-(1-7) include the inhibition of pro-inflammatory pathways derived from NF-κB or NLRP3 inflammasome activation, as well as the induction of essential cytoprotective systems such as the Nrf2-heme oxygenase-1 (HO-1) axis or the anti-aging protein klotho. Furthermore, Ang-(1-7) attenuates the senescence-associated secretory phenotype (SASP) characteristic of senescent cells and, when infused to animal models, it ameliorates endothelial dysfunction, a hallmark of vascular aging and disease. Although further research is needed to better understand the anti-aging properties of Ang-(1-7) on the vasculature, this heptapeptide arises as a promising pharmacological tool for preventing vascular aging and frailty.

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miRNA as potential modulators of cardiovascular function of estradiol

Novella, S.[1,2], Pérez-Cremades, D.[1,2], Paes A.B.[2], Rosales-Ariza, C.[1], Descals-Betrán, B.[2], Hermenegildo, C[1,2].

[1]University of Valencia, [2]INCLIVA Biomedical Research Institute, Valencia, Spain.

E-mail: susana.novella@uv.es

Estrogens, primarily 17β-estradiol (E2), have been reported to have beneficial effects on vascular biology through direct actions on the endothelium. Various studies have demonstrated the role of transcription factors in the protective action they exert on the vascular function. Alongside transcription factors, the small non-coding RNA microRNAs (miRNAs) are the major regulators of gene expression and signalling networks as they can regulate hundreds of genes.

As the miRNA biosynthesis machinery is modulated by E2 in endothelial cells (1), our focus is on identifying specific miRNA–transcription factor–downstream gene networks to delve into the role of miRNAs in the regulatory mechanisms of E2 in the endothelium. To achieve this, we conducted an integrative bioinformatic analysis of miRNA (2) and mRNA (3) microarray data obtained from human umbilical vein endothelial cells (HUVEC) exposed to a physiological concentration of E2 (1 nM). Gene set enrichment analysis was also performed.

Our results indicate significant regulation of E2 in the cardiac hypertrophy and hypoxia canonical signalling pathways in the cardiovascular system, both closely related to the observed sex differences in pathologies such as heart failure or myocardial infarction (4). For example, the transcription factor JUN, which plays a role in cellular response to stress, inflammation, and growth factor signalling, and is involved in cardiovascular diseases such as atherosclerosis and arterial hypertension, would be inhibited by miRNA-30b-5p.

Therefore, a deep understanding of the miRNA pathways modulated by E2 in the vascular endothelium, as well as the involvement of its estrogen receptors ERα, ERβ and GPER, expands our potential for intervention to achieve the cardiovascular beneficial effects of estrogens.

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Acknowledgements

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Estrogen Receptor in Cardiovascular Protection: The Good, The Bad and The Ugly

Ana Paula Dantas

Laboratory of Endothelial Signaling in Aging and Disease, Department of Biomedical Sciences, School of Medicine and Health Sciences, University of Barcelona, Spain.
Fundació Clínic Recerca Barcelona - Institut d`Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain

E-mail: adantas@recerca.clinic.cat

Estrogen has wide-ranging effects on the cardiovascular system. It plays a protective role against heart disease in pre-menopausal women, and its positive effects may explain the delayed onset of heart disease in women compared to age-matched men. As menopause approaches, estrogen levels decline, and women become more susceptible to cardiovascular risk factors such as hypertension and high cholesterol. Although the exact mechanisms of estrogen's action on the cardiovascular system are still not fully understood, studies have shown that estrogen mediates its influence on the cardiovascular system through estrogen receptors (ERα, Erβ and GPER). Estrogen receptors can modulate the expression and activity of a myriad of pathways, favoring an antioxidant and anti-inflammatory environment, and thereby decreasing their detriment cardiovascular system. Despite much evidence in animal models and observational studies indicating that estrogen promotes cardiovascular protection, placebo-controlled clinical trials, including the Women´s Health Initiative (WHI), have shown no benefit or have suggested an increased risk of coronary heart disease during the first years after randomization. Concerns raised in those clinical trials include that the estrogens used most of them were not naturally occurring and thus may not act identically to naturally occurring estrogen. Furthermore, the average age of women entering the WHI was 65. Thus, the WHI studied a population of women that were estrogen deficient for, on average, 10 years before hormone replacement was initiated. Moreover, the surprising negative cardiovascular results of WHI and other trials may in part, be explained by the complexity of estrogen receptor (ER) action. The cellular responses of estrogen receptor activation are specific to the ligand and dependent not only on the relative levels of ERα, ERβ and GPER in each tissue, but also on how ligand-induced conformational changes in those receptors lead to differential recruitment of coregulators molecules depending upon the cell type. Furthermore, cardiovascular responses to estrogen receptors ligands (estrogens and SERMs) can vary based on different physiological and pathophysiological situations, such as during aging and the nature of the pre-existent cardiovascular risk due to alterations in the expression in estrogen receptor subtypes and their splicing variants under these various conditions. These discrepancies in clinical trials and observational and basic science studies demonstrate the ultimate need to understand the cell and tissue specific mechanisms underlying the differential effects of estrogens and SEMRs action in the cardiovascular system. Findings from these studies will shed light on discrepancies between experimental and clinical studies of estrogen replacement therapy and may lead to the development of new therapies for the treatment of vascular diseases in women.

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Platelet hyperactivity as a trigger of platelet-leukocyte-endothelium interactions and subsequent cardiovascular complications in early-stage COPD

Marques, P[1,2]; Bocigas, I[3]; Domingo, E[1,2]; Francisco, V[1]; Piqueras, L[1,2,4]; González, C[2,3]; Sanz, MJ[1,2,4]

[1]Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain
[2] INCLIVA Biomedical Research Institute, Valencia, Spain
[3] University Clinic Hospital of Valencia, Valencia, Spain
[4] CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Madrid, Spain

E-mail: patricegmarques@gmail.com

Background: Increased cardiovascular diseases (CVDs) are one of the major consequences of chronic obstructive pulmonary disease (COPD) and are intimately associated with systemic inflammation. COPD is usually provoked by long-term cigarette smoking. However, little is known about the systemic inflammatory status of mild-severity COPD patients (classified as GOLD 1). Therefore, we assessed leukocyte-endothelium interactions and characterized the inflammatory state of GOLD 1 patients compared with non- and long-term smoker volunteers without COPD. **Material and Methods:** Blood samples from 27 GOLD 1 patients, 27 smokers and 10 non-smokers were extracted and complete blood counts were analyzed. Leukocyte adhesion to TNFα-stimulated (20 ng/mL, 24h) arterial 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 percentage of platelet-leukocyte aggregates ($CD41^+$ leukocytes) were assessed by flow cytometry. **Results:** Enhanced platelet-leukocyte adhesiveness to TNFα-stimulated endothelium was found in GOLD 1 patients vs. smokers or non-smokers. Moreover, greater platelet reactivity (platelet count and activation) and higher frequency of platelet-leukocyte aggregates were detected in GOLD 1 patients than in the other two groups. Notably, augmented platelet-monocyte aggregates were also encountered in smokers compared with non-smokers. Inasmuch, increased leukocyte counts were found in GOLD 1 patients and smokers compared with non-smokers. According to our data, the inflammatory state of smoker subjects seems to be a midpoint between those of GOLD 1 patients and non-smokers in their likelihood of developing CVDs. **Conclusions**: We have characterized the early changes in the COPD-associated inflammatory state. Our findings indicate that long-term cigarette smoking may be responsible for platelet hyperreactivity, increased leukocyte and platelet-monocyte aggregate frequencies, which in turn lead to elevated platelet-leukocyte-endothelium interactions and subsequent cardiovascular complications in COPD. Therefore, immunophenotypic monitoring, mainly of platelet count, activation and platelet-monocyte aggregates, might be useful in the prevention of CVDs development in early-COPD.

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Novel role of hydrogen sulfide in erectile dysfunction: Regulation of guanylyl cyclase - redox state.

Miguel A. Olivencia [1,2,3], Erika Esposito [4], Vincenzo Brancaleone [5], Sigismondo Castaldo [6], Giuseppe Cirino [4,7], Raffaella Sorrentino [7,8], Roberta d'Emmanuele di Villa Bianca [4,7], Emma Mitidieri [4,7], Francisco Pérez-Vizcaino [1,2,3].

1. *Department of Pharmacology and Toxicology, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain.*
2. *CIBER Enfermedades Respiratorias, Madrid, Spain.*
3. *Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain.*
4. *Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, 80131 Naples, Italy.*
5. *Department of Science, University of Basilicata, Potenza, Italy.*
6. *U.O.C.Ricerca Formazione & Cooperazione Internazionale, A.O.R.N." Antonio Cardarelli", Naples, Italy.*
7. *Interdepartmental Centre for Sexual Medicine, University of Naples Federico II, 80131 Naples, Italy.*
8. *Department of Molecular Medicine and Medical Biotechnology, School of Medicine and Surgery, University of Naples Federico II, 80131 Naples, Italy.*

E-mail: mioliven@ucm.es

Background: Erectile dysfunction (ED) has been associated with lower levels of the H_2S-generating enzymes cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS), and altered response to L-cysteine, the hydrogen sulfide endogenous precursor. However, the cause-effect relationship and the molecular mechanisms implicated remain unknown.

Material and methods: This study examines the role of hydrogen sulfide in the NO-sGC-cGMP pathway by analyzing ED in adult male CSE knockout mice ($CSE^{-/-}$). Erectile function was assessed in vitro in isolated mice corpora cavernosa (CC) mounted in a myograph. Protein expression, hydrogen sulfide, nitrates plus nitrites, cAMP and cGMP measurements were also performed.

Results: $CSE^{-/-}$ CC showed a significant decrease in the relaxant response to acetylcholine, to the PDE5 inhibitor sildenafil, and to the sGC stimulator riociguat. In addition, a reduction in the expression of p-eNOS, PDE5 and the β1 sGC subunit was observed. In contrast, the relaxation to cinaciguat, the sGC activator independent on the oxidative state of sGC, was unaltered in CC from CSE knockout mice. The response to isoprenaline was unchanged as were cAMP levels, as opposed to cGMP levels which were reduced in the CC of the $CSE^{-/-}$ animals. In addition, we found a decrease in CYP5R3, a reductase involved in the regulation of the redox state of sGC. Lastly, in vitro treatment with a H2S donor and sGC reductor (Na_2S), improved the responses to riociguat in $CSE^{-/-}$ CC.

Conclusions: Our results demonstrate that CSE knockout mice show ED in vitro due to an alteration of the NO-sGC-cGMP pathway with reduced response to PDE5i and changes in the redox state of sGC. Our study suggests that sGC activators or co- treatment of PDE5i with H_2S donors may be effective in ED patients that are non-responders to PDE5i.

Acknowledgements

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Session 4. Lectures by 2020-2023 SEF Awards to young researchers

Chairwoman: Mercè Pallas. Universidad de Barcelona

6/9/2023 Paraninfo Envases de Cartón

17:30-18:00. 2020. Marta Toral. **Aortic disease in Marfan syndrome is caused by overactivation of sGC-PRKG signaling by nitric oxide**.

18:00-18:30. 2021. Jesús Cosín. **Role of metabolite-sensing GPCRs in Inflammatory Bowel Disease: Relevance of Intestinal Microbiota**.

18:30-19:00. 2022. Gema Mondéjar. **Investigating the Risk of Prolonged Repolarization and Arrhythmogenesis Associated with Fentanyl Abuse Using Human iPSC-derived Cardiomyocytes.**

19:00-19:30. 2023. Iñaki López-Vera. **Translocation of gut microbiota induce trained immunity in bone marrow progenitor cells.**

Aortic disease in Marfan syndrome is caused by overactivation of sGC-PRKG signaling by nitric oxide

Marta Toral[1,2,3,6], Andrea De la Fuente-Alonso[1,2,6], María Jesús Ruiz-Rodríguez[1,2,4], Iván Alarcón-Ruiz[1,2], María José Méndez-Olivares[1,2], Miguel R. Campanero[2,5,7], Juan Miguel Redondo-Moya[1,2,7]

[1]Cardiovascular Risk Factors & Brain Function Department, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain. [2]Centro de Investigación Biomédicaen Red de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain. [3]Department of Pharmacology, University of Granada. Granada, Spain. Instituto de Investigación Biosanitariade Granada, ibs.GRANADA, Granada, Spain. [4]Yale School of Medicine. Yale University, NewHaven, United States. [5]Centro de Biología Molecular Severo Ochoa (CBMSO), ConsejoSuperior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain. [6]MT and AdlF-A contributed equally to this work. [7]MRC and JMR jointly directed this work.

E-mail: martatoral@ugr.es

Despite advances in the genetics of thoracic aortic aneurysm and dissection (TAAD), current pharmacological treatments neither effectively retard aortic expansion nor prevent catastrophicfailure in these diseases, including Marfan syndrome (MFS). We have described Nos2 as a critical mediator in the development of aneurysms, whose expression is induced in MFS. However, the mechanisms by which NOS2 contributes to TAAD in MFS remain unclear. We aim to investigate if NO-sGC-PRKG1 signaling is implicated in MFS aortopathy and identify novel targets that could improve MFS diagnosis, treatment and/or prognosis.

We show that increased NOS2-derived NO levels stimulate sGC–PRKG1 pathway in Marfan patients and mice, as evidenced by increased plasma cGMP and aortic staining of pVASP-S239. Our data also show that inhibitors of either sGC or PRKG1, or lentiviral-mediated silencing of Prkg1 in the aorta, regress the aortopathy of MFS mice. Nitrated proteinlevels are higher in plasma from MFS patients and mice and in aortic tissue from Marfan mice,indicating elevated circulating and tissue NO and suggesting that NO also mediates pathophysiological processes in a cGMP-independent fashion through mechanisms involving protein nitration.

These results show that NO-sGC-PRKG1 signaling mediates aortopathy in MFS mice and is activated in MFS mice and patients. Our findings also identify potential therapeutic targets for intervention in human MFS as well as circulating biomarkers for monitoring and clinical follow up of MFS disease.

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Role of metabolite-sensing GPCRs in Inflammatory Bowel Disease: Relevance of Intestinal Microbiota

Jesús Cosín-Roger[1]

[1]*Departamento de Farmacología, Facultad de Medicina, Universidad de Valencia*

E-mail: jesus.cosin@uv.es

Inflammatory Bowel Disease (IBD) is a chronic inflammatory autoimmune disease which affects the gastrointestinal tract. The two major subtypes of IBD are Ulcerative Colitis (UC) and Crohn´s Disease (CD) which differ in the disease manifestation. The aetiology of both diseases is still unknown and it is assumed that a combination of several environmental factors,intestinal microbiome composition, genetic factors and the mucosal immune response triggersthese gastrointestinal disorders

A critical protagonist in the pathogenesis of IBD is the intestinal microbiota. For years, it has been widely assumed that a microbial dysbiosis is associated to this disease. In fact, IBD patients suffer a reduction in the commensal and "good" microbial diversity (*Bifidobacterium longum, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis*) in parallel with an increase in the pathogenic microorganisms (*Bacteroides fragilis, Ruminococcus, Escherichia Coli* and *Fusobacterium*). In addition, intestinal microbiota act as an important source of a wide range of metabolites, since it interacts with the host by releasing these molecules. Given the wide range of these microbial-derived metabolites, the crosstalk betweenthe microbial dysbiosis and the specific metabolic profiles in IBD patients is a hot topic whichis currently being studied.

In recent years, G protein-coupled receptors (GPCRs) have emerged as key protagonists in immune response, inflammation regulation, cell proliferation and the maintenance of intestinalbarrier acting as sensors of a wide range of molecules. GPCRs constitute the largest and most diverse receptor family with approximately 800 different receptors described in humans so far. Their activity has been associated in several inflammatory and metabolic pathologies, including IBD. Although the most widely described ligands for GPCRs are neurotransmitters, hormones and chemokines, increasing evidence has demonstrated that several metabolites can act as signalling effectors and regulate their function. Specifically, till present, a total of 19 GPCRs have been described as possible targets for some metabolites such as amino acids, hydroxicarboxylic acids, bile acids, short, medium and long chain fatty acids. The specific roleof these metabolite-sensing GPCRs in intestinal inflammation has been studied and literature point to a dual role showing some of them a proinflammatory effect whereas others exert an anti-inflammatory action.

Our research line aimed to analyse the expression of metabolite-sensing GPCRs, to quantify the levels of the metabolites which can selective activate those GPCRs and to characterize the mucosa-associated microbiota in surgical resections from both UC and CD patients. Finally, inorder to assess the specific role of one metabolite-sensing GPCR, we have studied the role of GPR109a and its metabolite β-hydroxybutyrate in intestinal inflammation and fibrosis.

Investigating the Risk of Prolonged Repolarization and Arrhythmogenesis Associated with Fentanyl Abuse Using Human iPSC-derived Cardiomyocytes

Mondéjar-Parreño G[2], Zhao SR[2], Liu Y[2], Cao X[2], Yang JY[2], Wu JC[1,2*]

[1]Division of Cardiovascular Medicine, Department of Medicine, Stanford University
[2]Stanford Cardiovascular Institute, Stanford University School of Medicine.

E-mail: gemondej@stanford.edu; joewu@stanford.edu

A significant increase in synthetic opioid use, mainly fentanyl, has boosted the opioid epidemic which surged dramatically during COVID-19 pandemic. Fentanyl, 50-100 times stronger than morphine, is a legal prescription drug used for anaesthesia and pain. Opioid induced-cardiac arrest is the most dramatic manifestation of opioid use disorder. Fentanyl acts mainly in nervous system binding to opioids receptors (OR); however, under abuse condition pulmonary and cardiovascular systems can be depressed. Evidence confirms fentanyl safety in relation to cardiac electrical activity under anaesthetics procedures. The proarrhythmic effects of other opioid misuse are known; however, the cardiac electrophysiological consequences of fentanyl abuse have not been studied. Hence, we studied whether fentanyl abuse may have any consequences on cardiac electrophysiology using human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). The analysis of 19 toxicology studies determined a fentanyl blood concentration mean of 89 nM in cases of abuse/overdose, concentration ~3-fold higher than in fentanyl-treated patients for chronic pain (over 3 months) or ~7.5-fold higher than patients occasionally exposed to fentanyl. iPSC-CMs from 3 healthy donors were exposed to 3 doses of 89 nM fentanyl during 5 days. Microelectrode array (MEA) assay revealed that fentanyl exposure prolonged significantly field potential duration (cFPD) recorded at day 5, whereas fentanyl has not an acute effect on cFPD. Supporting the FPD prolongation, the action potential duration (cAPD) of iPSC-CMs recorded using patch-clamp technique was significantly prolongated after fentanyl exposure. Calcium transients showed an increase in transient decay (τ) by fentanyl exposure leading to a rise in the arrhythmia-like cell percentage. These data suggest fentanyl could alter the activities of ion channels implicated in cardiac repolarization. Consistently, a drastic reduction in hERG current was found in fentanyl-treated iPSC-CMs compare to control, suggesting that fentanyl is inhibiting hERG channel function. No changes in KCNH2 mRNA or hERG protein expression were found, indicating that fentanyl-induced hERG current inhibition may be due to a direct interaction between fentanyl-hERG structures or by a second regulator able to inhibit hERG channel function. To investigate the underlying mechanism, we performed an *in-silico* docking study to explore a possible direct interaction between fentanyl-hERG structure. Only one cavity for fentanyl reached a possible, but not optimal, interaction binding energy inside hERG structure. This finding along with the fact fentanyl has not an acute effect on FPD, and the recognized cardiac safety of fentanyl under anesthetic procedures, point to an indirect hERG function inhibition mechanism. To explore in-depth the alteration of intracellular pathways that could lead to proarrhythmic effect of fentanyl abuse, bulk RNA sequencing was performed. Several pathways that may disturb hERG channel function were found altered in fentanyl-treated iPSC-CMs. In summary, fentanyl abuse has substantial consequences on cardiac electrophysiology and calcium handling of iPSC-CMs leading to an increase of arrhythmia-like events. Consequently, fentanyl abuse could prolong repolarization and induces arrhythmogenesis, besides respiratory depression, which may contribute to opioid-associated cardiac arrest.

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Translocation of gut microbiota induce trained immunity in bone marrow progenitor cells.

Iñaki Robles-Vera[1], Aitor Jarit-Cabanillas[1], Paola Brandi[1], María Martínez-López[1,6], Sarai Martínez-Cano[1,2], Cristina González-Correa[3], Javier Moleón[3], Juan Duarte[3], Laura Conejero[2], Pablo Mata-Martínez[4], Carmen María Díez-Rivero[2], Marta Bergón-Gutiérrez[4], Iván Fernández-López[1], Manuel J. Gómez[1], Ana Quintas[1], Ana Dopazo[1], Fátima Sánchez-Cabo[1], Esther Pariente[1], Carlos del Fresno[4], José Luis Subiza[2], Salvador Iborra[5], David Sancho[1,7]

1Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain. 2Inmunotek S.L., Alcalá de Henares, Spain. 3Department of Pharmacology, School of Pharmacy, University of Granada, IBS14 Granada, Centro de Investigaciones Biomédicas (CIBM), CIBER-Enfermedades Cardiovasculares (CiberCV), Granada, Spain. 4 Immunomodulation Lab, Innate Immune Response Group, IdiPAZ, La Paz University Hospital, Madrid, Spain. 5Department of Immunology, Ophthalmology and ENT, School of Medicine, Universidad Complutense de Madrid, 12 de Octubre Health Research Institute (imas12), Madrid, Spain. 6 Current address: Champalimaud Research, Champalimaud Centre for the Unknown. Lisbon, Portugal

E-mail: inaki.robles@cnic.es

The interaction between gut microbiota and the immune system is maintained by a delicate balance. However, the precise impact of gut microbiota on systemic trained immunity (TI) in innate immune cells remains incompletely understood. In this study, we demonstrate that bone marrow-derived macrophages (BMDM) from mice treated with dextran sulfate sodium (DSS), which induces the translocation of microbiota throughout the body, exhibit increased production of TNFα when exposed to various Toll-like receptor (TLR) agonists, indicating TI. This effect is nullified by antibiotic treatment and is not observed in germ-free mice subjected to DSS treatment. However, germ-free mice reconstituted with a complete gut microbiota and subsequently treated with DSS display enhanced training of BMDM. Furthermore, conventionally housed mice subjected to lethal irradiation exhibit elevated TNFα production upon in vivo challenge with lipopolysaccharide (LPS) when reconstituted with bone marrow from DSS-treated mice, but not from untreated mice. These findings suggest that bacterial translocation of gut microbiota to distant tissues, such as the bone marrow, has the ability to prime precursor cells of the innate immune system. We are currently investigating the key taxa that are translocated and may mediate this priming effect, as well as the receptors on innate immune cells that recognize these bacteria. This research will contribute to identifying specific gut microbiota taxa that could serve as potential inducers of TI, thereby improving our understanding of their implications in both health and disease.

Acknowledgements

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Session 5. Nanopharmacology: a new era in therapeutics.

Chairman: Valentín Ceña. Universidad de Castilla- La Mancha

7/9/2023 Paraninfo Envases de Cartón

9:30-10:00. Helena Tomás. University of Madeira. **Dendrimer-based nanomaterials for anticancer drug delivery.**

10:00-10:30. Barbara Klajnert-Mackulewicz. University of Lodz. **Dendrimers in neurodegenerative disorders – an overview.**

10:30-11:00. Jean-Pierre Majoral. CNRS. **Phosphorus dendrimers as nanocarriers or drugs active per se. Selected recent advances.**

11:00-11:30. Valentín Ceña. Universidad de Castilla-La Mancha. **Role of siRNA and nanoparticles in glioblastoma treatment**.

Role of siRNA and nanoparticles in glioblastoma treatment.

Ceña, V. **Rodríguez-Clemente, I., De la Torre, C., Játiva, P.**

Unidad Asociada Neurodeath, Universidad de Castilla-La Mancha, Albacete, Spain andCIBER, ISCIII, Madrid, Spain

E-mail: valentin.cena@gmail.com

Glioblastomas (GBMs), Grade IV gliomas, are the most common primary brain tumours in adults. Median survival is approximately 14 months since the primary diagnosis, and 2-year survival is about 25 %. Thus, new therapeutic alternatives are indeed needed to further improveGBM patient's survival. Nanoparticles are compounds in the nanometer range that have a very large surface to volume ratio which provides them with great potential to be used in different applications including biomedicine. In this work, we have studied the ability of different chemical families of nanoparticles (cyclodextrins, dihydropyridine derivatives, and dendrimers) to deliver siRNA toGBM cells to knock-down proteins that are involved in proliferation and survival mechanisms of these tumoral cells. By blocking those proteins, an increased toxicity on GBM cells and/or apotentiation of temozolomide (TMZ) antitumoral actions would be expected.

Specific siRNAs were designed to be vectorized by different nanoparticles, in order to lower the levels of p42-MAPK, p44-MAPK, Rheb, and O^6-methylguanine-DNA-methyltransferase(MGMT) in a human GBM cell line (T98G), GBM cells isolated from patient's tumors, and GBM cells obtained from patient-derived organoids. Nanoparticle-mediated siRNA transfection indeed proved to be efficient by extensively knocking-down p42-MAPK, p44- MAPK and Rheb in T98G and patient derived GBM cells to levels about 15-25% of the untreated GBM cells levels. Only very low toxic effects appeared when any of these siRNAs or SCR siRNA were used at a concentration of 100 nM. Glioblastoma cells used herein responded very poorly to TMZ. Only at very high concentrationssome significant toxicity was detected. When nanoparticle:siRNA nanoplexes were used in addition to TMZ, we observed a clear potentiation in toxicity in some cases. In particular, TMZtoxicity was potentiated by knocking-down p42-MAPK alone or combined with p44-MAPK orRheb, increasing TMZ-induced cell death. Altogether, these results suggest that the toxicity exerted by TMZ can be potentiated in GBM cell lines by knocking-down different proteins, thus disturbing different cell pathways involved in GBM cell proliferation and survival.

Preliminary in vivo experiments were conducted in mice to characterise biodistribution of someof the nanoparticles labelled with a fluorescent probe and also the transport of fluorescence- labelled siRNA by nanoparticles to the brain. These experiments showed no toxicity, ubiquitous distribution of both nanoparticles and siRNA, and an evident accumulation in the liver, which faded away with time. When a transferrin-decorated version of a cyclodextrin was used instead, a slightly different biodistribution profile was observed, increasing the amount reaching the brain.

Acknowledgements

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Dendrimers in neurodegenerative disorders - an overview

Barbara Klajnert-Maculewicz[1], Josep Cladera[2]

Department of General Biophysics, University of Lodz, Poland, [2]Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Catalonia, Spain

E-mail: barbara.klajnert@biol.uni.lodz.pl

Dendrimers are a unique type of polymers known for their precise molecular structure. They have a spherical shape and are created step-by-step by attaching branched monomer units. The generation of a dendrimer refers to the number of layers it possesses. Many dendrimers exhibit a strong affinity for proteins and peptides, with the nature of these interactions being complex. Typically, electrostatic attraction plays a crucial role in determining the strength of these interactions. The interactions between dendrimers and proteins have numerous medical applications. For example, dendrimers can be employed in treating neurodegenerative disorders by disrupting harmful plaques called amyloids found in the brain. Promising results have shown that dendrimers have potential therapeutic activity against prion diseases, with the first publication on this topic dating back to 1999 [1]. It was discovered that amino-terminated dendrimers, particularly those with increasing amino surface groups per generation, are vital for their effectiveness in eliminating PrP^{Sc} from ScN2a cells.
In our laboratory, we utilized an alternative method to cell-based assays for screening antiprion dendrimers. Truncated prion peptides were exposed to destabilizing factors, mimicking the conditions that lead to the formation of fibrils. We monitored the accumulation of amyloids by measuring changes in the fluorescence of thioflavine T, a dye sensitive to the presence of amyloid fibrils. Fourier transformed infrared spectroscopy and electron microscopy were also employed to examine the impact of dendrimers on the secondary structure of peptides and the morphology of fibrils [2]. Dendrimers also hold promise as potential agents for treating other neurodegenerative disorders, including Alzheimer's disease (AD), due to similarities between the two pathologies. AD, the leading cause of disability and death in the elderly, is characterized by the formation of amyloid plaques in the brain. The primary component of these plaques is a peptide called β-amyloid peptide (Aβ) consisting of 40-42 residues. Aβ has a propensity to aggregate, forming oligomers, protofibrils, and fibrils. These aggregated forms act as potent neurotoxic agents, triggering a cascade of cellular events that result in neuronal damage and the clinical manifestation of AD. Consequently, the clearance of Aβ from the brain and the prevention of its aggregation are crucial therapeutic strategies for AD patients. Studies have demonstrated that different types of dendrimers can either inhibit or accelerate the production of Aβ fibrils [3]. Based on the obtained results, a hypothesis regarding the mechanism of interaction between dendrimers and peptides was formulated. Several strategies for inhibiting fibril formation by dendrimers were proposed, including binding to α-helical or β-sheet monomers, binding to oligomers, blocking free ends of fibrils, and disrupting existing fibrils.

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Phosphorus Dendrimers as nanocarriers or drugs active *per se* Selected recent advances

Majoral J.P

Laboratoire Chimie de Coordination, CNRS 205 route de Narbonne 31077 Toulouse Cedex 04 France

Email : jean-pierre.majoral@lcc-toulouse.fr

Dendrimers – nano-sized symmetrical macromolecules with nearly monodisperse structures – are used for many applications in different fields due to the fine tuning of their physicochemical and biological properties. Among the families of dendrimers, phosphorus dendrimers were found one of the most efficient nanodevices notably in nanomedicine both as nanocarriers or as drugs active *per* se. For example they were used as antiprions agents, stimulators of human natural killer (NK) cells, as anti-inflammatory compounds depending on the type of their terminal: neutral, anionic, cationic or metalated (Cu, Au, Ti, Ru, Zr, etc) groups or against Alzheimer and Parkinson diseases. Outstandingly, modification of the dendrimer terminal groups effectively changes the therapeutic domains, all these results allowing us to propose a novator concept of dendrimer space for innovative nanomedicine Phosphorus dendrimers are also used in a variety of other fields ranging from nanosciences to catalysis or for the formation of hybrid organic inorganic materials and unprecedented bioplastics.

Novel multistep synthesis in high yield of original stable and not sensitive to hydrolysis of a variety of neutral, cationic or anionic phosphorus dendrimers will be reported. Several recent applications will be illustrated in different domains using these phosphorus dendrimers as antituberculosis, anticancers, antimicrobial, or anti inflammatory compounds. Their contribution in the DNA-based multiplexing technology for accurate diagnosis of pathogens in the fields of respiratory diseases, sexual diseases diagnosis will be presented.

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Dendrimer-based nanomaterials for anticancer drug delivery

Helena Tomás

CQM-Centro de Química da Madeira, MMRG, Universidade da Madeira, Campus da Penteada, 9020-105 Funchal, Portugal

E-mail: lenat@staff.uma.pt

Dendrimers are highly branched synthetic molecules that have been extensively studied for their potential use as drug delivery vehicles in cancer therapy [1]. These nanomaterials have unique properties, such as their well-defined size and shape, high surface area, and ability to conjugate or encapsulate drugs. They also offer the possibility of precise control over drug release and targeted delivery to cancer cells due to their easy surface functionalization. Depending on their composition, dendrimers can also be biodegradable and give rise to non-toxic products that will be excreted from the body without causing toxicity. Furthermore, hybrid nanomaterials based on dendrimers can be developed that combine the unique properties of dendrimers with other materials in order to obtain optimal systems with improved efficacy. In fact, dendrimers offer a high degree of flexibility in their design, which allows for the creation of materials with tailored properties for specific applications, including in the field of cancer therapy.

The purpose of this presentation is to showcase various strategies being explored at CQM for delivering anticancer drugs, all of which rely on dendrimers. For instance, the drug delivery performance of biodegradable dendrimers based on bis-MPA (2,2-bis-(hydroxymethyl)propionic acid) can be fine-tuned by partially functionalizing the dendrimers at the periphery (generations 4 and 5) [2]. Another approach involves combining a lower generation of these dendrimers with fucoidan, a natural and biologically active polymer, to form an anti-angiogenic nanometric system that can deliver cisplatin to cancer cells. Additionally, thin films made from poly(amidoamine) dendrimers and dsDNA, which exhibit excellent physical properties and cytocompatibility, can be produced and utilized for delivering anticancer drugs.

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Acknowledgements

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Session 6. Teaching and creativity in Pharmacology.

Chairwoman: Rosario Jiménez. Universidad de Granada.

7/9/2023 Sala de Grados Sabatini

9:30-10:00. Michael Spedding. IUPHAR. **50 years of young pharmacology: managing and guarding creativity.**

10:00-10:30. Carmen Montesinos Mezquita. Universidad de Valencia. **Benefits of a collaborative teaching network for improved Pharmacology education.**

10:30-11:00. Francisco Pérez Vizcaíno. Universidad Complutense de Madrid. **Database for multiple choice tests in Pharmacology. Platform for the automatic generation of exams.**

11:00-11:30. Francisco Nieto. Universidad de Granada. **Demonstrating the placebo effect to pharmacology students.**

Fifty years of young pharmacology: managing and guarding creativity.

Michael Spedding

Spedding Research Solutions SAS, 6 rue Ampere, 78110 Le Vesinet, France.

michael@speddingresearchsolutions.fr

Standard pharmacology teaching does not normally include how to succeed, and survive(!), in the pharmaceutical industry and academia, while guarding creativity, and adapting management of both yourself, and of different sized groups, throughout a career. Management has to be different as a PhD student, or a research centre director, but nevertheless there are many common issues. MS celebrates 50 years of experimentation, 10 years of managing his own little company, building two research centres and putting 12 compounds into clinical development, while being 35 years in pharma research committees, seeing almost every way drugs can pass or fail. Much of this experience has been hard-won and I dedicate it to all the people I have worked with (and still do). I propose to give examples and advice for a research career in pharmacology, managing your 'boss', or groups at different levels, and how to retain creativity. For example, we have to take advantage of human evolution in drug discovery, which is quite different from laboratory animals,– and in MS' case this has allowed him to run 122,000 kms as an athlete, and work as a pharmacologist. Human running decline is absolutely precise, directly related to loss of VO2max, which has implications for ageing research: in contrast, brain function is relatively protected.

Benefits of a collaborative professorial network for improved Pharmacology education

Montesinos, M.C.[1,2], Ferrándiz, M.L.[1,2], D'Ocón, M.P.[1], Ivorra, M.D.[1]

[1]*Faculty of Pharmacy, University of Valencia, Spain*
[2]*IDM, Valencia, Spain*

E-mail: m.carmen.montesinos@uv.es

The Inter-University Teaching Innovation Network in Pharmacology was created with the purpose of establishing a common "virtual" space where university professors could share and exchange innovative teaching experiences, research results and materials of Pharmacology for different Health Science degrees at both pre- and post-graduate levels. The fundamental pillars of this initiative are: teaching innovation, research, and transmission of knowledge. The University of Valencia has funded eight consecutive projects that created and supported this network, with its overall focus on improving Pharmacology education by promoting active learning, improved communications and teamwork among academia. By learning from and applying other professors' teaching methods and activities, where needed, the student experience and learning is significantly improved.

From the enthusiastic efforts of the 45 professors who started the network, the collaborative work method and network were implemented. The common Moodle platform set up in the Virtual Classroom of the University of Valencia serves as the repository of authenticated materials, together with a critical analysis, highlighting strengths and weaknesses of said material to maximize the teaching and learning process. To date, 8 meetings have been held, in which the professors' innovative teaching experiences have been shared and discussed. Currently, the Network is composed of 131 professors from 22 Spanish, 1 Portuguese, 2 Chilean and 1 Mexican universities, from different Health Science Degrees (Nursing, Pharmacy, Medicine, Veterinary Medicine, Biomedical Sciences, Optics, Nutrition…).

Each year, the educational innovation project proposal focuses on different aspects: active learning methodologies, evaluation processes and systems, development of new materials that complement the master lectures and that could be used in face-to-face and/or hybrid teaching, and more recently the simulation method of teaching by using robot patient and/or standardized patient to resolve clinical cases. The collective work of this network constitutes a powerful tool to shape, revise and strengthen the fundamentals taught in Pharmacology, based on the material and teaching resources collaboratively created, implemented and shared through this Network.

Since the granting of the first project in September 2015, numerous innovative activities and methodologies have been shared in the network. This collaborative action indicates that the exchange of teaching experiences among different universities has a significant impact on innovation and quality training in university education.

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Demonstrating the placebo effect to pharmacology students

González-Cano, R., Artacho-Cordón, A., Tejada, M.A, Huerta, M.Á., Baeyens, J.M., Cobos, E.J., Nieto, F.R.

Faculty of Medicine and Institute of Neuroscience, Biomedical Research Center, University of Granada, Granada, 18016, Spain

Biosanitary Research Institute ibs.Granada, 18012 Granada, Spain

E-mail: fnieto@ugr.es

The placebo effect contributes to the global effect of medicines. Drug effects during clinical development are usually compared with placebo through randomized double-blind placebo-controlled trials (RCTs). The use of placebos makes possible to discern which part of the observed effect of a medication is due to its pharmacodynamic effectiveness (specific effects) and which part is due to the placebo effect (unspecific effects), generally caused by the expectations of clinical improvement of the patients [1].

We present our experience with a practical activity, whose goal is to demonstrate placebo analgesia to university students, by using a local anaesthetic (EMLA®) cream and a placebo cream. As secondary goals, the mechanism of action of local anaesthetic drugs is reviewed, and students acquire some practical skills in sensory evaluation.

At the beginning of the class, the activity is explained to the students (omitting that there is a placebo cream), and they are informed that it has been approved by the local ethic committee (648/CEIH/2018). An information sheet and an informed consent are provided to the students that they must sign if they like to participate, being a voluntary activity. Students are randomly distributed in three groups in a single-blinded manner, balanced in number and sex. The experience consists of evaluating the analgesic/anaesthetic activity of “two different anaesthetic creams” (students do not know that one of them is a placebo), and of a known control cream, which will be applied to the index finger of one of the students' hands. After application, it is necessary to let the creams act for 1 hour, a time that is used, among other things, to review the pharmacological properties of local anaesthetics (to maximize the expectations of the efficacy of the analgesic/anaesthetic effect). Then, the students carry out different sensory evaluations on the finger where the cream was applied (the contralateral untreated index finger is also evaluated as a control): response to thermal stimuli, recording the latency to finger withdrawal induced by cold (0ºC) and hot (42ºC, 46ºC, and 50ºC) stimuli; response to a pinprick stimulus (Neuropen®) and recording of perceived pain using a visual analog scale (VAS); determination of the tactile threshold (von Frey filaments); and discrimination between two points (Diskcriminator®). The results of the different sensory tests were recorded and later graphed and analyzed (ANOVA) in a second session with the students. Placebo analgesia is usually evident in painful stimuli. The effect of the anaesthetic cream is always greater than that of the placebo cream and also more evident in painful stimuli. It is then revealed that one of the creams was a placebo and a seminar on the mechanisms of the placebo effect is given, including its importance in clinical trials.

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Session 7. Teaser poster session.

Chairman: Antonio Rodríguez-Artalejo. Universidad Complutense de Madrid.

7/9/2023 Paraninfo Envases de Cartón 17:30

17:30-17:35. J. Rodrigues. University of Madeira. **Dendritic Ruthenium-based Anticancer Nanosystems: multi-step synthesis procedures coming to life.**

17:35-17:40. I. Valencia. Universidad Autónoma de Madrid. **Vascular smooth muscle cells-derived extracellular vesicles are conveyors of NLRP3 inflammasome and mediate inflammation in diabetes.**

17:40-17:45. C.González-Correa. Universidad de Granada. **The probiotic Lactobacillus fermentum CECT5716 increases the antihypertensive response of hydrochlorothiazide in spontaneously hypertensive rats.**

17:45-17:50. E. Domingo. Universidad de Valencia. **Deletion of CCR3 receptor in apoE−/− mice subjected to an atherogenic diet accelerates atherosclerosis.**

17:50-15:55. A.L. Martínez. Universidad de Santiago. **A Novel Model for HTS of Drugs for Post-Acute Sequelae of COVID-19 -Related Neuronal Damage.**

17:55-18:00. A. Bellver Sanchis. Universidad de Barcelona. **Neuroprotective effects of G9a inhibition through modulation of PPARγ/GADD45α-dependent pathways by miR-128.**

18:00-18:05. J. E. Baños. Universidad de Vic. **The (mis)use of scientific discourse to legitimize drug promotion in the opioid crisis in the US. An analysis for teaching purposes using TV series Dopesick.**

18:05-18:10. A. Antolín. Bellvitge Biomedical Research Institute – IDIBELL. **The major metabolite of the PARP inhibitor rucaparib exhibits unique PLK2 inhibition unlocking a new anti-Parkinson strategy.**

18:10-18:15. R. Muñoz-García. Universidad de Sevilla. **Dietary Oleacein attenuates Lupus Nephritis in Balb/C Pristane-Induced.**

18:15-18:20. Gayo-Abeleira. Universidad de Alcalá. **Ménière disease and interleukin 17A.**

18:20-18:25. R.M. Giner. Universidad de Valencia. **Transferosomes loaded with cyanocobalamin effectively reduce oedema associated with atopic dermatitis in an in vivo assessment using a hypersensitivity mouse model.**

18:25-18:30. C. Costas. Universidad de Lugo. **Neurotoxic Shellfish Poisoning: Brevetoxin 3 acute toxicity in vivo.**

Session 8. New perspectives in neuropharmacology.

Chairwoman: Mercè Pallás. Universidad de Barcelona.

8/9/2023 Paraninfo Envases de Cartón

9:30-10:00. Enrique J. Cobos. Universidad de Granada. **Sigma-1 receptors and pain: modulation of peripheral sensitization.**

10:00-10:30. Julia García-Fuster. Universidad de les Illes Balears. **Characterizing novel antidepressants for adolescent psychopathology from a preclinical perspective.**

10:30-11:00. Mercè Pallas. Universidad de Barcelona. **I2 imidazoline receptors: validation as a target for Alzheimer's disease treatment.**

11:00-11:15. Inmaculada Posadas. Universidad de Castilla-La Mancha. **Engineered Neutral Phosphorous Dendrimers Protect Mouse Cortical Neurons and Brain Organoids from Excitotoxic Death.**

11:15-11:30. Antonio Rodríguez-Artalejo. Universidad Complutense de Madrid. **From chromaffin cells to primary nociceptive neurons: a round trip from stress to pain.**

Sigma-1 receptors and pain: modulation of peripheral sensitization

Enrique J Cobos

Departamento de Farmacología, Centro de Investigación Biomédica e Instituto de Neurociencias, Universidad Granada e Instituto de Investigación Biosanitaria ibs.GRANADA

ejcobos@ugr.es

The sigma-1 receptor is a chaperone protein. In response to the increase of intracellular Ca^{2+}, it migrates from intracellular locations to the plasma membrane, where it modulates the action of several protein partners, including G-protein coupled receptors such as μ-opioid receptors (MOR), and ion channels such as NMDA receptors. It is well described that NMDA receptor activity decreases MOR signalling, and that sigma-1 receptors mediate the communication between NMDA receptors and MOR at central levels to modulate the effect of opioid drugs. In fact, sigma-1 antagonism is known to increase opioid analgesia, and S1RA, a selective sigma-1 antagonist undergoing phase IIa clinical trials, has an intended indication as an adjuvant to opioid drugs. Although the role of the sigma-1 receptor in pain has been studied mainly at central levels, this receptor has a much higher peripheral presence, as it is expressed by all peripheral sensory neurons, and not only in mice, but also in human samples.

Peripheral sensory neurons can detect chemicals generated during inflammation, including lipid mediators such as prostaglandin E2 (PGE2) or neurotrophins such as nerve growth factor (NGF), among others. These substances increase the excitability of peptidergic C-nociceptors (TRPV1+ sensory neurons), promoting pain development through the process known as peripheral sensitization.

We recently described that the hyperalgesia induced by the intraplantar injection of PGE2 or NGF is completely reversed by sigma-1 receptor antagonism [1]. Interestingly, this effect is abolished by administration of naloxone methiodide, a peripherally-restricted opioid antagonist. These results indicate the involvement of the peripheral opioid system in the effects induced by sigma-1 antagonism, even in the absence of the administration of any opioid drug. We detected immunoreactivity against the endogenous MOR agonist endomorphin-2 in TRPV1+ nociceptors, and the administration of an anti-endomorphin-2 antibody to a sensitized paw reversed the antihyperalgesia induced by sigma-1 antagonists. Therefore, the effect induced by sigma-1 antagonism appears to be mediated by the enhancement of neuronally derived endogenous opioid analgesia. Using recombinant proteins, we showed that the sigma-1 receptor participate in TRPV1-MOR crosstalk in a manner analogous to the communication between NMDA receptor and MOR previously described at central levels. Finally, we show that PGE2 increases calcium currents induced by capsaicin, the prototype TRPV1 agonist, in cultured sensory neurons. This effect is reversed by S1RA, and in a naloxone-sensitive manner, consistent with the opioid-mediated effects of sigma-1 antagonism observed *in vivo*.

In summary, sigma-1 antagonism harnesses endogenous opioids produced by peptidergic C nociceptors to reduce hyperalgesia in a mechanism which involves the enhancement of MOR activity at the pain site. The modulation of peripheral endogenous opioid analgesia by sigma-1 receptors might have potential clinical application for pain treatment.

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Characterizing novel antidepressants for adolescent psychopathology from a preclinical perspective

Ledesma-Corvi, S.[1], Jornet-Plaza, J. [1], Gálvez-Melero[1], L., García-Fuster, M.J. [1,2]

[1] *IUNICS and IdISBa, Palma, Spain.*
[2] *School of Medicine, University of the Balearic Islands, Palma, Spain*

E-mail: j.garcia@uib.es

There is an urgent need to characterize novel therapeutical options for adolescent major depression, since it affects up to 5-6% of the adolescents and its treatment, besides a psychological approach, is limited to the pharmacological use of fluoxetine. Moreover, to later warrant the best translational outcome to the clinic, sex should be incorporated as a biological variable in all preclinical studies, given the lack of data including both sexes. In this context, we will be presenting data on the antidepressant-like effects induced during adolescence by three therapeutical options with differential mechanisms of action, such as cannabidiol, ketamine, or electroconvulsive seizures. The results will demonstrate that all options are efficacious and safe to be administered during adolescence at given particular conditions, however, some clear sex disparities exist between sexes that deserve further studies, especially for female adolescents, which generally rendered less responsive.

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I_2 imidazoline receptors: validation as a target for Alzheimer's disease treatment

Pallàs, M.[1,3]; Vasilopoulou, F.[1]; Taboada-Jara, T.[1]; Bagan, A.[2]; Escolano, C.[2]; Griñán-Ferré, C.[1,3]

[1]Pharmacology Section, Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institut de Neurociències (UBNeuro), University of Barcelona, Barcelona, Spain
[2]Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Barcelona, Spain
[3]Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.

E-mail: pallas@ub.edu

Identifying imidazoline receptors (IR) as a new class of receptors has raised several research questions regarding their potential pharmacological and therapeutic properties. Among them, I2-IR receptors are widely distributed in the central nervous system (CNS), with a predominant localization in glial cells. At the same time, the prevalence of Alzheimer's disease (AD) is increasing rapidly due to increasing life expectancy in developed countries, further highlighting the urgency of identifying new targets to halt the progression of the disease and provide an effective treatment. Because, it is accepted the complexity of AD, the diversity of the pathology and the dynamic interactive network of components that influence the disease progression, it is suggested that to halt its progression, potential drugs should interfere with more than one pathogenic step responsible for the clinical symptoms, such as extracellular A-beta plaques, NFTs formation, inflammation, or oxidative stress. Notably, I_2-IR ligands have neuroprotective properties, although the exact molecular mechanisms are not fully described. Here, our objective is to contribute to the scientific knowledge of the pharmacological characteristics of I_2-IR receptors and their feasibility as a new target for neurodegenerative diseases with unmet medical needs, such as AD. To this end, we have validated known chemical entities described as I_2-IR ligands (2-BFI, LSL60101), as well as developed new ones (MCR5, MCR9), as effective tools to prevent the characteristic signs of AD in 5XFAD and SAMP8, mouse models of early-onset and late-onset AD, respectively. We demonstrated a delay in the cognitive and behaviour endpoints after I_2-IR ligands *"in vivo"* that point out I_2-IR ligands as a pharmacological tool to face AD. Indeed, we went deep on molecular mechanisms affected after treatment with I_2-IR ligands, which can modify detrimental processes characteristic of neurodegeneration. To this end, we characterized changes in neuroinflammation and oxidative stress markers, neurotrophic pathway as well as amyloid processing and tau hyperphosphorylation mechanisms. In conclusion, we demonstrated that I_2-IR ligands treatment addressed most of the key etiological processes in two preclinical AD models, preventing them from the generalized cognitive downfall and modifying specific molecular AD markers, including AD histological and biochemical ones, allowing us to validate I_2-IR as a new pharmacological target for AD.

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Engineered Neutral Phosphorous Dendrimers Protect Mouse Cortical Neurons and Brain Organoids from Excitotoxic Death

Posadas, I.[1,2] Romero-Castillo, L.[1,2] Ronca, R.[1], Karpus, A.[3], Mignani, S.[3], Majoral, J- P.[3], Muñoz-Fernandez, MA.[2,4], Ceña, V.[1,2]

[1]Unidad Asociada Neurodeath, Facultad de Medicina, Universidad de Castilla-La Mancha, 02006 Albacete, Spain; [2]Centro de Investigación Biomédica en Red (CIBER), Instituto de Salud Carlos III (ISCIII), 20029 Madrid, Spain; [3]CNRS, Toulouse, France; [4]Hospital General Universitario Gregorio Marañón, Madrid, Spain
inmaculada.posadas@uclm.es

Excitotoxicity, mainly mediated by the activation of the ionotropic NMDA-activated glutamate receptors (NMDAR), plays a significant role in the pathophysiology of neurodegenerative diseases, such as Alzheimer's or Parkinson's disease. Overactivation of NMDAR leads to excitotoxic death by increasing intraneuronal calcium levels, triggering mitochondrial potential collapse, increasing free radicals, inducing endoplasmic reticulum stress, and generating an inflammatory response (1,2).

Nanomedicine is a relatively new science that uses nanoscale materials in diagnosis, drug delivery, and as therapeutic agents. Dendrimers are well-defined nano-sized structures able to deliver small drugs or siRNA into different cell types. In addition, phosphorous dendrimers have shown noteworthy biological activities, including anti-inflammatory properties (3,4).

In the present work we have studied the effect of two neutral phosphorous dendrimers, G3b and G4b, that have previously displayed a marked anti-inflammatory action (4) both in vitro and in vivo, on a well-established in vitro model of excitotoxicity such as NMDA-mediated toxicity both in primary mouse cortical neurons and in human brain organoids.

To this end, we determined toxicity by measuring the percentage of LDH released to culture medium spectrophotometrically; the cellular redox status by measuring the mitochondrial membrane potential, the mitochondrial and total ROS production by fluorescence microscopy; the activation of the intrinsic apoptotic pathway and the activity of caspases -3,-9 and -12 by fluorometry; and finally, we determined the UPS activation by studying the expression of different proteins involved in ER stress by western blot.

We found that both phosphorous dendrimer markedly decreased NMDA-mediated excitotoxicity in primary cortical neurons by decreasing mitochondrial ROS production, ER stress, and UPR responses. The dendrimers also showed a marked neuroprotective action in NMDA-treated human brain organoids by decreasing NMDA-induced caspase -3, -9, and -12 activation as well as neuronal death. These data strongly suggest that neutral phosphorous dendrimers can penetrate not only neurons in culture but also a 3D structure, such as the brain organoid. Therefore, this family of dendrimers might represent a useful scaffold to design new NP-based therapeutic agents for treating neurodegenerative diseases.

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From chromaffin cells to primary nociceptive neurons: a round trip from stress to pain

L.A. Olivos-Oré, M. Arribas-Blázquez, C. Llorente Sáez, A. Quintero Pérez, M.V. Barahona Gomariz, A.R. Artalejo

Instituto Universitario de Investigación en Neuroquímica. Departamento de Farmacología y Toxicología. Facultad de Veterinaria. Universidad Complutense de Madrid

E-mail: artalejo@ucm.es

Stress and pain are responses to external and internal stimuli that individuals perceive as threats, carrying a significant emotional component. Furthermore, stress and pain can interact and influence each other. Consequently, stress modulates pain perception, either attenuating or exacerbating it. Simultaneously, pain activates the hypothalamus-pituitary-adrenal cortex axis and the sympathetic nervous system. However, the impact of pain, particularly chronic pain, on the function of the adrenal medulla, which is part of the sympathetic nervous system, remains poorly understood. In this communication, we review the evidence gathered by our research group concerning the changes experienced by the adrenal medulla in a model of chronic neuropathic pain resulting from peripheral nerve injury. This model displays increased cholinergic innervation, heightened expression of alpha9 nicotinic receptors, P2X3 and P2X7 purinergic receptors, TRPV1 and T-type voltage-dependent Ca^{2+} channels of chromaffin cells, leading to an elevation in the quantal secretion of catecholamines. Importantly, some of these alterations in receptor and channel expression are also observed in primary nociceptive neurons. Lastly, we will also report on compounds (e.g., phenylethanolamine N-methyltransferase inhibitors and peripherally acting $alpha_2$-adrenergic receptor agonists) that exhibit an antinociceptive effect by inhibiting adrenomedullary catecholamine secretion.

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Session 9. Pulmonary fibrosis: new challenges.

Chairwoman: María Jesús Sanz. Universidad de Valencia.

8/9/2023 Sala de Grados Sabatini

9:30-10:00. Ana Montes. Bellvitge Biomedical Research Institute – IDIBELL. **Pathobiological features of idiopatic pulmonar fibrosis.**

10:00-10:30. Javier Milara. Universidad de Valencia. **Emerging Therapeutic Targets for idiopatic pulmonar fibrosis.**

10:30-11:00. Paula Montero. Universidad de Valencia. **Experimental Models in idiopatic pulmonar fibrosis.**

11:00-11:15. C. Griñán Ferré. Universidad de Barcelona. **Discovery of a Dual-Action of G9a Inhibitors for the Treatment of Alzheimer's Disease.**

11:15-11:30. María Jesús Sanz. Universidad de Valencia. **Normalization of metabolic and inflammatory parameters in vitro and in vivo in ob/ob mice by a novel pan-PPAR agonist (BP-2).**

Pathobiological features of Idiopathic Pulmonary Fibrosis.

Ana Montes Worboys

Instituto de Biomedicina de Bellvitge (IDIBELL)

E-mail: amontesw@idibell.cat

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and usually, lethal disorder of unknown etiology, minimal therapeutic options, and survival is limited to 3–5 years post-diagnosis. It is an age-related interstitial lung disease, the majority of IPF patients are males and above 60 years of age at presentation. The disease is characterized by progressive scarring of lung tissue, which reduces gas exchange and leads to progressive respiratory failure, and by the aberrant activation of epithelial cells, which secrete numerous mediators resulting in the expansion of the fibroblast/myofibroblast population with the subsequent exaggerated accumulation of extracellular matrix and the destruction of the lung architecture. Most of the hallmarks that characterize the aging process have been identified in IPF lungs, and several environmental and occupational exposures have been reported as risk factors, but smoking is the most consistently recognized risk factor for developing both the sporadic and the familial forms of IPF. Decades of research have revealed a complex underlying pathophysiology of IPF with alterations in many molecular aspects and cellular physiology, including genetics, epigenetics, microRNAs, developmental reprogramming, cell-signaling pathways, apoptosis, metabolism, and autophagy. The involvement of multiple pathogenetic pathways may account for the heterogeneity of the clinical behavior of IPF. Some patients experience a slow decline, whereas others decline rapidly and die within a few months from the time of diagnosis. Taken together, IPF is a rare incurable disease characterized by a high burden of disease, with evolving encouraging unraveling of the underlying pathobiology, hopefully leading to novel therapeutic options in the future.

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Emerging therapeutic targets for idiopathic pulmonary fibrosis.

Javier Milara[1,2]

[1]*Uuniversity of Valencia, Department of pharmacology*
[2]*Valencia General Hospital Consortium, Pharmacy Service*

E-mail: Presenting author: xmilara@hotmail.com

Idiopathic pulmonary fibrosis (IPF) is a severe chronic lung disease characterized by progressive scarring and thickening of the lung tissue, leading to impaired lung function and death within 5 years of diagnosis. Over the past two decades, significant progress has been made in the development of therapies that can slow down the progression of the disease. Currently, there are two approved drugs for IPF treatment, nintedanib and pirfenidone. However, the limited survival rates and the need for more effective treatments have spurred interest in identifying new therapeutic targets.

In recent years, there have been advancements in understanding the underlying mechanisms of IPF, leading to the exploration of various drug targets and therapies in preclinical and early-stage clinical trials. These investigations aim to identify novel approaches that can halt or reverse the fibrotic process and improve patient outcomes.

To develop precise and effective therapies for IPF, it is crucial to consider the complex pathogenesis of the disease, as well as the variability in disease course and individual responses to treatment. Precision medicine approaches, such as transcriptomics and the use of serum biomarkers, are emerging as essential tools in guiding future drug development and therapeutic decision-making. By analyzing gene expression patterns and identifying specific biomarkers, researchers can gain insights into the underlying mechanisms of IPF and develop targeted therapies tailored to individual patients.

Combination therapy is another area of interest in IPF research. Given the multifaceted nature of the disease, targeting multiple pathways simultaneously may yield better therapeutic outcomes. By combining drugs with complementary mechanisms of action, researchers hope to achieve synergistic effects and improve treatment efficacy.

In conclusion, while significant progress has been made in IPF therapeutics over the past two decades, there is still a need for more effective treatments to improve patient survival and quality of life. Ongoing research is focused on identifying new drug targets and developing precision-based approaches that consider the heterogeneity of IPF. By leveraging advanced technologies and adopting combination therapy strategies, researchers aim to open new therapeutic avenues for this challenging lung disease.

Experimental Models in idiopathic pulmonary fibrosis

Montero, P[1], Roger, I[1,2]

1University of Valencia, Valencia, Spain

2 CIBERES Health Institute Carlos III, Madrid, Spain

E-mail: paulamonmag@gmail.com; paula.montero@uv.es

Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease characterized by the formation of fibrotic tissue within the lungs, resulting in impaired gas exchange and debilitating symptoms. Despite the availability of pirfenidone and nintedanib as approved therapies, which have exhibited efficacy in slowing IPF progression in clinical trials, the prognosis for IPF patients remains poor, with a high mortality rate within 2 to 5 years followingdiagnosis. The complex pathogenesis of IPF and the absence of safe and effective treatments necessitate innovative approaches to address the unmet medical needs of patients.Consequently, extensive research has been conducted utilizing various in vivo and ex vivo experimental models to study IPF. In this oral session, we present an overview of the differentanimal models employed, including pulmonary fibrosis induction via bleomycin, viral vector- mediated transgene delivery (e.g., TGF-β1 and Interleukin-1β), cytokine overexpression models, and rodent models utilizing silica instillation. Additionally, we explore the utility of *exvivo* models such as precision-cut lung slices (PCLS) and three-dimensional (3D) cell culture systems like lung spheroids and organoids. By evaluating the advantages and limitations of each model, we aim to facilitate a better understanding of their suitability for studying IPF pathogenesis and evaluating potential therapeutic interventions.

Discovery of a Dual-Action of G9a Inhibitors for the Treatment of Alzheimer's Disease

Griñán-Ferré, C.[1,3], Bellver-Sanchis, A.[1]; Sánchez-Arfelis, A.[2]; Irisarri, A.[1]; Tic, I.[1]; Vázquez, S.[2]; Pérez, B.[3]; Leandro Martínez Rodríguez, A.[4], Brea, J.[4,5], Loza, M. Escolano, C.[2]; & Pallàs, M.[1,3].

[1]Department of Pharmacology and Therapeutic Chemistry, Faculty of Pharmacy and Food Sciences, Institut de Neurociències, Universitat de Barcelona, Avda. Joan XXIII, 27, 08028 Barcelona, Spain.
[2]Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII, 27-31, E-08028, Barcelona, Spain.
[3]Department of Pharmacology, Therapeutics and Toxicology, Institute of Neuroscience, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.
[4]Innopharma screening platform, Biofarma research group. Centro de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS), Departamento de Farmacología, Farmacia y Tencología Farmacéutica. Universidad de Santiago de Compostela, Santiago de Compostela, Spain.
[5]Health Research Institute of Santiago de Compostela (IDIS), University Hospital of Santiago de Compostela (SERGAS), Trav. Choupana s/n, 15706 Santiago de Compostela, Spain.
[6]Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.

E-mail: christian.grinan@ub.edu

Alzheimer's disease (AD) is the most common cause of dementia, making the disease a global health crisis that must be addressed. Until now, none of the approved AD treatments turned out to be a success. AD is unknown but involves a combination of genetic, biochemical, and environmental factors, being one of the reasons why single-target-directed drugs have failed to reach clinical trials. As a new strategy in drug discovery for AD, multifunctional molecules avoid drug–drug interactions, off-target adverse effects, poor patient compliance, and high development costs compared to combination therapies. Multiple lines of evidence suggest that epigenetic alterations and tau pathology are two of the crucial causes of AD. Strikingly, overexpression of G9a and the other protein serve as drivers of the cognitive impairment, leading to synaptic plasticity reduction, autophagy dysfunction, increasing Tau pathology, OS and neuroinflammation. Here, we synthesized the compound AMC-1, a new chemical scaffold with high potency micromolar (μM) to inhibit both targets. Moreover, other interesting characteristics are that AMC-1 is selective to G9a respect GLP (another histone/lysine methyltransferase), exhibits a high PAMPA-BBB permeability, no presented hERG toxicity and a good drug metabolism and pharmacokinetics. Besides, treatment with AMC-1 in SAMP8 mice rescued cognitive decline measured via NORT. G9a is responsible for methylating Histone 3, being capable to repress the expression of genes related to learning and memory formation, we evaluated several repressive histone marks in the SAMP8 mice model. Furthermore, we evaluated the Tau phosphorylation, observing that AMC-1 was able to reduce its levels in SAMP8. In addition, the density of dendritic spines and the length of dendritic branches were evaluated, showing an increase in the treated group. Therefore, our dual approach is an innovative and promising multifaceted therapeutic strategy for AD treatment.

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Normalization of metabolic and inflammatory parameters *in vitro* and *in vivo* in *ob/ob* mice by a novel pan-PPAR agonist (BP-2)

Sanz, M.J.[1,2,3], Marques P.[1,2], Villarroel-Vicente, C.[1,2], Collado, A.[1,2], García, A.[1,2], Vila, L.[2], Piqueras, L[1,2,3]., Cortes, D.[1,2], Cabedo, N.[1,2]

1Faculty of Medicine and Faculty of Pharmacy, University of Valencia, Valencia, Spain.
2Institute of Health Research INCLIVA, Valencia, Spain.
4CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Madrid, Spain.

E-mail: maria.j.sanz@uv.es

Background: Selective peroxisome proliferator-activated receptors (PPARs) are widely used to treat metabolic complications. However, given that selective-PPARα agonists present limited effect on glucose metabolism and selective-PPARγ agonists showed serious adverse effects, novel pan-PPAR agonists are a promising therapeutic approach to improve the treatment of metabolic disorders [1]. Since we synthesized a new prenylated benzopyran, 2-(ethyl 4'-methylheptenoate)-6-(*p*-fluorobenzyloxy)-2-(methyl)-benzodihydropyran (BP-**2**) with pan-PPAR properties (strong PPARα activity, moderate PPARβ/δ activity and weak PPARγ activity), we evaluated its possible anti-inflammatory effects and the impact on metabolic derangements.
Material and Methods: Parallel-plate flow chamber was employed to investigate the effects of BP-**2** on TNFα-induced (20 ng·ml^{-1}, 24h) mononuclear cell-endothelium interactions. Flow cytometry was used to determine its effects on TNFα-induced expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and transmembrane fractalkine/CX$_3$CL1 in human umbilical vein endothelial cells (HUVEC). The impact of BP-**2** administration (10 or 30 mg/kg/d, 15 days) on metabolic abnormalities and inflammation was evaluated in *ob/ob* mice. T cell and macrophage infiltration in the liver or white adipose tissue were quantified by immunofluorescence.
Results: *In vitro*, our results showed that BP-**2** concentration-dependently reduced TNFα-induced endothelial mononuclear cell adhesion through the downregulation of ICAM-1, VCAM-1 and fractalkine/CX$_3$CL1 via PPARβ/δ–RXRα interactions. *In vivo*, BP-**2** administration improved circulating levels of glucose and triglycerides in *ob/ob* mice as well as those of hepatic transaminases. Moreover, BP-**2** also reduced plasma levels of TNFα and suppressed T-cell and macrophage infiltration in the liver and white adipose tissue in *ob/ob* mice.
Conclusions: BP-**2** emerges as a novel pan-PPAR lead candidate capable of normalizing glycemia/triglyceridemia and minimize inflammation in metabolic disorders, likely preventing the development of further cardiovascular complications.

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Poster sessions

RECEPTORS AND ION CHANNELS

PO-000

Effects of empagliflozin on human cardiac sodium and Kir2.1 channels

Rapún J, Cámara-Checa A, Crespo-García T, Gil-Cabezudo C, Tamargo J, Gómez R, Caballero R, Delpón E.

Dpt. Pharmacology & Toxicology. School of Medicine. Universidad Complutense de Madrid. 28040-Madrid. CIBERCV.

E-mail: rcaballero@med.ucm.es

Empagliflozin (Empa) is a sodium-glucose cotransporter 2 inhibitor (SGLT2i) used for the treatment of type 2 Diabetes Mellitus (T2DM). Empa reduces morbidity and mortality in heart failure (HF) patients with reduced or preserved ejection fraction, regardless of the presence or absence of T2DM. Nav1.5 channels encoded by the *SCN5A* gene carry the Na^+ current (I_{Na}) which is responsible for cardiac action potential (AP) depolarization and, thus, is a critical determinant of cardiac excitability and conduction velocity. Kir2.1 channels encoded by the *KCNJ2* gene carry the inward rectifier K^+ current (I_{K1}) that controls resting membrane potential and the duration of the final repolarization. Our group has demonstrated that Empa, incubated for 24 hours at therapeutically relevant concentrations, increased I_{Na} and I_{K1} in human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CMs),[1] effects that could participate in the beneficial actions of the drug on HF patients, where the expression of Nav1.5and Kir2.1 channels is reduced. The increase did not involve Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) or the Na^+/H^+ exchanger. Moreover, we demonstrated that Empa directly augmented currents generated by Nav1.5 ($I_{Nav1.5}$) and Kir2.1 ($I_{Kir2.1}$) channels in heterologous systems. Our aim was to elucidate the molecular mechanisms underlying the Empa-induced increase on cardiac I_{Na} and I_{K1}. We recorded macroscopic and unitary currents in CHO cells incubated or not with Empa expressing Nav1.5 or Kir2.1 channels by using the whole-cell and cell-attached configurations of the patch-clamp technique, respectively. Furthermore, luciferase assays were conducted to determine putative effects of the drug at the transcriptional level and flow cytometry experiments to measure putative effects on themembrane expression of the channels. In all cases, Empa (1 μM) was added to the culture mediaand incubated for 24 h. Whole-cell patch-clamp recordings demonstrated that Empa produced a concentration-dependent increase of $I_{Nav1.5}$ (EC_{50}=0.8±0.01 μM) and $I_{Kir2.1}$ (EC_{50}=0.9±0.01 μM). Single channel recordings demonstrated that Empa augmented mean open time (MOT, from 0.96±0.04 to 1.42±0.13 ms, P<0.05) and open probability (*Po*, from 0.03±0.001 to 0.065±0.009) of Nav1.5 channels, without modifying mean current amplitude (-2.9±0.1 pA at -20 mV, P>0.05). On the other hand, Empa increased MOT (from 98.8±19.1 to 279±33 ms, P<0.05) and *Po* (from 0.56±0.06 to 0.78±0.02, P<0.05) of Kir2.1 channels, without modifying the current amplitude (-2.7±0.3 pA at -120 mV) or slope conductance (ϒ=28±0.06 pS)(P>0.05). Luciferase assays carried out in CHO cells transfected with vectors encoding the minimal promoters of the *SCN5A* and *KCNJ2* genes demonstrated that incubation with Empa does not modify their transcriptional activity. Flow cytometry experiments in HEK-293 cells, showed that Empa treatment slightly but significantly increased the expression of Kir2.1 channels at the membrane. In conclusion, our results demonstrated that Empa increased cardiac I_{Na} and I_{K1} by producing remarkable effects on single Nav1.5 and Kir2.1 channel properties and suggested that Empa directly interacts with the respective channel proteins.

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PO-001

Effects of dapagliflozin on human cardiac sodium and Kir2.1 channels

Cámara-Checa A, Crespo-García T, Rapún J, Gil-Cabezudo C, Tamargo J, Gómez R, Delpón E., Caballero R.

Dpt. Pharmacology & Toxicology. School of Medicine. Universidad Complutense de Madrid. 28040-Madrid. CIBERCV.

E-mail: edelpon@med.ucm.es

Dapagliflozin (Dapa) is a sodium-glucose cotransporter 2 inhibitor (SGLT2i) used for the treatment of type 2 Diabetes Mellitus (T2DM) that improves cardiovascular outcomes, as it significantly decreases hospitalizations and mortality in patients with heart failure (HF) with reduced or preserved ejection fraction, even in the absence of T2DM. Previous data suggested that Dapa may produce antiarrhythmic actions which eventually lead to a decrease incidence of ventricular arrhythmia and fatal or resuscitated sudden cardiac death. The Na^+ current (I_{Na}) carried by Nav1.5 channels (encoded by the *SCN5A* gene) is responsible for cardiac action potential (AP) depolarization, while the inward rectifier K^+ current (I_{K1}) carried by Kir2.1 channels (encoded by *KCNJ2*) controls resting membrane potential and the duration of the final repolarization. Therefore, these currents determine cardiac excitability and refractoriness. In HF patients, the expression of Nav1.5 and Kir2.1 channels is reduced, leading to a decrease of ventricular excitability that enhances the arrhythmic risk. In human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CMs) and in heterologous expression systems, we recently described that 24-h incubation with Dapa, at therapeutically relevant concentrations, increased I_{Na} and I_{K1}.[1] In the present work, we tried to identify the molecular mechanisms underlying the effects of Dapa on cardiac I_{Na} and I_{K1}. To this end, we recorded macroscopic or unitary currents in CHO cells expressing Nav1.5 by using the whole-cell and cell-attached configurations of the patch-clamp technique, respectively. Moreover, we conducted luciferase assays and flow cytometry analyses to identify effects at the transcriptional level or on the expression level of the channels at the cell membrane, respectively. In each experimental approach, Dapa was added to the culture media at the corresponding concentration and incubated for 24 h. Dapa produced a concentration-dependent increase of $I_{Nav1.5}$ (EC_{50}=0.6±0.02 μM), while it did not modify $I_{Kir2.1}$ at any of the concentrations tested. Single channel recordings using the cell-attached patch-clamp configuration showed that Dapa did not modify mean open time (MOT, 0.96±0.04 vs 0.84±0.03 ms, P>0.05) or mean current amplitude (-2.9±0.1 vs -3.2±0.2 pA at -20 mV, P>0.05) of single Nav1.5 channels, but produced a profound effect on channel gating characterized by a marked increase of channel reopening and the number of traces with openings, which eventually led to a significant increase of P_o (from 0.03±0.001 to 0.048±0.003, P<0.05). Dapa did not modify the transcriptional activity of the minimal promoters of the *SCN5A* and *KCNJ2* genes, as demonstrated by luciferase assays conducted in CHO cells. Flow cytometry experiments in HEK-293 cells, demonstrated that Dapa increased the membrane expression of Kir2.1 channels when both Kir2.1 and Nav1.5 channels were co-expressed. We conclude that Dapa increased cardiac I_{Na} by a dual mechanism involving a direct effect on Nav1.5 channel gating and, probably, enhancing Nav1.5 trafficking to the cell membrane. In contrast, its effects on I_{K1} seem not to be due to a direct effect on the channel gating but mediated by the positive reciprocal Nav1.5-Kir2.1 modulation.

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PO-002

Distinct coupling with transducers of natural genetic variants of fractalkine receptor CX_3CR1 associated with disease

Paz, A.[1,2], García Silva, A.[1,2], Blázquez, P.[1,2], de la Fuente, R.A.[1,2], García Izquierdo, A.[1,2],Halls, M.[3], Castro M.[1,2]

[1] Molecular Pharmacology of GPCRs research group, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), Universidade de Santiago de Compostela, Spain [2] Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Spain
[3] Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia

E-mail: *albapaz.castro@usc.es*

CX_3CR1, the sole human receptor for the chemokine fractalkine (CX_3CL1) [1], is a key playerin inflammation/immunity and neuron-microglia communication. Natural variants of CX_3CR1 of not well-known pharmacology have been found associated to disease. We aimed at investigated the signalling and trafficking of CX_3CR1 and two of its natural genetic variants: the polymorphic variant CX_3CR1-V249I/T280M and the rare variant CX_3CR1-A55T [2].

Fractalkine promoted recruitment of β-arrestin 1 and 2, and GRK2 by CX_3CR1 transfected intoHEK293 cells, as assessed by BRET-based assays. The receptor variant CX_3CR1-V249I/T280M displayed increased efficacy at engaging β-arrestins and GRK2 over wild-type receptor in these assays, without changes in fractalkine potency. On the other hand, the CX_3CR1-A55T variant, proposed to be deficient in G protein coupling, still retained capacity to recruit β-arrestins while resulted deficient in GRK2 recruitment. The three receptor variants investigated trafficked from the plasma membrane to intracellular compartments in response tofractalkine, although with subtle qualitative differences in subcellular trafficking.

Fractalkine elicited extracellular-signal regulated kinase (ERK) 1/2 signalling with different kinetics and amplitude in the cytosolic and nuclear compartments of HEK293 cells transfected with CX_3CR1, assessed by using FRET-based biosensors of ERK1/2 activity. Both cytosolic and nuclear ERK activation were abolished by disruption of $G_{i/o}$ signalling with pertussis toxin. Whereas cytosolic ERK signalling was abolished by downregulation of β-arrestins, nuclear ERK signalling was only partially dependent on β-arrestins. Interestingly, only nuclear ERK signalling was dependent on dynamin function.

Our results suggest different signalosomes contributing to compartmentalized ERK signalling by CX_3CR1, with possibly distinct cellular consequences. The altered engagement of signallingtransducers by natural genetic variants of CX_3CR1 might have pathophysiological implications.

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PO-003

Voltage-gated calcium channels characterization in neuronal differentiation in the immortalized F11 cell line

López, D[1,2,3], Martínez, AL[1,2,3], Brea, J[1,2,3] and Loza, MI[1,2,3]

[1]BioFarma Research Group, Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS), Universidad de Santiago de Compostela.
[2]Instituto de Investigaciones Sanitarias (IDIS), Santiago de Compostela.
[3]Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela (USC).

E-mail: daniellopez.fernandez@usc.es

Neuronal differentiation is a complex process through which newborn neurons acquire the morphology of mature neurons and become excitable. Despite that the mechanisms of neuronaldifferentiation are not well known, it has been demonstrated that ion transients play a major role on this process. Previously, we studied the role of sodium transients in neuronal differentiation employing the immortalized DRG neuronal cell line F11 (1). This cell line, oncedifferentiated, acquires a sensorial neuron phenotype, which allows the study of diseases like neuropathic pain (2).

Our hypothesis is that calcium channels may play a role in neuronal differentiation. So, our aimis to characterize the role of calcium transients during differentiation and to disclose which calcium channels are involved in neuronal differentiation of F11 cells.

We performed a transcriptomic study of the expression of calcium channels in the F11 cell linebefore and after differentiation by employing RT-qPCR. The results showed that the expressionof calcium channels was increased after differentiation ($p<0.0001$, ANOVA followed by a Sidak's post-hoc test). However, three subtypes of Ca_V1 were not expressed and one of them was only present in differentiated cells.

Afterwards, we performed a pharmacological study using selective blockers (nitrendipine, felodipine and nifedipine) of these channel types during the differentiation to evaluate the impact of blocking those channels on the excitability and on neurite length of differentiated F11cells. The blockade of Ca_V1 channels during differentiation induced a decrease at least of 20% in the excitability ($p<0.001$, ANOVA followed by Dunnett's post-hoc test). In neurite outgrowth, nitrendipine at 10nM induce a decrease of 45% of neurite length ($p<0.0001$, ANOVA followed by Dunnett's post-hoc test).

These results support the hypothesis that Ca_V1 voltage-gated calcium channels play a significant role in the regulation of neuronal differentiation of F11 cells, since Ca_V1 antagonistshinder the acquisition of neuronal phenotypic features.

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PO-004

Glucocorticoids exert distinct barrier modulatory effects in intestinal epithelial cells *in vitro*

Ceacero-Heras, D[1]; Ruiz-Henares, G[1]; Seguí-Pérez, A[1]; Tena-Garitaonaindia, M[1]; Martínez-Augustin, O[1]; Sánchez de Medina, F[1]

[1]*Faculty of Pharmacy, University of Granada, Granada, Spain*

E-mail: dch@ugr.es

Glucocorticoids (GC) are important antiinflammatory and immunosuppresive agents in the management of inflammatory bowel disease. However, GC also display deleterious effects on the intestinal barrier which may limit their clinical benefit. In this regard, DSS-induced colitis in mice show a worse general status when treated with prednisolone. This may be associated to antiproliferative effects on the epithelium.
Our objective is to evaluate GC potency and deleterious effects in vitro.
The antiproliferative effects of GC were evaluated on IEC4.1 cell line carrying out a wound healing assay. Epithelial paracellular permeability was determined in IEC4.1 and CACO-2 cell lines in Transwell systems with budesonide. Effects of GR stimulation were appraised using jejunal organoids from mice carrying an inducible *Nr3c1* KO in intestinal epithelial cells. Organoids were stimulated overnight with corticosterone then GR target gene expression was assessed by RTqPCR.
GC inhibited the epithelial response in the wound healing assay in a concentration-dependent manner. The response was somewhat higher with dexamethasone and budesonide. This correlated with GR translocation to the nucleus. However, budesonide-stimulated cells show a reinforcement in epithelial integrity in Transwell system. Finally, intestinal organoids from GR KO mice showed an attenuated expression of some proteins related to epithelial permeability (*Tjp1* and *Occludin*). In turn, WT, but not KO organoids, showed an attenuated expression of genes related to cell proliferation (*Pcna*, *Cyclin D1*) and steroidogenesis (*Cyp11a1* and *Cyp11b1*) when stimulated with corticosterone.
GC exert antiproliferative effects both in WT organoids and intestinal cell lines in basal conditions. Unexpectedly, budesonide may reinforce epithelial barrier and GR KO in intestinal organoids results in downregulation of tight junction proteins, suggesting the effect of GC on barrier function may be context dependent.

PHARMACOGENOMICS AND TOXICOLOGY

PO-005

Voluntary ingestion of Diarrhetic Shellfish Toxins a promising alternative to oral gavage in mice for toxicity studies

Louzao, M. C., Costas, C., Rodríguez-Santos, L., Raposo-García, S., Vale, C., Vieytes, M.R., Botana, L. M.

Universidad de Santiago de Compostela, Lugo, Spain

E-mail: mcarmen.louzao@usc.es

Okadaic acid (OA), dinophysistoxins 1 (DTX1) and (DTX2) are polyether compounds of marine origin synthetised by dinoflagellates. They can accumulate in filter-feeding organisms like bivalve molluscs, though they can spread across the food network up to human consumption. Diarrhetic Shellfish Poisoning is caused by seafood contaminated with OA-group toxins, which comprises incapacitating gastrointestinal signs like vomiting and diarrhoea. Symptoms onset can be as early as 30 min and last between 2-3 days, with no fatalities registered. Currently, OA group of toxins are regulated and constantly monitored in the European Union. Oral toxicological information is essential to provide information for risk assessment of these toxins in edible seafood. During animal experimentation for the administration of precise amounts of toxin, oral gastric feeding needles are typically used (oral gavage). However, it has been discussed that this administration route could lead to fast absorption, therefore, encouraging studies with voluntary feeding of the toxin. We aimed to compare OA, DTX1 and DTX2 toxicity by oral gavage and voluntarily fed administration. Swiss female mice were habituated to the food in which the toxin was administered. Each toxin was freshly diluted in saline solution and mixed with the food. Animals ingested the whole product in 5 min, assuring the consumption of the prepared toxin dose. Fast diarrhoea onset has been reported as early as 25-30 min after toxin oral treatment. Compared with oral gavage, voluntary food ingestion of OA-group toxins is less stressful to the animal, less technical demanding for the operator, without any loss in drug uptake and efficacy and with similar onset of symptoms.

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PO-006

Epigenetic modifications implicated in idiosyncratic drug-induced liver injury

Villanueva-Paz M.[1], de los Santos-Fernández R.[1], Álvarez-Álvarez I.[1,2], Niu H.[1,2], Sanabria-Cabrera J. [1,3], Stephens C.[1,2], González-Jiménez A.[1], Matilla-Cabello C.[1], Medina-Cáliz I.[1]*, Andrade R.J.[1,2*], Lucena M.I.[1,2,3]*.

[1] Servicios de Aparato Digestivo y Farmacología Clínica, Hospital Universitario Virgen de la Victoria, Instituto de Investigación Biomédica de Málaga y Plataforma en Nanomedicina-IBIMA Plataforma BIONAND, Universidad de Málaga, Málaga, Spain.
[2] Centro de Investigación Biomédica en Red Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain.
[3] Plataforma de Investigación Clínica y Ensayos Clínicos UICEC-IBIMA, Plataforma ISCIII de Investigación Clínica, Madrid, Spain.

E-mail: rldlsf@hotmail.com

Background: Drug-induced liver injury (DILI) is a complex pathology involving pharmacological, genetic, and environmental factors. This study aimed to analyze the genome methylation status during an idiosyncratic DILI episode in a cohort of well-defined DILI cases from the Spanish DILI Registry.

Material and methods: DNA from peripheral blood samples was extracted from 32 well-characterized DILI cases enrolled in the Spanish DILI Registry and 32 healthy controls. Genome-wide methylation analysis was performed by bisulfite conversion using the EZ-96 DNA Methylation kit (Zymo Research, Irvine, CA). DNA methylation analysis was carried out by microarray assays using Infinium MethylationEPIC BeadChip Kit (Illumina, San Diego, CA). Whole-genome amplification and hybridization were done with BeadChip microarray (Illumina). Cytosine methylation state was assessed by single-base extension and analysis using the HiScan SQ module (Illumina). DNA methylation for each CpG site was represented by beta values ranging from 0 to 1, corresponding to fully unmethylated and fully methylated. Finally, an analysis of differentially methylated regions between groups was performed.

Results: Control and DILI groups samples showed similar beta values density distribution. A total of 43861 CpG sites were identified (FDR $\leq$ 0.05), from which 213 manifested significative differential methylation ($|\Delta\beta| \geq$ 0.1 and FDR $\leq$ 0.05) between groups with an overall tendency towards hypomethylation within the DILI cohort. Candidate genes with the most significative differentially methylated CpG sites between groups were identified, resulting in 14 hypomethylated genes (ZNF350-AS1, LOC349408, P2RY13, GPR109B, SUMO1B1, LOC285626, FAM200B, LOC284276, TEX28, STS, KLRK1, LOC101928100, TLR8, CSTA) and one hypermethylated gen (FAM163B) in DILI group compared to the control group.

Conclusions: This first exploratory analysis has shown a general tendency towards hypomethylation within the DILI cohort compared to the control group. Further research must be conducted to unveil the relationship between DNA methylation and DILI phenotypes.

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CANCER

PO-007

Dendritic Ruthenium-based Anticancer Nanosystems: multi-step synthesis procedures coming to life.

Nunes[1], N., Shi[1,2], X., Rodrigues[1], J.

[1]CQM-Centro de Química da Madeira, MMRG, Universidade da Madeira, Campus da Penteada, 9020-105 Funchal, Portugal
[2]College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai, 201620, China

E-mail: joaor@uma.pt

The interplay of nanotechnology and chemotherapy enabled us previously [1,2] to synthesize and incorporate a promising ruthenium-based anticancer drug (RuCp) onto polynitrile Poly(alkylidenimine) and, later, onto PAMAM dendritic scaffolds, producing a family of metallodendrimers as pre-clinical anticancer candidates with potent antitumor efficacy, specificity to a broad set of tumors, and good biosafety.
In brief, our *in vitro* results have shown that the PAMAM G3-metallodendrimer with thirty-twoterminal Ru-moieties (G3-CNRu) was the most cytotoxic compound for all the tested cancer cell lines, including the one with acquired cisplatin resistance (A2780*cis*R), with IC_{50} values ranging from 14 to 30 nM. In contrast, it was ≈ 5 to 11-fold less toxic for the human BJ fibroblasts and non-toxic for human erythrocytes. The *in vivo* studies confirmed the antitumor efficacy of G3-CNRu on a metastatic breast tumor model (4T1). This metallodendrimer presents good biosafety and mainly accumulates inside the tumor, highly inhibiting its growth (71.6% of inhibition rate). Furthermore, an apoptosis rate of 56% was found in tumor sections.On the other hand, throughout *in vitro* testing, the identified breast cancer (MCF-7 cells) cell death promoted by G3-CNRu relied on the stress-induced premature senescence triggered by high levels of reactive oxygen species and cell cycle arrest in the G0/G1 phase, which indicatesthat its mechanism of action possibly involves multiple targets. In view of these results, two multi-step synthesis procedures were devised to prepare new ruthenium-based dendritic nanosystems with enhanced stability for long-term clinical formulations. These procedures aime to produce novel acetylide RuCp-PAMAM metallodendrimers and dendrons with great potential as highly effective anticancer drugs. So far, we have additionally prepared by the divergent method the polyacetylide RuCp metallodendrimer with eight terminal groups (G0- CCRu) after optimizing six sequential reactions and isolating four dendrimers during the process (G0-Boc, G0-OH, G0-BrPy, G0-CCH). As a result, good reaction yields (49 – 93%) were obtained for each purified compound, validating the efficiency of the designed multi-stepsynthesis procedure. In addition, all the compounds (nitrile and acetylide RuCp-PAMAM metallodendrimers and dendrons) were characterized by 1D/2D-NMR, FTIR, and MALDI- TOF MS. With this optimized multi-step synthesis procedure, higher generations of this acetylide RuCp-PAMAM metallodendrimers having sixteen and thirty-two active groups can now be prepared.

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PO-008

A diet lacking selenium induces anticancer activity in mice with metastasis

Patricia Díaz-Ortega, José Manuel Calderón-Montaño, Julio José Jiménez-Alonso, Emilio Guillén-Mancina, Víctor Jiménez-González, Estefanía Burgos-Morón, and Miguel López-Lázaro*

Department of Pharmacology, Faculty of Pharmacy, University of Seville, 41012 Sevilla, Spain

E-mail: patriciadiort@gmail.com

The essential micronutrients selenium, zinc, copper, and manganese are part of the enzymes peroxidases, thioreductases and superoxide dismutases. These enzymes play a key role in the elimination of reactive oxygen species such as hydrogen peroxide (H_2O_2). Since cancer cells produce higher levels of H_2O_2 than normal cells, and H_2O_2 induces cell death above a certain threshold, cancer cells are particularly vulnerable to strategies that further increase the cellular levels of H_2O_2 (1). If we temporarily eliminate selenium, zinc, copper, or manganese from the diet, the cellular levels of H_2O_2 may increase and cause selective toxicity towards cancer cells. To evaluate the in vivo anticancer activity of this strategy, we prepared 6 artificial diets from scratch, which contained normal levels of proteins (20%) and lipids (7%) but lacked each of these micronutrients. The control diet contained all micronutrients, diet C-S lacked selenium, diet C-Z lacked zinc, diet C-C lacked copper, diet C-M lacked manganese and diet C-SZCM lacked all four micronutrients. The anticancer activity of these diets was evaluated in an ovarian cancer model, which was established by injecting 5×10^6 ID8 *Trp53*$^{-/-}$ murine ovarian cancer cells into the peritoneal cavity of female C57BL/6JRj mice (2). Treatments started 15 days after the inoculation of the cancer cells. Treatments simply consisted of replacing their normal diet with one of the artificial diets for several weeks. Mice survival was markedly improved when their normal diet wasreplaced with the diet lacking selenium. Diets lacking zinc, copper or manganese did not significantly change mice survival. The diet lacking all four micronutrients increased mice survival,but only mildly. The anticancer activity of the artificial diet lacking selenium was confirmed in mice with metastatic colon cancer (BALB/cAnNRj mice inoculated in the tail vein with 10^5 CT26.WT cells) and in mice with metastatic triple negative breast cancer (female BALB/cAnNRj mice inoculated in the tail vein with 10^5 4T1 cancer cells) (3). These data suggest that diets lacking selenium have cancer therapeutic potential.

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PO-009

PAMAM and PPI dendrimers as nanocarriers for rose bengal in photodynamic therapy

Sztandera K.[1,2,3], Klajnert-Maculewicz B.[3]

[1] *CIBER, Instituto de Salud Carlos III, 28031 Madrid, Spain;*
[2] *Unidad Asociada Neurodeath,Facultad de Medicina, Universidad de Castilla-La Mancha, 02006 Albacete, Spain*
[3] *Department of General Biophysics, University of Lodz, Lodz, Poland*

E-mail: krzysztof.sztandera@uclm.es

Photodynamic therapy (PDT) is an alternative method for treating skin cancer that significantly reduces the side effects associated with commonly used techniques such as radiotherapy or chemotherapy. PDT involves the simultaneous use of a photosensitizer, a light source of an appropriate wavelength for activating the photosensitizing agent, and molecular oxygen [1]. When combined, these components generate reactive oxygen species leading to cell death [2]. One of the most important factors determining the success of the therapy is the photosensitizer. Its therapeutic effect may be limited by low solubility and specificity of the drug or insufficient accumulation within the tumor [3].

In the study we focused on evaluation of nanosystems composed of poly(amidoamine) PAMAM or poly(propyleneimine) PPI dendrimers with rose bengal (RB) in terms of: (a) biophysical properties, such as hydrodynamic diameter, zeta potential, spectral properties and also the ability to form complexes with RB; (b) singlet oxygen generation, intracellular reactive oxygen species production, intracellular transport and phototoxic properties

The results showed that the generation- and structure-dependent binding of the dye by the dendrimers increased the cellular uptake and production of singlet oxygen and intracellular reactive oxygen species, leading to an increase in phototoxicity of rose bengal.

In conclusion, cationic PAMAM and PPI dendrimers can serve as efficient carriers of RB in photodynamic therapy. Due to their structural properties, the patterns of interaction with RB, and the characteristic features of the dendrimer complexes with RB complexes, PPI dendrimers outperform PAMAM dendrimers by exhibiting the most efficient uptake in the case of PPI G4 and significantly increasing generation of singlet oxygen in the case of PPI G3.

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CARDIOVASCULAR

PO-010

Resolvin E1 mitigates doxorubicin-induced endothelial senescence through the modulation of NLRP3 inflammasome activity

Shamoon, L.[1,2] Espitia-Corredor, JA.[1,2,3] Dongil, P.[1,2] Menéndez-Ribes, M.[1,2] Romero, A.[1,2] Valencia, I.[1,2] Díaz-Araya, G.[3,4] Sánchez-Ferrer, CF.[1,2] Peiró,C.[1,2]

1 Fac. Medicine, Universidad Autónoma de Madrid, Madrid, Spain.2 Instituto de Investigaciones Sanitarias (IdiPAZ), Madrid, Spain.
3 Fac. Chem. Sci. and Pharm., Universidad de Chile, Santiago, Chile.
4 Advanced Center for Chronic diseases ACCDiS, Universidad de Chile, Santiago, Chile.

E-mail: licia.shamoon@uam.es

Vascular aging is associated with endothelial cell senescence, favouring low-grade inflammation, endothelial dysfunction, and cardiovascular diseases(1). Cell senescence arises from a wide variety of endogenous and exogenous stressors including some anticancer agents such as doxorubicin(2). Recently, doxorubicin was linked to the innate immunity component NLRP3 inflammasome which is implicated in many vascular inflammatory disorders(3). Thereis a need for therapeutic tools to help cancer patients who have been exposed to cardiovascular toxic chemotherapy to avoid premature vascular complications. We investigated whether resolvin E1 (RvE1), an endogenous lipid mediator of the inflammation resolution phase(4), could prevent doxorubicin-induced senescence in cultured human umbilical veins endothelial cells (HUVEC) with focus on a potential involvement of the NLRP3 inflammasome.

Cell senescence was quantified by senescence-associated-β-galactosidase (SA-β-gal) staining. The expression of senescence markers (γH2AX, p21, p53) and inflammatory markers (pP65, NLRP3) was determined via Western blot. NLRP3 inflammasome activation was analysed by visualizing the formation of ASC specks by indirect immunofluorescence.

Doxorubicin (25 nmol/L) augmented the number of SA-β-gal positive HUVEC and the levels of γH2AX, p21, and p53 which were all reduced by RvE1 (10 nmol/L). In doxorubicin- treated cells, RvE1 further reduced the expression of pP65 and NLRP3 proteins and the formation of ASC specks as did the inflammasome assembly inhibitor MCC950 (1µmol/L). Additionally, both MCC950 and interleukin-1 receptor inhibitor anakinra diminished SA-β- gal positive staining induced by doxorubicin.

RvE1 offers a novel therapeutic approach against doxorubicin-induced cardiovascular toxicity and subsequent age-related vascular disorders by counteracting endothelial senescence through the modulation of NLRP3-inflammasome activation.

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PO-011

miRNA Cluster miR-99b/let-7e/miR-125a as Modulator of Endothelial Function in Myocardial Infarction

Hermenegildo, C.[1,2], Mompeón, A.[1], Paes, A.B.[2], Rosales-Ariza, C.[1], Descals-Beltrán, B.[2], Pérez-Cremades, D.[1,2], Dantas, A.P.[3], Novella, S.[1,2]

[1]University of Valencia, [2]INCLIVA Biomedical Research Institute, Valencia, [3]University of Barcelona, Barcelona, Spain.

E-mail: carlos.hermenegildo@uv.es

MicroRNAs (miRNA) are related to intercellular communication and have been associated to cardiovascular diseases. In a previous study (*Mompeón et al., 2022. Cells 11: 1823*), we analysed the miRNA expression profile in non-ST-segment myocardial infarction (NSTEMI) patients and confirmed a decrease in circulating levels of let-7e-5p during the acute phase of NSTEMI, which subsequently reverted to control levels after one-year follow-up. Our aim is to study the expression of circulating miRNA within the miR-99b/let-7e/miR-125a cluster and evaluate their function in endothelial cells where intercellular communication involving circulating molecules is especially relevant.

Total circulating RNA was isolated from 400 µL aliquot of non-haemolysed serum from control (n = 41), acute phase of NSTEMI (n = 46), and one-year follow-up (n = 24) patients using the miRCURY RNA Isolation Kit for Biofluids (Exiqon) following the manufacturer's instructions. TaqMan miRNA Reverse Transcription Kit was used for reverse transcription (RT) and TaqMan MicroRNA Assays (Applied Biosystems) for amplification by qPCR. The expression was calculated according to the $2^{-\Delta\Delta Ct}$ method. To assess the endothelial function, we analyse the adhesion, proliferation and vasculogenesis of human umbilical vein endothelial cells HUVEC (Lonza) transfected with miRNA inhibitors and mimics (Qiagen).

Our results show a decrease in the levels of all miRNA composing the cluster miR-99b/let-7e/miR-125a ($p < 0.05$) in serum from NSTEMI patients in the acute phase in comparison with control. After one-year follow-up the circulating levels of these miRNA were reverted to control levels. In cell culture, the miRNAs were expressed in endothelial cells, suggesting a role in cell to cell communication. HUVEC transfected with let-7e-5p mimic showed a 20% increase in adhesion capacity and let-7e-5p inhibitor increased the number of tube-like structures, whereas miR-125a-5p inhibitor decrease HUVEC proliferation. miR-99b-5p did not change the analysed endothelial functions.

Thus, the components of miR-99b/let-7e/miR-125a cluster are related to the acute manifestation of NSTEMI, and let-7e-5p and miR-125a-5p affect the endothelial function in a different way, indicating different roles of each miRNA of the same cluster in controlling endothelial processes. As each miRNA can regulate multiple genes, it is important to understand their regulation, effects and biological functions of miRNA clusters.

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PO-012

Vascular smooth muscle cells-derived extracellular vesicles are conveyors of NLRP3 inflammasome and mediate inflammaging in diabetes

Valencia I[1], San Hipólito-Luengo A[1], Martínez MC[2], Andriantsitohaina R[2], Sánchez-Ferrer CF[1], Peiró C[1].

[1]Department Pharmacology and Therapeutics, School of Medicine, Universidad Autónoma de Madrid, Spain.
[2] PhyMedExp, Vésicules extracelullaires et maladies métaboliques, University of Montpellier, INSERM, CNRS, Montpellier, France

E-mail: inesvalencia.5@gmail.com

NLRP3 inflammasome and its main product interleukin (IL)-1β are relevant contributors to thechronic inflammation underlying atherosclerosis and cardiometabolic diseases. Considering that several of the IL-1β-induced effects are exacerbated in hyperglycemic conditions and that cardiovascular risk may be shrinked by inhibiting this cytokine, IL-1β is presented as a promising pharmacological target in diabetes.

We have previously observed that IL-1β directly induces inflammation in human vascular smooth muscle cells (HASMC) and promotes cell senescence in human umbilical vein endothelial cells (HUVEC). However, whether IL-1β-induced inflammaging response could be spread between the layers of the vascular wall is unknown. Extracellular vesicles (EVs), key mediators of intercellular communication, have been growingly recognized as biomarkers and mediators of vascular dysfunction in human disease. Thus, we aimed to characterize the EVs derived from HASMC (HASMC-EVs) in response to IL-1β and high glucose to evaluate theirrole as potential mediators of inflammaging in the vasculature.

In response to IL-1β and hyperglycemic conditions, both HASMC-derived large EVs (100-1000 nm) and small EVs (30-150 nm) were isolated by differential ultracentrifugation, and theircontent in NLRP3 inflammasome components was evaluated. Naïve HASMC and HUVECwere exposed to lEVs and sEVs and inflammatory and pro-senescence effects were analysed. HASMC-lEVs and sEVs concentration were significantly increased by 3-fold and 2-foldrespectively in response to IL-1β, which was further intensified in presence of high glucose.Treatment of naïve HASMC with EVs from IL-1β-treated HASMC induced paracrine inflammation and fully activation of NLRP3 inflammasome. Furthermore, HUVEC exposed toHASMC-EVs undergone senescence. Differences in the cargo of lEVs and sEVs were identified, with mature IL-1β only detected within sEVs. Accordingly, we observed that bothautocrine and paracrine sEVs effects could be prevented by IL-1 receptor blocker anakinra.

Taken together, our results show that IL-1β induce the release of NLRP3 inflammasome- enriched EVs from HASMC, perpetuating inflammaging in an autocrine and paracrine manner within the cellular layers of the vascular wall.

PO-013

Angiotensin II Receptor Blockers Reduce Tau/Aβ42 Ratio: A Cerebrospinal Fluid Biomarkers' Case – Control Study

García-Lluch, G[1,2]; García-Zamora, M[1,2]; Peña-Bautista, C[1]; Moreno, L[2,3]; Baquero, M[1,2,4]; Cañada-Martínez, AJ[5]; Álvarez, L[1,4]; Ferré, L[1]; Pardo, J[2,6] and Cháfer-Pericás, C[1,2]

1 Research Group in Alzheimer Disease, Instituto de Investigación Sanitaria La Fe, 46026 Valencia, Spain
2 Cátedra DeCo MICOF-CEU UCH, Universidad Cardenal Herrera-CEU
3 Department of Pharmacy, Universidad Cardenal Herrera-CEU
4 Neurology Unit, Hospital Universitari i Politècnic La Fe
5 Data Science and Biostatistics Unit, Health Research Institute La Fe
6 Department of Mathematics, Physics and Technological Sciences, Universidad Cardenal Herrera – CEU

E-mail: *Mar.garcia1@alumnos.uchceu.es*

The role of antihypertensives in Alzheimer's Disease (AD) prevention is controversial. This case-control study aims to assess whether antihypertensive medication has a protective role in AD. For this purpose, the association between this drugs and abnormal levels of amyloid and tau has been studied. Furthermore, it suggests a holistic view of the involved pathways between renin-angiotensin drugs and the tau/amyloidß42 ratio (tau/Aß42 ratio).
The medical records of the participant patients were reviewed, with a focus on prescribed antihypertensive drugs and clinical variables, such as arterial blood pressure. The patients were divided in two groups depending on the CSF biomarkers levels. On the one hand, patients with AD diagnosis (cases) and, on the other hand, cognitively healthy patients (control). Each drug was classified by The Anatomical Therapeutic Chemical classification.
The total number of participants was 280, 223 in the AD group and 57 in the control group. The study shows that age and high systolic blood pressure are associated with a higher risk of developing AD. In addition, combinations of angiotensin II receptor blockers are associated with a 30% lower t-tau/Aß42 ratio than plain angiotensin-converting enzyme inhibitor consumption.
In conclusion, angiotensin II receptor blockers may play a potential role in neuroprotection and AD prevention. Likewise, several mechanisms, such as the PI3K/Akt/GSK3ß or the ACE1/AngII/AT1R axis, may link cardiovascular pathologies and AD presence, making its modulation a pivotal point in AD prevention. The present work highlights the central pathways in which antihypertensives may affect the presence of pathological amyloid and tau hyperphosphorylation.

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PO-014

Vorinostat is neuroprotective in hypertensive stroke promoting central and peripheral vessel protection

Díaz-Pérez. A[1,2,3], Pérez. B[1,3], Navarro. X[2,3], Penas. C[2,3], Jiménez-Altayó. F[1,3]

[1] Departament de Farmacologia, Terapèutica i Farmacologia, Facultat de Medicina, Universitat Autònoma de Barcelona
[2] Departament de Biologia Cel·lular, Fisiologia i Inmunologia, Grup de Neuroplasticitat i Regeneració, Facultat de Medicina, CIBERNED, TERAV, Univesitat Autònoma de Barcelona
[3] Institut de Neurociències, Univesitat Autònoma de Barcelona

E-mail: andrea.diaz@uab.cat

Stroke is a major global health disease which constitutes the second leading cause of worldwide death and the third cause of disability. An elevated percentage of stroke global burden is attributable to risk factors, especially hypertension.[1] Currently, endovascular thrombectomy and/or intravenous administration of recombinant tissue plasminogen activator are the only available approved treatments, but have several side effects and less than a half of patients can benefit from these therapies. Histone deacetylases inhibitors have emerged as potential novel therapeutic agents to treat acute injuries to the central nervous system. In particular, Vorinostat (*Suberoylanilide hydroxamic acid*), which reduces histone acetylation levels, has been postulated as a potential neuroprotective drug after ischemic brain damage.[2] Hence, the aim of this study is to provide evidence on the potential neuro- and vasculoprotective effects of Vorinostat (50mg/kg; i.p.) in spontaneously hypertensive rats (n=60) submitted to transient middle cerebral artery occlusion (90 minutes occlusion/ 24 hours reperfusion). The drug was administered at different time points during reperfusion (1, 4 and 6 hours). Infarct volume was assessed by 2,3,5-triphenyltetrazolium chloride staining and functional outcome was evaluated by a nine-point neurological test and the rotarod assay. The histone 3 acetylation levels were assessed by Western blot. Main pathophysiological mechanisms involved in stroke damage such as oxidative stress and cytokine profile were studied in brain and plasma. Angiogenesis was studied in Matrigel-cultured middle cerebral arteries. Moreover, structural, mechanical, and myogenic properties of mesenteric resistance arteries were studied "*ex vivo*" using pressure myography. In addition, an analysis of nuclei distribution by confocal microscopy and total collagen content by picrosirius red staining was performed in these arteries. Results show that Vorinostat induced an increase of histone 3 acetylation levels during 6 hours after its administration. After ischemia/reperfusion, Vorinostat was neuroprotective within 4 hours after the onset of stroke, showing a significative reduction of brain infarct (*Total Infarct, mm^3, n=7-8, Mean ± SEM; Veh: 239,9±50,76; Vor1h: 81,86±32,81; Vor4h: 41,00±20,93; Vor6h: 160,2±56,30*) and neurological disability at 24 hours after the ischemic insult. Mechanistically, the treatment reduced oxidative stress and inflammation levels in the brain and plasma, inducing a favourable cytokine profile. Furthermore, Vorinostat reverted the altered angiogenic response and prevented the hypertrophic remodelling induced by ischemia/reperfusion in mesenteric resistance arteries. These effects were mediated by the ability of the treatment to mitigate the increased arterial collagen accumulation after stroke. Taken together, the present study suggests that Vorinostat is an encouraging neuro- and vasculoprotective drug to treat hypertensive ischemic stroke-induced damage.

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PO-015

Erythrocyte-derived extracellular vesicles from type 2 diabetes patients induce endothelial dysfunction through arginase 1

Collado, A[1]; Humoud, R[1]; Eldh, M[1]; Jiao, T[1]; Domingo, E[2,3]; Kontidou, E[1]; Yang, J[1]; Mahdi, A[1]; Gabrielsson, S[1]; Eriksson, P[1]; Zhou, Z[1], and Pernow, J[1,4]

[1]Karolinska Institute, Stockholm, Sweden; [2]University of Valencia, Valencia, Spain; [3]Institute of Health Research INCLIVA, University Clinic Hospital of Valencia, Valencia, Spain; [4]Karolinska University Hospital, Stockholm, Sweden.

E-mail: aida.collado.sanchez@ki.se

The mechanisms driving the development of cardiovascular injury in type 2 diabetes (T2D) remain incompletely understood. We recently demonstrated that red blood cells (RBCs) from patients with T2D (T2D-RBCs) act as mediators of endothelial dysfunction, but the mechanisms underlying this interaction are not clarified. We found that RBCs via upregulation of arginase 1 attenuate nitric oxide bioavailability and endothelial function. It is increasingly clear that extracellular vesicles (EVs) are actively secreted by all cell types, including RBCs, and represent a novel mechanism of intercellular communication. This study aimed to determine whether EVs derived from T2D-RBCs are involved as mediators in vascular injury through the signaling of arginase 1.

T2D-RBCs and RBCs from age-matched healthy controls (H-RBCs) were isolated and incubated with Krebs-Henseleit buffer (20% hematocrit). Following 18h incubation, the conditioned medium was collected for EV isolation using sequential ultracentrifugation and membrane affinity column. EV concentration was measured by nanoparticle tracking analysis. Aortas isolated from wild-type mice were incubated with EVs derived from T2D-RBCs and H-RBCs for 18h. Endothelium-dependent and -independent relaxation (EDR and EIDR, respectively) of the aortas were evaluated in a wire myograph. The involvement of arginase was investigated by the addition of the arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH) either to the 18h co-incubation of EVs with the aortic segments to selectively investigate the contribution of EV-derived arginase (ABH, 10 mM) or to the aortas following the 18h co-incubation to selectively target vascular arginase (ABH, 100 μM).

The concentration of RBC-derived EVs from T2D patients (T2D-RBCs EVs) was ten times lower than those from healthy controls. T2D-RBCs EVs significantly impaired EDR, whereas EIDR was not affected. This effect was observed irrespective of if the same volume or concentration of EVs were administered. Inhibition of the uptake by heparin during the co-incubation prevented the impairment of EDR induced by T2D-RBCs EVs. The uptake of T2D-RBCs EVs by endothelial cells was 2-fold greater than that of EVs from H-RBCs, and this uptake was inhibited by the addition of heparin. Inhibition of arginase with ABH during the co-incubation with EVs completely prevented the impairment of EDR induced by the T2D-RBCs EVs. Administration of ABH to the aortas following the co-incubation also attenuated the impairment of EDR. Further, immunohistochemical staining revealed upregulation of arginase 1 but not arginase 2 in the vasculature following incubation with T2D-RBCs EVs.

Overall, T2D-RBCs EVs induce endothelial dysfunction. In addition to the increased uptake of EVs in endothelial cells, the signaling behind this effect of EVs on endothelial function is mediated by arginase 1. These results shed new important light on the mechanism underlying vascular injury mediated by RBCs in T2D.

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PO-016

Neurovascular alterations in a mouse model of Marfan syndrome, a connective tissue disease, are greater in females than males

Manich, G.[1], Crespí, MM.[1], Sánchez-Bernadó, P.[2], Pérez, B.[2], Díaz-Matés, B.[2], Rodríguez-Rovira, I.[3], Rojas, S.[1], Egea, G.[3], Jiménez-Altayó, F.[2]

[1]Department of Morphological Sciences, School of Medicine, Universitat Autònoma deBarcelona, Cerdanyola del Vallès, Spain
[2]Department of Pharmacology, Toxicology and Therapeutics, Neuroscience Institute, School of Medicine, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[3]Department of Biomedical Sciences, University of Barcelona, School of Medicine, and HealthSciences and IDIBAPS, Barcelona, Spain

E-mail: francesc.jimenez@uab.cat

Background: Mutations in the fibrillin-1 (FBN1) gene lead to connective tissue disease and are the major cause of Marfan syndrome (MFS), an autosomal dominant rare disease happeningin about 1 in 5,000 people. MFS can lead to life-threatening aortic aneurysm development, especially in men, but encompasses other multiple manifestations that are less explored. Patients with MFS are suspected to be at higher risk for cerebrovascular alterations than the general population, but contradicting clinical data and the absence of solid preclinical evidencemakes difficult to confirm the true involvement of neurovascular problems in these patients. In addition, it is not known how sex may impact cerebrovascular alterations in MFS. **Material and methods:** We used 3- to 13- months-old male (n = 21) and female (n = 21) mice harboringthe most frequent mutation (*Fbn1*$^{C1039G/+}$) found in MFS patients compared to age- and sex- matched wild-type (WT) C57BL/6 mice (male, n = 35; female n = 20). We studied: i) basilar artery angiography and brain ventricles anatomy by Time-of-Flight magnetic resonance imaging; ii) brain and plasma superoxide anion and malondialdehyde levels by high performance liquid chromatography and a colorimetric assay, respectively; iii) brain inflammation (glial fibrillary acidic protein, GFAP, and Iba-1) by immunostaining; iv) basilar artery properties by pressure myography; and v) basilar artery nuclei distribution and total collagen content by confocal microscopy and picrosirius red staining, respectively. **Results:** There were no differences in body weight, blood pressure, and macroscopic basilar artery morphology between WT and MFS mice at any age. The volume of brain lateral ventricles wassignificantly smaller in 3-months-old MFS *versus* WT female mice. From 3 to 13 months of age, despite a decrease in brain oxidative stress levels was observed, brains from MFS mice showed significantly higher GFAP expression indicative of astrocyte activation, whereas Iba-1(microglia) expression was not altered. Notably, MFS females showed a broader range of increased GFAP immunoreactivity across different several brain regions (cortex, striatum, hippocampus, corpus callosum) as compared to MFS males (cortex, corpus callosum). Basilararteries from 3-months-old MFS females showed hypertrophic remodeling, whereas no differences were observed in males at any age. These alterations in MFS females were ascribedto increases in adventitial volume and were attenuated at 13 months, at least in part, because ofa parallel increase in arterial wall distensibility. **Conclusions**: Altogether, these results show the presence of neurovascular alterations in a mouse model of MFS at the early stage of the disease, which are more pronounced in female mice. This evidence supports the necessity to investigate the mechanisms involved in neurovascular disease susceptibility, the determinants of sex differences, and the potential pathological implications.

PO-017

The probiotic *Lactobacillus fermentum* CECT5716 increases the antihypertensive response of hydrochlorothiazide in spontaneously hypertensive rats

González-Correa, C[1,2]; Miñano, S[1]; Moleón, J[1,2]; Toral, M[1,2,3]; Robles-Vera, I[4]; Sánchez, M[1,2]; Jiménez, R[1,2,3]; Olivares, M[5]; Romero, M[1,2]; Gómez-Guzmán, M[1,2]; Duarte, J[1,2,3]

[1]University of Granada, Granada, Spain. [2]Instuto de Investigación Biosanitaria de Granada, ibs. Granada, Granada, Spain. [3]Ciber de Enfermedades Cardiovasculares (CIBERCV), Spain. [4]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain. [5]Biosearch Life, Granada, Spain

E-mail: cristinagoncor@gmail.com

Hypertension is the main cardiovascular risk factor and ten to thirty percent of hypertensive subjects exhibit drug-resistant hypertension[1]. Hypertension has been shown to be linked to gut dysbiosis, which was characteristic of both rodent hypertensive models and human hypertensive patients. It is known that orally administration of *Lactobacillus fermentum* CECT5716 (LC40) reduced hypertension and endothelial dysfunction development in spontaneously hypertensive rats (SHR) by increased butyrate-produced bacteria, gut integrity and permeability and restored the Th17/Tregs balance in mesenteric lymph nodes[2]. Endotoxemia derived from leaky gut, as result of higher gut sympathetic drive, seems to be involved on the development of hypertension. Evidence in SHR supports the action of the ACE inhibitor captopril[3] and angiotensin II type 1 receptor blockers losartan[4] may be partially dependent on the gut microbiota. These first-line antihypertensive drugs reduced sympathetic tone, improved gut integrity and gut dysbiosis. However, it is unknown if diuretics, such as hydrochlorothiazide (HCTZ), improved gut integrity and reduced endotoxemia. We hypothesize that LC40 might improve gut integrity and increase the antihypertensive effects of HCTZ. Twenty-weeks-old male SHR were randomly divided in 6 groups (n=8): a) untreated SHR (SHR, 1 mL of vehicle day^{-1}), b) SHR treated with LC40 10^9 CFU day^{-1} (SHR-LC40), c) SHR treated with HCTZ10 mg kg^{-1} day^{-1} (SHR-HCTZ10), d) SHR treated with HCTZ 50 mg kg^{-1} day^{-1} (SHR-HCTZ50), e) SHR treated simultaneously with HCTZ 10 mg kg^{-1} day^{-1} and LC40 10^9 CFU día^{-1} (HCTZ10+LC40), f) SHR treated simultaneously with HCTZ 50 mg kg^{-1} day^{-1} and LC40 10^9 CFU día^{-1} (HCTZ50+LC40). Both LC40 and HCTZ were administered by oral gavage during five weeks. As expected LC40 induced a small drop in systolic blood pressure (SBP), reduced gut sympathetic drive and improved gut integrity. HCTZ, in a dose-dependent manner improve endothelial dysfunction and reduced SBP but did not reduce the gut sympathetic tone (higher colonic levels of noradrenaline). Co-administration of HCTZ, at both doses, with LC40 triggered a further reduction in SBP. Additionally, aortae from treated groups showed increased endothelium-dependent vasodilation to acetylcholine, which was further increased by co-administration with LC40, and linked with higher reduction of NADPH oxidase activity. In addition LC40 reduced the colonic NA content. In conclusion, we have found for the first time that probiotic LC40 co-administration increased antihypertensive effect of HCTZ, at least in part, by endothelial dysfunction improvement.

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PO-018

Mitochondrial oxidative stress, endothelial dysfunction and enhanced mitochondrial respiration of kidney preglomerular arteries in obesity.

Contreras, C.[1], Muñoz M.[1], Gómez del Val A.[1], Sánchez A.[1], Olmos L.[1], Rodríguez-Prados, C[1]., Fernandes V.S.[1], Benedito S.[1], Rivera L.[1], Sáenz-Medina J.[2], Prieto D.[1]

[1]Complutense University of Madrid, Madrid, Spain. [2]University Hospital Puerta de Hierro-Majadahonda, Madrid, Spain.

E-mail: *criscont@ucm.es*

Obesity is a public health problem of increasing prevalence worldwide, and it is now recognized as a risk factor for the development of chronic kidney disease independent of other comorbidities such as diabetes and hypertension (Wang et al, 2008; de Vries et al., 2014). Mitochondria, organelles producing energy as ATP via oxidative phosphorylation, are the main source of reactive oxygen species (ROS) in the cell, and increased production of mitochondrial ROS (mtROS) has been postulated as a cause of metabolic and vascular disorders (Forte et al., 2019). On the other hand, the kidney is an organ with a high energy demand and subsequent high mitochondrial activity to obtain ATP through fatty acid β-oxidation. Accordingly, mitochondrial dysfunction has recently been shown to play an important role in obesity-related kidney disease (Zseto et al., 2016; Tang et al., 2016), while increased oxidative stress is involved in renal endothelial dysfunction in obesity (Muñoz et al., 2019). While some researchers have demonstrated that mitochondrial ROS can promote renal injury resulting in decreased mitochondrial energy metabolism (Zhao et al., 2021), others have reported preserved mitochondrial respiration in obesity while augmented mitochondrial ROS production (Ruggiero et a., 2011). Involvement of mitochondrial respiration in kidney vascular function in obesity is not completely understood. Therefore, the aim of this work was to assess whether mitochondria play a role in renal vascular dysfunction associated to diet-induced obesity.

Male Wistar rats were fed either a high fat diet (HFD, 60% lipids) or a standard diet (STD, 4% lipids) in control animals. Renal endothelial function was assessed by the relaxing responses to the endothelial agonist acetylcholine (ACh) in preglomerular interlobar arteries precontracted with phenylephrine, with or without the mitochondrial antioxidant MitoTempo (0.01 µM) in order to assess the invol vement of mtROS. mtROS production in renal arteries was determined by using the fluorescent dyes MitoSox and AmplexRed. Mitochondrial oxygen consumption ratio (OCR) and extracellular acidification rate (ECAR) were measured with an Agilent Seahorse XF Pro analyzer and a Mito Stress Assay (Oligomycin, 50 µM; FCCP, 50 µM; and Rotenone/Antimycin A, 10 µM) in intact preglomerular kidney microarteries.

Endothelial relaxation induced by ACh was impaired in renal arteries of HFD rats, an effect that was reversed by incubation with mitoTempo, thus suggesting the production of mtROS with a vasoconstrictor effect. MitoSox results showed that mtROS were increased in renal arteries of HFD rats, but not in renal cortex, while the AmplexRed data showed that H_2O_2 levels were decreased, both in arteries and renal cortex of HFD. In line with the elevated mtROS production, Mito stress assay demonstrated that OCR was augmented in renal arteries from obese animals, with increased basal respiration, basal ATP production, and maximal respiration, that were associated to ECAR rise in HFD preglomerular arteries.

These results suggest that there is a compensatory increase in mitochondrial respiration in kidney preglomerular arteries from obese animals, resulting in altered REDOX balance, with an overproduction of contractile superoxide anion ($O_2^{-\bullet}$) of mitochondrial origin, and a decreased production of relaxing H_2O_2, which might contribute to endothelial dysfunction in kidney disease associated to obesity.

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PO-019

Activation of mitoK$_{ATP}$ channels induces vasodilation and inhibits mitochondrial respiration mediated by endothelial NO in penile arteries

Gómez del Val A., Contreras, C., Muñoz M., Rodríguez, C, Olmos L., Rivera L., Sánchez A.[1], Prieto D.[1]

[1]*Complutense University of Madrid, Madrid, Spain.*

Mitochondrial ATP-sensitive K^+ channels (mitoK$_{ATP}$) located in the inner mitochondrial membrane are involved in the protection of tissues from ischemia and oxidative stress, and its activity is regulated by endogenous factors such as the ADP/ATP ratio, peroxynitrite and superoxide anions (*Paggio et al., Nature. 2019; 572: 609*). Penile erection is a complex neurovascular event initiated by sexual stimuli releasing NO from parasympathetic nerves and relaxing erectile tissue, with a subsequent increased blood-induced shear stress that further releases endothelial NO to sustain erection (*Prieto et al., Int J Impot Res 2008, 20 (1): 17*). In addition to its essential role in the control of blood flow, NO has been demonstrated to regulate O_2 consumption in skeletal and cardiac muscle and the kidney, and in vascular endothelial cells endogenous NO regulates O_2 consumption and mitochondrial respiration (*Palacios-Callender et al., (2004) Proc. Natl. Acad. Sci. USA 101, 7630)*. The aim of the present study was to assess the role of K_{ATP} channels in vascular tone and mitochondrial dynamics of penile arteries, and to determine whether NO is involved. Dorsal penile arteries from male Wistar rats were mounted in microvascular myographs and the effects of the selective mitoK$_{ATP}$ activators BMS-191095 (BMS) and diazoxide were assessed on vascular tone, and on mitochondrial oxygen consumption ratio (OCR) and extracellular acidification rate (ECAR), measured by Agilent Seahorse XF Pro analyzer with a Mito Stress Assay. BMS and diazoxide concentration-dependently relaxed penile arteries, BMS being one order of magnitude more potent than diazoxide. BMS-induced relaxations were reduced by mechanical endothelium removal and NOS blockade, and by the blockers of mitoK$_{ATP}$ channels 5- HD (1 μM), plasmalemmal mitoK$_{ATP}$ channels glibenclamide (0.1 μM) and BK$_{Ca}$ channels iberiotoxin (0.1 μM). 5-HD, glibenclamide and iberiotoxin also reduced relaxations induced by the NO donor SNAP. Mito Stress Assay demonstrated that both BMS (0.1 μM) and exogenous NO inhibited OCR. Thus, in the presence of BMS, basal respiration, ATP production and ECAR were significantly reduced, while SNAP inhibited basal respiration, ATP production and proton leak, and increased spared respiratory capacity of intact penile arteries. These results demonstrate that selective activation of mitoK$_{ATP}$ channels causes penile vasodilation and inhibits mitochondrial respiration by releasing endothelial NO that relaxes adjacent vascular smooth muscle (VSM) and reduces O_2 consumption and mitochondrial dynamics. This mechanism might couple blood flow to metabolism in the sustained phase of erection, by reducing mitochondrial respiration and preserving ATP in VSM when blood inflow to the penis is maximum.

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PO-020

Gut microbiota of lupus patients with hypertension contributes to the development of endothelial dysfunction

Moleón J[1,2], González-Correa C[1,2], Miñano S[1], Jiménez-Moleón I[3], Sabio JM[2,4], Martín-Armada M[4], Toral M[1,2], Gómez-Guzmán M[1,2], Sánchez M[1,2], Romero M[1,2], Jiménez R[1,2,5], Duarte J[1,2,5]

[1] Department of Pharmacology, University of Granada, Granada, Spain.
[2] Instituto de Investigación Biosanitaria de Granada, ibs.GRANADA, Granada, Spain.
[3] Department of Rheumatology, Hospital Universitario Campus de la Salud, Granada, Spain.
[4] Systemic Autoimmune Diseases Unit, Department of Internal Medicine, Hospital Universitario Virgen de las Nieves, Granada, Spain
[5] Ciber de Enfermedades Cardiovasculares (CIBERCV), Spain.

E-mail: javiermm95@ugr.es

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease associated with cardiovascular diseases, being hypertension more prevalent in SLE patient than aged matched non SLE subject (Liu & Kaplan, *Curr. Opin. Rheumatol*. 2018, *30*, 441-48, Bartels et al., *J. Rheumatol*. 2014, *41*, 680-87). Lupus has been associated with several changes in gut microbiota, which could be correlated with the manifestations of the pathology (He et al., *Gut Pathog*. 2016, *8*, 64). Despite the strong information linking gut dysbiosis and autoimmunity in SLE, scarce investigations demonstrated the role of microbiota in the development of SLE hypertension in murine models. In the present study, we tested the hypothesis that gut microbiota from hypertensive SLE patients is different to normotensive SLE and would be involved in the raise of blood pressure (BP). Therefore, we investigated whether microbiota from normotensive and hypertensive SLE patients can induce changes in BP when is administered to mice. This study was approved by the biomedical research ethics committee of the Junta de Andalucía (Andalusian Regional Government; ref. 1664-N-21). Faecal samples were obtained from healthy controls subjects (SN), normotensive SLE patients (LN), and hypertensive SLE patients (LH). Normotensive ten-week-old C57Bl/6J female mice were used as recipient mice. These mice were administered with 0.1 mL ceftriaxone sodium (400 mg/mL) once daily for 5 consecutive days by oral gavage, as previously described (Toral et al., Mol. Nutr. Food Res. 2018, e1800033). Forty-eight hours after the last antibiotic treatment recipient mice were orally gavaged with donor faecal contents (0.1 mL) for 3 consecutive days, and once every 3 days for a total period of 6 weeks. Animals were randomly assigned to 3 different groups (n = 8): Control with control microbiota from SN, control with microbiota from LN, and control with microbiota from LH. An assessment of BP, by the tail cuff method, were performed every week. Disease activity (splenomegaly and plasma anti-ds-DNA) was unaffected by SLE microbiota inoculation. Moreover, no significant changes in systolic BP were observed in mice after FMT in any experimental group. Aortic relaxation induced by acetylcholine were impaired in mice receiving microbiota from hypertensive SLE patients as compared to healthy subjects, whereas no significant changes were induced by FMT from normotensive SLE patients. These results could be related with a higher production of reactive oxygen species (ROS) from NADPH oxidase in the vascular wall in LH groups. In addition, when we incubated the aortic rings with VAS2870, a NADPH oxidase inhibitor, the relaxation was similar among groups. Higher proportion in mesenteric lymph nodes and higher aortic infiltration of proinflammatory lymphocytes Th1 and Th17 was found after FMT from LH patients as compared to SN subjects.
In conclusion, the microbiota of SLE patients with hypertension is involved in the development of endothelial dysfunction through immunoregulation.

PO-021

Characterization of 5-HT receptors that regulates the vascular sympathetic neurotransmission in female rats

Terol-Úbeda AC[1,2], Fernández-González JF[1,2], García-Domingo M[1,2], Martín ML[1,2],Morán A[1,2], García-Pedraza JA[1,2].

[1] Laboratorio de Farmacología, Departamento de Fisiología y Farmacología, Facultad deFarmacia. Universidad de Salamanca, 37007. Salamanca, Spain.
[2] Instituto de Investigación Biomédica de Salamanca (IBSAL). Paseo San Vicente 58-182,37007, Salamanca, Spain.

E-mail: aniter99@usal.es

Cardiovascular functionality is influenced by the peripheral serotonergic system; our research team has demonstrated that 5-hydroxytryptamine (5-HT) modulates noradrenergic vascular neurotransmission, inhibiting it via 5-$HT_{1A/1D}$ receptors and potentiating by 5-HT_3 activation inmale Wistar rats [1,2]. However, the pharmacological profile of 5-HT sympatho-modulation infemale rats remains unknown. The aim of this study was to determinate the 5-HT receptor typesinvolved in the serotonergic vascular sympatho-regulation in female rats. To do so, female Wistar rats were anaesthetized, pithed, pretreated with atropine and d-tubocurarine and prepared to stimulate the sympathetic outflow (0.1; 0.5; 1 and 5 Hz; 15±3V), as previously described [3]. Mean blood pressure (MBP) and heart rate were continuously monitored, and electrical stimulation of the entire spinal cord was performed to obtain frequency-dependent increases in MBP. Selective 5-HT receptor agonists were continuously i.v. infused (1 mL/h), evaluating the modification on electrical-obtained vasopressor responses. In females, 5-CT (5-$HT_{1/5/7}$ agonist) exerted an inhibitory effect, whereas 5-HT_2 and 5-HT_3 receptors agonist, α- methyl-5-HT and 1-phenylbiguanide, respectively, significantly increased electrically inducedvasopressor responses. Neither cisapride nor AS-19 (5-HT_4 and 5-HT_7 receptor agonists, respectively) modified noradrenergic vasopressor responses. The 5-CT-produced inhibition was not reversed by the i.v. pretreatment with a 5-HT_5 antagonist (SB699551). In conclusion, in female rats 5-HT maintains the modulatory role on sympathetic outflow, inhibiting via 5- HT_1 receptor activation and potentiating via 5-$HT_{2/3}$ activation.

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PO-022

Cardioprotection by metoprolol relies on circadian variability

Clemente-Moragón, A[1,2], Gómez, M[3], Suárez-Barrientos, A[1], López-Palomar, LP[1], Fuster,V[1,4], Oliver, E[5], Ibáñez, B[1,2,6]

[1] Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), [2] Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), [3]Centro Nacional de Investigaciones Oncológicas Carlos III (CNIO), [4] Icahn School of Medicine atMount Sinai, [5]Centro de Investigaciones Biológicas (CIB) Margarita Salas, [6] Instituto de Investigación Sanitaria (IIS)-Fundación Jiménez Díaz

E-mail: agustin.clemente@cnic.es

Background. In the clinical trial METOCARD-CNIC, it was shown that the intravenous (i.v.)pre-reperfusion adminis<tration of the β1-selective antagonist metoprolol reduces IS andimproves long-term cardiac function in patients after acute myocardial infarction (AMI) (1). However, the small sample size precludes a definite conclusion. Despite not having demonstrated a solid clinical benefit in terms of hard endpoints reduction, the clinical guidelines for the management of ST-Elevation Myocardial Infarction (STEMI) recommend the early administration of i.v. β-blockers in patients undergoing Primary Coronary Intervention. Additionally, our group has recently described that the beneficial effect of metoprolol against myocardial I/R injury is due to neutrophil stunning (2), which represents a one-of-a-kind property not shared by other i.v. β-blockers, such as atenolol or propranolol (3).

Purpose. Given the fact that IS has been shown to be significantly larger with STEMI onset in the dark-to-light transition period (from 6 to noon) and neutrophils exhibit a complex chonobiological profile, here we explore whether the cardioprotection exerted by metoprolol through neutrophil activity disruption is dependent on circadian variability.

Methods. Patients included in the METOCARD-CNIC trial were classified into 4 groups depending on the period of time for the onset of symptoms of AMI. Additionally, to evaluate whether neutrophil dynamics alteration by metoprolol follows an oscillatory pattern, sterile inflammatory models were performed in C57BL/6 wild-type adult mice (e.g. myocardial I/R injury, thioglycolate-induced peritonitis, 2D intravital microscopy) at different ZT times.

Results. Our multivariate regression analysis showed that the cardioprotective effect of metoprolol at 7 d was preserved only in patients undergoing AMI in the morning (from 6 to noon). In patients having suffered from AMI in this period, cardioprotection was still evident at 6 months post-AMI. These patients exhibited a better long-term left ventricle ejection fraction and attenuation of the extent of microvascular obstruction. The results from our mouse models showed that neutrophil dynamics alteration by metoprolol follows the same oscillatory pattern as in cardioprotection.

Conclusions. Our results confirm a time-window of cardioprotection for metoprolol which is independent of the time of drug administration. All the data presented refines cardiovascular pharmacotherapy, and have major implications for the prospective clinical trial design aiming at positioning metoprolol as the β-blocker of choice to reduce hard endpoints in STEMI patients.

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PO-023

Beta3-adrenergic agonists as a potential therapy for pulmonary arterial hypertension

[3]Rocha, S.F., [1]Sierra-Palomares, Y., [3]Spaczynska-Kwiatkowska, M., [3]Diaz-Guerra, A., [2,3]Macías, A., [2,3,4]García-Alvarez, A., [2,3]Ibañez, B., [1,2,3]Oliver, E.

[1]Centro de Investigaciones Biológicas Margarita Salas (CIB), CSIC, Madrid, Spain; [2]Centro de Investigaciones Biomédicas en Red de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain; [3]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; [4]Institut Clínic Cardiovascular-Hospital Clínic (IDIBAPS), Barcelona, Spain.

E-mail: eduardo.oliver@cib.csic.es

The role of β3-adrenergic receptor (β3-AR) in heart and vessels has been widely studied by our group as a therapeutic strategy in certain cardiovascular diseases such as hypertension, myocardial infarct and heart failure (1-4). Following these discoveries, we aimed to investigate the potential use of this family of clinically available drugs to treat pulmonary arterial hypertension (PAH), a rare and devastating disease for which current treatments are still not curative. PAH is originated by an aberrant vascular remodelling characterized by endothelial dysfunction and vascular cell proliferation. These changes lead to an increase in pulmonary arterial pressure followed by right ventricular hypertrophy, heart failure and premature death. To clarify the role of β3-AR in PAH and whether it might be a potential therapeutic target, we have used two animal models: hypoxia- and monocrotaline-induced PAH in mice and rats, respectively. In addition, we have analysed both β3-AR knockout and transgenic mice, with conditional cell-specific restoration of β3-AR expression in a β3-AR knockout background. We found that loss of β3-AR aggravates the PAH phenotype while its restoration in endothelial cells (EC), but not in cardiomyocytes nor in vascular smooth muscle cells (SMCs), leads to an ameliorated pathophysiology. This amelioration is reflected in a decrease in RVSP, RV hypertrophy, arterial remodelling, SMC proliferation and a recovered endothelial dysfunction (reflected by a decreased ectopic vWF expression and normalized NO production). Accordingly, pharmacological activation of β3-AR with mirabegron (2 and 10 mg/kg·day in drinking water), both in mice and rats also leads to better hemodynamic and pathophysiological parameters. In vitro experiments, with human pulmonary artery EC and SMC, demonstrate that activation of endothelial β3-AR induces NO production, which acts indirectly on SMCs to regulate vasodilation and proliferation. Concurrently, pharmacological activation of β3-AR ceases to have any beneficial effect in eNOS knockout mice exposed to hypoxia. Additionally, β3-AR regulates ROS levels and shows a role in controlling endothelial cellular stress and mitochondrial fitness. In conclusion, β3-AR stands as a new therapeutic target and β3-agonists as a potential therapeutic strategy to fight against PAH and other pulmonary vascular diseases in which endothelial dysfunction plays a relevant role.

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PO-024

AhR-HIF interplay in a model of hypertension associated to chronic intermittent hypoxia

Pimpão, AB[1], Melo Junior, A[1], Coelho, NR[1,2], Sousa C[1], Costa, PM[3], Teixeira-Santos, L [1], Morello, J[1], Pereira, SA[1], Monteiro, EC[1]

[1]NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisboa, Portugal; [2]Egas Moniz School of Health and Science, Caparica, Portugal; [3]NOVA School of Science and Technology, NOVA University of Lisbon, Lisboa, Portugal

E-mail: antonio.pimpao@nms.unl.pt

Chronic intermittent hypoxia (CIH) is responsible for the development of systemic hypertension (HTN) in patients with obstructive sleep apnea (OSA). OSA subjects frequently display a non-dipping pattern of blood pressure (BP) and resistant HTN [1,2]. We previously reported in a rat model that the activation of the aryl hydrocarbon receptor (AHR)-Cyp1a1 pathway underlies CIH-HTN [3]. The AhR is a ligand-activated transcription factor from the Per-Arnt-Sim (PAS) superfamily of proteins and the use of the AhR antagonist CH-223191 was found to decrease blood pressure [3]. Herein we aim at investigating the AhR-HIF-1α interplay in CIH conditions, besides the chronopharmacology of the antihypertensive efficacy of the AhR blocker CH-223191 and its effect in the transcriptome of the kidney.

This study was approved by NMS's Ethics Committee and followed national and international regulations. Male wistar rats were subjected to CIH conditions (21% to 5% O_2, 5.6 cycles/h, 10.5 h/day, in inactive period of Wistar rats) for 1, 7, 14, 21 and 35 days. To evaluate AhR-HIF-1α interplay, VEGF and CYP1A1 protein expression in the renal cortex was assessed by Western Blot and the VEGF/CYP1A1 ratio was calculated. Moreover, wistar rats were subjected to CIH for 21 days and then administered with the AhR blocker CH-223191 (5 mg/kg/day, gavage, in vegetable oil) for 14 days, concomitantly with CIH exposure. BP was measured by radiotelemetry, at 8 am (active phase) and at 6 pm (inactive phase) of the animals. Transcriptomic analysis using Next-Generation RNA-Seq and bioinformatics was performed in the kidney cortex of the animals (n=3 for each group). Differentially-expressed genes (DEGs) were assessed comparing CIH+CH vs. CIH+CV, using FDR-corrected $p<0.05$ as main criterion. Additionally, to investigate the circadian variation of AhR activation in the kidney in normoxia, a group of male rats was maintained in normoxic conditions. AhR activation in the renal cortex of this animals was assessed by measuring CYP1A1 (hallmark of AhR activation) protein levels.

An acute exposure to IH (1 and up to 7 days) increased the expression of VEGF in the kidney, as shown by increased VEGF/CYP1A1 ratio. CIH increased BP after 21 days, established HTN, and CH-223191 administration was able to revert this increase in the active period of the animals. However, the same was not observed in the animals´ inactive period wherein the exposure to CIH occurs and despite the drug administration before this period. Moreover, CH-223191 administration altered the transcriptome, by increasing the levels of genes associated with arterial HTN (*e.g.*, Alad, Ceacam1, Scap).

AhR-HIF interplay is affected by CIH and its chronicity. The blockade of AhR decreases blood pressure in the active period of the animals, but is not able to revert the BP non-dipping profile associated with CIH. These results suggest that the CIH model is suitable to investigate mechanisms of circadian variation, AhR-HIF interplay and chronopharmacologic properties of the CH223191.

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PO-025

Persons with uremic profiles rich in Galectin-3 and low in Klotho are at higher risk of poor peritoneal dialysis outcomes.

Sousa, C.[1,2], Calça, R.[1,2,3], Martins, A.R.[3], Teixeira-Santos, L. [1,2], Mateus, C.[3], Jervis, M.J.[3], Gomes, D.P.[3], Azeredo-Lopes, S.[1,4], Civantos, E.[5], Mas-Fontao, S.[5], Gaspar, A.[3], Ramos, S.[3], Morello, J.[1], Nolasco, F. [1], Rodrigues, A.[6,7] and Branco, P.[1,2,3*] Pereira, S.A.[1,2*]

*[1] NOVA Medical School|Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal; [2] Centro Clínico Académico de Lisboa, Lisboa, Portugal; [3] Hospital de Santa Cruz, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal; [4] Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; [5] IIS-Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain; [6] UMIB—Unidade Multidisciplinar de Investigação Biomédica, ITR—Laboratory for Integrative and Translational Research in Population Health, Porto, Portugal; [7] ICBAS—Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Centro Hospitalar Universitário do Porto, Porto, Portugal *equal contribution*

E-mail: sofia.pereira@nms.unl.pt

Peritoneal dialysis (PD) is a renal replacement therapy that is based on the dialytic properties of the peritoneal membrane (PM) and is a self-care treatment performed at home, facilitating patient's autonomy [1]. However, loss of PM integrity and progression to fibrosis is still a major complication. The PM microenvironment is variable among patients and reflects their systemic uremic profile, which might be a factor for PM integrity, patient survival and long-term PD outcomes, e.g. major cardiovascular events (MACE) and loss of residual renal function. Thus, the aim of this study was to investigate PM status, clinical data, and aging-related molecules, such as α-klotho and galectin-3, as predictors of PD long-term outcomes.

A 5-year prospective study was conducted at the PD Unit of Santa Cruz Hospital, Centro Hospitalar de Lisboa Ocidental, where 58 incident patients with biopsy at the study baseline were included. This study was approved by the Ethics Committee of the NOVA Medical School, Faculdade de Ciências Médicas, NOVA University of Lisbon (Approval number 50/2019). The following endpoints were evaluated:(a) PD failure and time until PD failure, (b) MACE and time until MACE. PM histomorphology and aging-related indicators were assessed before the start of PD and investigated as predictors of study endpoints.

Fibrosis of the PM was associated with MACE occurrence and earlier MACE, but not with the patient or membrane survival. Serum α-Klotho bellow 742 pg/mL was related to the submesothelial thickness of the PM. This cutoff stratified the patients according to the risk of MACE and time until MACE. Uremic levels of galectin-3 were associated with PD failure and time until PD failure.

To sum up, this work points out galectin-3 and α-klotho as molecular indicators to tailor patient management in this home-based renal replacement therapy and potential therapeutic targets. However, further studies are needed to better understand the subjacent mechanisms.

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PO-026

Description of the cardiovascular properties of aqueous extracts of roasted geisha coffee beans

Abrego-González, K. [1,2] Vega, A. [1] Sánchez-Martínez, H. [2] Morales, A. [2,3] Morán-Pinzón, J. [2,3] López-Pérez, J. [2,4] Guerrero, M.F. [5] Doncel, V. [5] Del Olmo, E. [4] Guerrero, E. I. [2,3,6]

[1] Programa de Maestría en Ciencias Químicas, Universidad Autónoma de Chiriquí, Panamá. [2] Centro de Investigaciones Psicofarmacológicas, Universidad de Panamá, Panamá. [3] Departamento de Farmacología, Universidad de Panamá, Panamá. [4] Departamento de Ciencias Farmacéuticas, Facultad de Farmacia, Universidad de Salamanca, España. [5] Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá-Colombia [6] Sistema Nacional de Investigación, SENACYT-Panamá.

E-mail: guerrerodleon@gmail.com

According to general phytochemical studies, Panamanian Geisha coffee has a high content of chlorogenic acids[1], which have antioxidants properties. Based on these findings, we have proposed to study the antioxidant, vascular and antihypertensive activity of the aqueous extract of Geisha coffee (AEGC). In vivo experiments, 24 male Spontaneously hypertensive rats (SHR) were randomly divided into groups that received AEGC (300 and 600 mg/kg/day). Positive-control animals received Enalapril (ENA, 10 mg/kg/day) and a negative-control group received distilled water. All treatments were administered by gavage for 3 weeks. Systolic blood pressure (SBP) was recorded in vivo by non-invasive methods. In vitro vasoactive effects of AEGC (100 to 3000 µg/ml) were assessed on phenylephyne (PE) and KCl contractile response in aorta rings with endothelium from Sprague-Dayley rats. The AEGC (0.48-1000 µg/ml) was evaluated on inhibition of lipid peroxidation using egg yolk assay. ENA led to significantly lower blood pressure in SHR animals for first week and was sustained on time (137.01 ± 0.66 mmHg); meanwhile the lowest doses of AEGC (300 mg/kg) significantly decreased SBP compared with negative-control (140.63 ± 0.90 vs. 157.17 ± 1.02 mmHg, respectively; $P < 0.05$). The AEGC caused vascular relaxation in a concentration-dependent manner reaching Emax= 59.39 ± 15.03%) in aortic rings contracted with PE. The effect of extract against KCl contraction was minor (31.33 ± 5.91%). Regarding to antioxidant effect of AEGC against lipid oxidation, the extract caused a high inhibition (73 ± 1.7%) at the concentration of 15.6 µg/ml, effect that was similar to curcumin (89.3± 1.7%). These results suggest that AEGC have a potential use as nutraceutical in hypertension. The reduction in SBP may be attributed in part to vasorelaxant effect and antioxidant activity, however further research is needed to understand the mechanisms underlying this antihypertensive effect.

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PO-027

The dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 receptor agonism reduced abdominal aortic aneurysm development in ApoE$^{-/-}$ mice

Marques P [1,2], Domingo E[1,2], de Alburqueque D[1,2], Real JT [2,3], Sanz MJ [1,2,3], Piqueras L [1,2,3]

1 Department of Pharmacology. University of Valencia, VALENCIA, SPAIN
2 Institute of Health Research-INCLIVA, VALENCIA, SPAIN
3 CIBERDEM: Diabetes and Associated Metabolic Diseases Networking Biomedical Research-ISCIII, MADRID, SPAIN

E-mail: Laura.Piqueras@uv.es

Background & aims:Abdominal aortic aneurysm (AAA) is a vascular degenerative disease characterized by a local dilatation of the abdominal aorta. Since AAA is associated with high morbidity and mortality in male over 65 years old, new effective treatments are needed to prevent AAA pathogenesis. Dual agonism of glucose-dependent insulinotropic polypeptide (GIP) and Glucagon like peptide-1 (GLP-1) receptors is a promising new approach currently in clinical development for the treatment of diabetes and obesity. In addition to improving glycaemic control they have shown efficacy in reducing major adverse cardiovascular events. However, the effect of these new drugs in AAA remains unknown.
Objective: The aim of this study is to evaluate the effect of treatment with tirzepatide on AAA induced by angiotensin-Il (Ang-Il) infusion for 28 days in apolipoprotein E knockout (apoE$^{-/-}$) mice.
Results: Ang-II infused apoE $^{-/-}$ mice developed AAA ($P < 0.05$). By contrast, treatment with the dual agonism of GIP and GLP-1 receptors, tirzepatide reduced Ang-II-induced AAA formation in apoE$^{-/-}$ mice ($P < 0.05$). Tirzepatide also decreased macrophage infiltration, neovessel formation and matrix metalloproteinase-2, matrix metalloproteinase-9 in suprarenal aortic walls of apoE$^{-/-}$ mice infused with Ang-Il.
Conclusion: Tirzepatide reduces dissecting AAA formation induced by Ang-II in apoE$^{-/-}$ mice and may constitute a novel therapeutic strategy to prevent AAA progression in humans.

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PO-028

Deletion of CCR3 receptor in apoE$^{-/-}$ mice subjected to an atherogenic diet accelerates atherosclerosis

Domingo E[1,2], Collado A[1,2], Marques P[1,2], Cervera M[1,2,3,4,5], Real JT[1,2,3,4,5], Piqueras L[1,2,4,5], Sanz MJ[1,2,4,5]

[1]Faculty of Medicine, University of Valencia, Valencia, Spain.
[2]Institute of Health Research 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.

E-mail: eldopre7@gmail.com

Background: Atherosclerosis is one of the leading causes of morbidity and mortality in Western countries and bears several histopathologic similarities to chronic inflammation. The early atherosclerotic lesion involves an inflammatory response consisting in the intimal accumulation of T lymphocytes and lipid-laden macrophages, and these events occur continuously throughout the entire atherogenic process. Eotaxin-1 (CCL11) expression has been detected in human and mouse atherosclerotic aortas [1], however, its role in the atherosclerotic lesion development remains elusive. Therefore, we evaluate the impact of an atherogenic diet in the lesion formation in apoE$^{-/-}$ mice (apoE$^{-/-}$CCR3$^{+/+}$ mice) versus those lacking eotaxin receptor (CCR3, apoE$^{-/-}$CCR3$^{-/-}$). **Material and Methods:** Two months old apoE$^{-/-}$CCR3$^{+/+}$ or apoE$^{-/-}$CCR3$^{-/-}$ mice were subjected or not to an hypercholesterolemic diet (10.8% fat, 0.75% cholesterol) during two additional months. Lesion formation, macrophage and T lymphocyte infiltration, collagen, necrotic core, vascular smooth muscle cells (VSMC) and eotaxin-1/CCL11 content were determined within the lesion through histological and immunohistochemical techniques. Statistical significance was determined using a Two-way ANOVA followed by a Bonferroni's post hoc test on raw data. **Results:** ApoE$^{-/-}$CCR3$^{+/+}$ and apoE$^{-/-}$CCR3$^{-/-}$ mice subjected to a hypercholesterolemic diet showed clear atherosclerotic lesion formation at the aortic sigmoid valve characterized by enhanced macrophage and T lymphocyte infiltration, collagen, necrotic core and VSMC proliferation than those subjected to a control diet. ApoE$^{-/-}$CCR3$^{-/-}$ mice subjected to an atherogenic diet showed increased lesion formation, augmented macrophage and T lymphocyte infiltration and collagen content within the lesion than CCR3 expressing apoE$^{-/-}$ mice (apoE$^{-/-}$CCR3$^{+/+}$). Eotaxin-1 expression within the lesion of apoE$^{-/-}$CCR3$^{+/+}$ mice in a hypercholesterolemic scenario was much higher than that detected in apoE$^{-/-}$CCR3$^{-/-}$mice. We characterized the diet-induced atheroma in cardiac valves of apoE$^{-/-}$CCR3$^{+/+}$ and apoE$^{-/-}$CCR3$^{-/-}$ mice. **Conclusions:** Our findings suggest that the eotaxin-1 (CCL11)/CCR3 axis may exert a protective effect in the development of the atherosclerotic process.

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PO-029

The role of the immune system in antihypertensive effects of dietary fiber

González-Correa, C[1,2]., Moleón, J[1,2]., Miñano, S[1]., Robles-Vera[4], I., de la Visitación, N[1],Jiménez, R[1,2,3]., Duarte, J[1,2,3]., Romero, M[1,2]

[1]University of Granada, Granada, Spain. [2]Instuto de Investigación Biosanitaria de Granada, ibs. Granada, Granada, Spain. [3]Ciber de Enfermedades Cardiovasculares (CIBERCV), Spain. [4]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.

E-mail: miguelr@ugr.es

A lower incidence of hypertension has been linked to dietary fiber supplementation. However, the mechanisms by which dietary fiber reduces blood pressure remain unclear. The aim of this study was to investigate whether dietary supplementation with two types of fiber-rich diets, inulin-type fructan soluble fiber (ITF, OraftiP95®) and resistant starch insoluble fiber (RS, SF11-025®), exerts a cardiovascular protective effect in the development of hypertension in an experimental model of genetic hypertension, focusing on the involvement of the sympathetic nervous system, the immune system, and the intestinal microbiota. Spontaneously hypertensive male rats (SHR) and their respective normotensive control, Wistar Kyoto rats (WKY), 6 weeks old, were used and randomly divided into 4 experimental groups (n=8): 1) control group (WKY
), 2) hypertensive group (SHR), 3) hypertensive group treated with OraftiP95® (250 mg/rat/day in drinking water) (SHR+ITF), 4) hypertensive group treated with SF11-025 diet (SHR+RS). The control group received a standard diet with 47.6% fiber. The treatment was maintained for 12 weeks. The evolution of blood pressure values was monitored every 2 weeks during the treatment using tail plethysmography. Additionally, at the end of the treatment, blood pressure values were determined through direct recording, endothelial function studies were conducted, vascular oxidative and inflammatory status, intestinal wall integrity, plasma levels of lipopolysaccharide (LPS), changes in lymphocyte populations in mesenteric lymph nodes and vascular infiltration, as well as sympathetic nervous system activity were measured. We observed that chronic treatment with the SF11-025 diet was able to prevent the increase in blood pressure and the development of endothelial dysfunction in SHR rats, as a result of a reduction in vascular production of reactive oxygen species, via NADPH oxidase and decreased vascular infiltration of proinflammatory cytokines. These protective effects are associated with reduced sympathetic activity, improved intestinal integrity, decreased plasma levels of LPS, and restoration of the balance between Th17/Treg lymphocyte populations in mesenteric lymph nodes and aorta. On the other hand, dietary supplementation with OraftiP95® fiber did not exhibit any cardiovascular protective effects in the development of hypertension and endothelial dysfunction observed in SHR rats. Furthermore, fecal microbiota transplantation from SHR+RS group to recipient SHR was able to exert the beneficial effects described above. In conclusion, our study demonstrates that dietary supplementation with insoluble RS fiber exerts a cardiovascular protective effect, resulting in reduced vascular oxidative and inflammatory status, improved intestinal wall integrity, decreased endotoxemia, reduced sympathetic activity, and improved immune response by increasing the accumulation of Treg lymphocytes in the vasculature and reducing Th17 lymphocytes. These protective effects were mediated, at least in part, by changes in the composition of the gut microbiota. However, a diet rich in soluble ITF fiber lacks beneficial cardiovascular effects in preventing the development of hypertension and endothelial dysfunction in SHR rats.

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PO-030

Different contributions of gut microbiota in the blood pressure lowering effects induced by first-line antihypertensive drugs

González-Correa C[1,2], Moleón J[1,2], Miñano S[1], Robles-Vera I[3], de la Visitación[4], Toral M[1,2,5], Martín-Morales N[6], O'Valle F[2,6], Gómez-Guzmán M[1,2], Sánchez M[1,2], Jiménez R[1,2,5], Romero M[1,2], Duarte J[1,2,5]

[1] Department of Pharmacology, University of Granada, Granada, Spain.
[2] Instituto de Investigación Biosanitaria de Granada, ibs.GRANADA, Granada, Spain.
[3] Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid 28029, Spain.
[4] Vanderbilt University Medical Center, Nashville, Tennessee, USA.
[5] Ciber de Enfermedades Cardiovasculares (CIBERCV), Spain.
[6]Department of Pathology, School of Medicine, University of Granada, Granada, Spain.

E-mail: javiermm95@ugr.es

Microbiota plays a key role in the host blood pressure (BP) regulation. This study analyses whether first-line antihypertensive drugs ameliorate the dysbiosis state in hypertension, and to test if this modification contributes to its BP reducing properties in a genetic model of neurogenic hypertension. Twenty-week-old male Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were untreated or treated with captopril, or amlodipine, or hydrochlorothiazide, for 5 weeks. A faecal microbiota transplantation (FMT) experiment was also performed by gavage of faecal content from donor SHR-treated groups to SHR recipients for 3 weeks. BP was assessed by tail-cuff plethysmography. Composition of the gut microbiota was determined through amplification of the V4-V5 region of 16S rRNA gene. T cells were analysed in mesenteric lymph nodes (MLNs), blood, and aorta by flow cytometry. Faeces from SHR showed gut dysbiosis, characterised by lower acetate- and higher lactate-producing bacteria, and lower strict anaerobic bacteria. All three drugs increased anaerobic bacteria proportion, captopril and amlodipine restored the proportion of acetate-producing bacteria populations to WKY levels, whereas hydrochlorothiazide decreased butyrate-producing bacteria. The amelioration of gut dysbiosis induced by captopril and amlodipine was associated with a decrease in gut pathology and permeability and an attenuated sympathetic drive in the gut. Both drugs decreased neuroinflammation and oxidative stress in the hypothalamic paraventricular nuclei. By contrast, hydrochlorothiazide was unable to reduce neuroinflammation, gut sympathetic tone and gut integrity. FMT from SHR-amlodipine to SHR decreased BP, ameliorated aortic endothelium-dependent relaxation to acetylcholine, and lowered NADPH oxidase activity, whereas FMT from SHR-hydrochlorothiazide did not have these effects in recipient SHR. The vascular changes induced by amlodipine after FMT were accompanied by decreased proportion of Th17 in MLNs, lower aortic Th17 infiltration and reduced neuroinflammation. This study demonstrates that first-line antihypertensive drugs induced different modifications of gut integrity and gut dysbiosis in SHR, which result in the absence of contribution of microbiota in the BP lowering effects of hydrochlorothiazide, whereas the vasculo-protective effect induced by amlodipine through adequate gut microbiota reshaping and gut-immune system communication.

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PO-031

Effect of the TLR4 inhibition on Cardiovascular and Renal Alterations Caused by Different Doses of Cisplatin: Modulation of the TLR4/MyD88/NLRP3 Axis Expression

González Ruiz, A[1,3]. Flaj Prados, S[1]. Fernández-Cabello, V[2]. Quesada del Sol, E[2,3]. Herradón Pliego, E[1,3]. López-Miranda González, V[1,3]

[1] Area de Farmacología y Nutrición y Bromatología, Dpto. CC Básicas de la Salud. FacultadCC Salud, URJC. Grupo de alto rendimiento PHARMAKOM [2] Instituto de Química Médica (IQM), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain. [3]Unidad Asociada I+D+i del Instituto de Química Médica (IQM), Consejo Superior de Investigaciones Científicas (CSIC)

E-mail: antonio.gonzalezr@urjc.es

Cisplatin (CPT), a widely used chemotherapeutic agent, is known to cause cardiovascular and renal toxicity, limiting its clinical efficacy. TLR4 activation has been proposed as a critical pathway involved in these toxicities. Consequently, inhibition of TLR4-induced inflammatoryresponse could help treat or prevent cardio-renal Cisplatin-induced complications.

The aims of the study are: 1) to evaluate whether modulation of TLR4 receptors by using a synthetic antagonist of the TLR4 receptor activation process (CAS [1202208-36-3]) canameliorate cardio-renal alterations induced in an experimental model of chronic administrationof Cisplatin in cycles at two different doses, 2 mg/Kg and 3 mg/Kg; 2) to analyze whether the administration of the antagonist modifies the alterations in the expression of TLR4, MyD88, and NLRP3 at cardiac, vascular and renal levels produced by chronic administration ofCisplatin in cycles at the two different doses assayed.

Adult male Wistar rats were divided into five experimental groups (n=8-10 rats/group): Control (Saline); CPT 2 (CPT 2 mg/Kg i.p); CPT 3 (CPT 3 mg/Kg i.p.). Cisplatin was administered once a week for five weeks; (CPT 2 +TLR4-inh): animals treated with CPT 2 mg/Kg in the mentioned schedule that during the last two weeks received a daily dose of 10 mg/Kg TLR4 inhibitor (i. p.); (CPT 3 + TLR4-inh): animals treated with CPT 3 mg/kg in the mentioned schedule above that during the last two weeks received a daily dose of 10 mg/Kg TLR4-inh (i.p.). Animals' body weight and feeding behavior were evaluated during experimental period. Atthe end of experimental, MDA and creatinine plasma levels, and general cardiovascularparameters (blood pressure and heart rate) were evaluated. In addition, *in vitro* basal cardiac function and vascular reactivity in the aorta and mesenteric bed were also analyzed. The expression of TLR4, MyD88, and NLRP3 in the heart, aorta, and kidney was determined by Western-Blot.

Treatment with TLR4 antagonist did not modify caloric intake or weight gain in Cisplatin- treated animals. However, TLR4 inhibition caused a significant decrease in plasma creatinine concentrations. At the cardiovascular level, it restored diastolic blood pressure, left ventricular function, and endothelial dysfunction in the aorta at the two doses of cisplatin tested. Moreover, TLR4 inhibition recovered mesenteric artery contractile function in the CPT 3 mg/Kg group. Likewise, TLR4 inhibition causes MyD88 and NLRP3 expression change at cardiac, vascular, and renal levels.

These findings suggest that the TLR4-PA1 antagonist may protect against CPT-induced cardiac, vascular, and renal toxicities through modulation of the TLR4/MyD88/NLRP3 signaling pathway.

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PO-032

Influence of chronic 5-HT$_2$ receptor blockade on renal sympatheticinnervation in diabetic rats

García-Pedraza JA[1,2], Fernández-González JF[1,2], Terol-Úbeda AC[1,2], Ordóñez JL[1,2], Martín ML[1,2], Morán A[1,2] and García-Domingo M[1,2].

[1] Laboratorio de Farmacología, Departamento de Fisiología y Farmacología, Facultad deFarmacia. Universidad de Salamanca, 37007. Salamanca, Spain.
[2] Instituto de Investigación Biomédica de Salamanca (IBSAL). Paseo San Vicente 58-182,37007, Salamanca, Spain.

E-mail: joseagp@usal.es

Sympathetic innervation plays an important role in cardiovascular and renal complications derived from diabetes[1]. It has been shown that the serotonergic system contributes to the control of cardiovascular homeostasis by modulating vascular and renal sympathetic neurotransmission in diabetes[2,3]. Considering that activation of the 5-HT$_2$ receptors is related to the development of cardiovascular disorders[4], the aim of this study was to determine whether 5-HT$_2$ receptor blockade could modify the 5-HT influence on renal sympathetic activity in diabetic animals. Experimental diabetes was induced by alloxan administration (150 mg/kg; s.c.) in male Wistar rats. After 14 days post-induction, the animals received, or not (non-treated diabetic group), oral treatment with sarpogrelate (5-HT$_2$ receptor antagonist; 30 mg/kg/day; sarpogrelate-treated diabetic group) for 14 days. During these 28 days, the glycemia and body weight were weekly controlled. A non-treated normoglycemic group was maintained for the same period to serve as age-matched non-diabetic controls. After this period, a group of the rats were placed in metabolic cages (measuring food and drink consumption, and urine production), and blood samples were collected to determine renal function markers such as plasma creatinine and bloodurea nitrogen (BUN). The rest of animals were anesthetized and prepared for the *in situ* autoperfusion of the kidney, monitoring blood pressure (BP), renal perfusion pressure (RPP), and heart rate (HR). Electrical stimulation of the renal sympathetic periarterial nerves (12.5 ± 2.5 V; 2, 4, and 6 Hz) produced frequency-dependent renal vasoconstrictor responses, evaluating the effect of 5-HT (i.a.) on the RPP increases. Sarpogrelate treatment did not modify glycemia, body weight, food intake, BP, HR or RPP in diabetic animals. However, the drink intake, urine excretion, plasma creatinine, BUN, and the increases in RPP obtained by renal sympathetic stimulation were significantly lower in diabetic rats treated with sarpogrelate; 5- HT exerts an inhibitory effect on renal sympathetic innervation. In conclusion, chronic blockade of 5-HT$_2$ receptors reduces kidney damage and the renal sympathetic hyperactivity in diabetic rats, maintaining the sympatho-inhibitory effect of 5-HT.

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PO-033

Contribution of selective activation of PKA and EPAC to cAMP endothelial barrier stabilization in LPS challenged HUVEC

Aitor Picos[1], Nuria Seoane[1], Ezequiel Álvarez[2,3,4], Dolores Viña[1,2,5], Manuel Campos-Toimil[1,2,5]

1. Pharmacology of Chronic Diseases, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela, Spain.
2. Department of Pharmacology, Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela (USC), Spain.
3. Health Research Institute of Santiago de Compostela (IDIS), USC University Hospital Complex (CHUS), SERGAS, Spain
4. CIBERCV, Institute of Health Carlos III, 28220 Madrid, Spain.
5. IDIS. Translational Research in Neurological Diseases (ITEN), Spain.

E-mail: manuel.campos@usc.es

Inflammation is tightly related to cardiovascular risk factors, such as diabetes, obesity, high blood pressure. Chronic exacerbated inflammation plays a critical role in atherosclerotic plaques formation, a key process in vascular degeneration.
Cyclic adenosine monophosphate (cAMP) regulates many aspects of cellular physiology, proliferation, and survival. It exerts its actions through its effectors: protein kinase A (PKA), the guanine-nucleotide-exchange factor Epac, and some cyclic-nucleotide-gated ion channels that are relatively specific for calcium. The complex compartmentalization of cAMP is crucial for the specificity of downstream pathways, relying on both on the location of synthesis by adenylyl cyclases, and degradation by phosphodiesterases. Also, A-kinase anchoring proteins (AKAP) are scaffolding proteins that bind PKA as well as other enzymes, forming multi-protein complexes physically tethered to some intracellular domain, which further increases the compartmentalization process. Regarding endothelial cells, cAMP microdomains and downstream effectors determine the positive or detrimental contribution to endothelial barrier function. Thus, the modulation of cAMP signaling pathway represents a promising strategy to improve endothelial function in both health and disease.
In our study, we evaluated the effect of forskolin and rolipram (cAMP-elevating agents), 8-pCPT-2'-Me-O-cAMP (Epac activator) and 6-Bnz-cAMP (PKA activator) in LPS challenged human umbilical vein endothelial cells (HUVEC).
Combined forskolin and rolipram shown a major effect in barrier function, rising trans-endothelial electric resistance (TEER) both in normal and inflammatory conditions, improving VE-cadherin levels and location, and diminishing JAK2/STAT3 pathway activation and cytokine production. Neither EPAC nor PKA activation fully mimicked the effect of cAMP rise in barrier stabilization, but their combination appears to have some impact on inflammatory status of HUVEC challenged with LPS.
In conclusion, loss of intracellular microdomains using cAMP analogs may be conditioning the contribution of downstream effectors Epac and PKA to endothelial barrier function. More studies are necessary to fully characterize the specificity on the endothelial response to both cAMP effectors due to temporal and physical compartmentalization.

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NEUROPHARMACOLOGY

PO-034

A Novel Model for HTS of Drugs for Post-Acute Sequelae of COVID-19 -Related Neuronal Damage

Martínez, A.L.; López, D.; Brea, J.; Loza, M.I.

Universidad de Santiago de Compostela, Santiago de Compostela, Spain.

E-mail: antonleandro.martinez@usc.es

Post-acute sequelae of COVID-19 (PASC) is a debilitating syndrome that affects more than 65 million people worldwide. It encompasses multiple symptoms affecting numerous organs, including nervous system. PASC-associated neurological symptoms affect both central and peripheral nervous system. Regarding the latter, paraesthesias have been observed in patients after recovery from acute COVID-19 (1). The ethiology of this damage is not clear, but it has been proposed that the high plasmatic levels of inflammatory cytokines may contribute to organ damage (2). We previously developed a translational phenotypic *in vitro* model for high-throughput screening (HTS) of novel drugs for neuropathic pain based on DRG neuron-like F11 cell line (3). Thus, F11 cells could be a valid model for HTS of novel drugs for preventing PASC. The aim of this work is to employ differentiated F11 cells for the screening of new drugs for inflammatory cytokine-induced damage on peripheral neurons.

We exposed differentiating F11 cells to several inflammation-related cytokines and we checked that, even though the exposition to 100 ng/mL IFN-γ and 50 ng/mL TNF-α does not have any repercussion on neurite length nor in the morphology of differentiated F11 cells, they induce a significant reduction in the excitability of those cells in response to KCl ($p<0.001$; ANOVA followed by Dunnett's post-hoc test). We screened Prestwick Chemical Library for the search of hits that could counteract the deleterious effect of these cytokines on the reduction of the excitability of F11 cells, identifying seven hits whose mechanism of action is related with the blockade of ion transients that protected differentiated F11 cells against the deleterious effect of IFN-γ and TNF-α with an activity higher to the 65% at 10 µM.

Thus, F11 cells constitute an *in vitro* phenotypic model for HTS of new drugs that could protect against the deleterious effect of inflammatory cytokines on sensory neurons in patients suffering from COVID-19.

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PO-035

Identifying putative I_2 Imidazoline Receptor Target proteins By Using Cellular Thermal Shift Assay In Mice

Taboada-Jara, T.[1]; Vasilopoulou, F.[1]; Bagan, A.[2]; Escolano, C.[2]; Griñán-Ferré, C.[1,3] & Pallàs, M.[1,3]

[1]*Pharmacology Section, Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institut de Neurociències (UBNeuro), University of Barcelona, Barcelona, Spain*
[2]*Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Barcelona, Spain*
[3] *Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.*

E-mail: pallas@ub.edu

2-BFI is a selective ligand for the I_2 imidazoline receptors (I_2-IR) that has attracted much attention in recent times. Furthermore, it has demonstrated a potential pharmacological and therapeutic effect on brain disorders in different animal models, such as Alzheimer's disease (AD). Likewise, it has been shown that I_2-IR receptors are widely distributed in the central nervous system (CNS), with a predominant localization in glial cells. However, although I_2-IR ligands such as 2-BFI have neuroprotective properties in several AD mice models or brain damage models, the exact mechanism of action (MoA) is not fully described. The I_2-IR has been associated with MAO, although its location on the enzyme and its role have never been elucidated. A poor correlation between pharmacological classification and physiological function leads to further studies of its MoA.

In the present study, we determine putative targets of 2-BFI in CD-1 mice hippocampus by using cellular thermal shift assay (CETSA). We treated protein extract from CD-1 hippocampus with 2-BFI (5mM) or with vehicle for 30 min. Samples were heat to the following ten temperatures 37, 41, 44, 47, 50, 53, 56, 59, 63 and 67 °C for 3 min. After ultracentrifugation at 100,000g, supernatant will be processed HPLC-MS to protein identification to determine the identity of the 2-BFI-targeted proteins. In the analysis, we found 9 proteins, such as REEP1, RUFY2, LARP4B, ARHGAP44, which were uniquely detected in the drug-treated sample at 59-63ºC. Those proteins are associated with AD, e.g. REEP1 decreases reactive oxygen species (ROS) accumulation in zebrafish; others such as ARHGAP44 are involved in synaptic plasticity, promoting dendritic spine morphology changes, thereby this result could explain improving long-term memory decline in AD mice models. Hence, we can establish a relation between these new target proteins and the effect exerted by de 2-BFI in the beneficial effects presented in AD.

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PO-036

Discovery of a Dual-Action of G9a Inhibitors for the Treatment of Alzheimer's Disease

Griñán-Ferré, C.[1,3], Bellver-Sanchis, A.[1]; Sánchez-Arfelis, A.[2]; Irisarri, A.[1]; Tic, I.[1]; Vázquez, S.[2]; Pérez, B.[3]; Leandro Martínez Rodríguez, A.[4], Brea, J.[4,5], Loza, M. Escolano, C.[2]; & Pallàs, M.[1,3].

[1]Department of Pharmacology and Therapeutic Chemistry, Faculty of Pharmacy and Food Sciences, Institut de Neurociències, Universitat de Barcelona, Avda. Joan XXIII, 27, 08028 Barcelona, Spain.
[2]Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII, 27-31, E-08028, Barcelona, Spain.
[3]Department of Pharmacology, Therapeutics and Toxicology, Institute of Neuroscience, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.
[4]Innopharma screening platform, Biofarma research group. Centro de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS), Departamento de Farmacología, Farmacia y Tencología Farmacéutica. Universidad de Santiago de Compostela, Santiago de Compostela, Spain.
[5]Health Research Institute of Santiago de Compostela (IDIS), University Hospital of Santiago de Compostela (SERGAS), Trav. Choupana s/n, 15706 Santiago de Compostela, Spain.
[6]Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.

E-mail: christian.grinan@ub.edu

Alzheimer's disease (AD) is the most common cause of dementia, making the disease a global health crisis that must be addressed. Until now, none of the approved AD treatments turned out to be a success. AD is unknown but involves a combination of genetic, biochemical, and environmental factors, being one of the reasons why single-target-directed drugs have failed to reach clinical trials. As a new strategy in drug discovery for AD, multifunctional molecules avoid drug–drug interactions, off-target adverse effects, poor patient compliance, and high development costs compared to combination therapies. Multiple lines of evidence suggest that epigenetic alterations and tau pathology are two of the crucial causes of AD. Strikingly, overexpression of G9a and the other protein serve as drivers of the cognitive impairment, leading to synaptic plasticity reduction, autophagy dysfunction, increasing Tau pathology, OS and neuroinflammation. Here, we synthesized the compound AMC-1, a new chemical scaffold with high potency micromolar (μM) to inhibit both targets. Moreover, other interesting characteristics are that AMC-1 is selective to G9a respect GLP (another histone/lysine methyltransferase), exhibits a high PAMPA-BBB permeability, no presented hERG toxicity and a good drug metabolism and pharmacokinetics. Besides, treatment with AMC-1 in SAMP8 mice rescued cognitive decline measured via NORT. G9a is responsible for methylating Histone 3, being capable to repress the expression of genes related to learning and memory formation, we evaluated several repressive histone marks in the SAMP8 mice model. Furthermore, we evaluated the Tau phosphorylation, observing that AMC-1 was able to reduce its levels in SAMP8. In addition, the density of dendritic spines and the length of dendritic branches were evaluated, showing an increase in the treated group. Therefore, our dual approach is an innovative and promising multifaceted therapeutic strategy for AD treatment.

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PO-037

Effects of Spinocerebellar Ataxia type 3 in early cerebellar morphogenesis

Celia Llorente-Sáez[1], Marina Arribas-Blázquez[1], Julia Serrano-López[1], Yaiza Trueba[1],Marina L. Pérez-Sanz[1], Luis Alcides Olivos-Oré[1], Rosa Gomez Villafuertes[1], Raquel Pérez-Sen[1], Esmerilda G. Delicado[1], Antonio R. Artalejo[1], Felipe Ortega[1],

[1]Instituto Universitario de Investigación en Neuroquímica. Universidad Complutense deMadrid. Avda. Puerta de Hierro s/n. 28040 Madrid, España

celillor@ucm.es

Spinocerebellar Ataxia type 3 or Machado Joseph disease (SCA3/MJD) is a neurodegenerativedisease caused by an expansion of the CAG triplet in the coding region of the ATXN3 gene, which leads to a polyglutamine stretch in the protein ataxin-3[1]. SCA3/MJD is autosomal and dominantly inherited, being the most prevalent ataxia worldwide, especially in Europe and theIberian Peninsula (20%-50% of the families)[2,3]. Most common clinical symptoms are progressive impairment of mobility, cognition, balance, and language skills, which result in severe disability and premature death of the patients; and there is no effective treatment available to cure or delay the development of the disease. Although the onset of SCA3/MJD normally occurs during adulthood, there is plenty of evidence that the cerebellum structure could be altered from earlier stages, as well as the existence of nonspecific symptoms several years before the clinical diagnosis[4]. Therefore, research on unveiling when neural alterations exactly appear are of utmost importance so as to establish treatments as early as possible. Thisis why, we hypothesize that mutant ataxin-3 protein could modify cerebellar morphogenesis during early postnatal development. For this purpose, we use a mouse model of SCA3/MJD which faithfully mirrors human hallmarks and progression of the disease, at postnatal day 0 and5.

Our research group has developed a methodology based on the isolation and culture of neural stem cells (NSCs) of the cerebellum to later analyse individually the fate of each NSC and its progeny by time-lapse video microscopy and single cell tracking[5], as well as electrophysiological characterization by patch-clamp and cell viability assays. We have also completed immunohistochemical analysis and gene and protein expression studies. Finally, weplan to further analyse the modulation of different components of the purinergic system, whichis known to have a neuroprotective effect on neuronal populations of the cerebellum[6].

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PO-038

Heteromer-selective agonists for the adenosine A_1-dopamine D_1 receptor heterotetramer

Alonso-Carrasco,A.[1,2], **Llopart, N.**[1,2], **Casadó-Anguera, V.**[1,2,3], **Moreno, E**[1,2], **Casadó, V.**[1,2]

[1]*Nanomedicine and Molecular Neuropharmacology, Consolidated Research Group of the Generalitat of Catalonia, Barcelona*
[2]*Institute of Biomedicine of the University of Barcelona, Barcelona, Spain*
[3]*Universitat Pompeu Fabra, Barcelona, Spain*

E-mail: aalonsca73@alumnes.ub.edu

Restless Legs Syndrome (RLS) is a neurological motor disorder that severely impacts patients' quality of life. Current treatment options using D_3 dopamine receptor (D_3R) agonists often lead to augmentation, where symptoms worsen over time after an initial period of improvement. In the context of the direct pathway of motor control in the basal ganglia, it has been observed that A_1 adenosine receptor (A_1R) agonists downregulate the neuronal activation mediated by D_1 dopamine receptor (D_1R) through previously described A_1R-D_1R heterotetramer. To address the issue of augmentation, identifying A_1R-D_1R heteromer-selective A_1R agonists shows significant potential as a therapeutic strategy for RLS.

Our primary goal was to identify A_1R-D_1R heteromer-selective A_1R agonists from a drug library. We employed radioligand binding assays and CODA-RET assays on HEK-293T cells transfected with A_1R alone or both A_1R and D_1R. Our secondary objective was to evaluate the potential negative crosstalk of these agonists using dynamic mass redistribution (DMR) assays, CODA-RET, and intracellular cAMP quantification.

Our results revealed significant differences in R-PIA, CCPA, GR79236, and ADAC A_1R agonist-induced activation of A_1R-A_1R homodimer and A_1R-D_1R heterodimer by CODA-RET. However, in radioligand binding assay, our study did not identify any A_1R-D_1R heteromer-selective ligand. Notwithstanding, the transition from a biphasic curve in the A_1R-A_1R homodimer to a monophasic curve in the A_1R-D_1R heterodimer when applying GR79236 indicated a loss of negative cooperativity, suggesting an improved affinity for the heteromer. Based on these findings, we selected R-PIA, CCPA, and GR79236 to investigate the potential negative allosteric interaction of these compounds over D_1R activation. Using intracellular cAMP accumulation, DMR and $G\alpha_s$-D_1R interaction analysis, we identified the presence of negative crosstalk of these A_1R agonists over D_1R.

These findings highlight the complex nature of drug interactions and provide insights into the potential for improving affinity and selectivity towards receptor heteromers. Through our research, we have identified A_1R-D_1R heteromer-selective A_1R agonists that have the potential to attenuate D_1R receptor activation in the direct pathway of the basal ganglia. By doing so, these agonists may alleviate motor activation in conditions like RLS.

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PO-039

The major metabolite of the PARP inhibitor rucaparib exhibits unique PLK2 inhibition unlocking a new anti-Parkinson strategy

Hu, H[1], Serra, C[2], Zhang, W[3,4], Scrivo, A[5,6], Fernández-Carasa, I[5,6], Consiglio, A[5,6,7], Aytes,A[3,4], Pujana, MA[3,4], Llebaria, A[2] and Antolin, AA[1,3,4,*]

[1] *The Institute of Cancer Research, London, UK*
[2] *Institut de Química Avançada de Catalunya (IQAC-CSIC), Barcelona, Spain.*
[3] *Catalan Institute of Oncology (ICO), Barcelona, Spain.*
[4] *Bellvitge Institute for Biomedical Research (IDIBELL), Barcelona, Spain.*
[5] *Bellvitge University Hospital-IDIBELL, Barcelona, Spain.*
[6] *University of Barcelona, Barcelona, Spain.*
[7] *University of Brescia, Brescia, Italy.*

E-mail: aantolin@idibell.cat

The (poly)pharmacology of drug metabolites is seldom comprehensively characterized in drug discovery and development. However, some drug metabolites can reach high plasma concentrations and display relevant *in vivo* activity, which may be distinct from its parent drug. Here, we use computational and experimental methods to comprehensively characterize the kinase polypharmacology of M324, the major metabolite of the FDA-approved PARP inhibitorrucaparib.[1] We experimentally demonstrate that M324 displays a distinct *in vitro* kinome profilefrom its parent drug, characterized by potent inhibition of GSK3A and PLK2 at clinically observed concentrations. These confirmed kinase activities of M324 could have potential implications for the efficacy and safety of rucaparib and therefore warrant further clinical investigation. Importantly, we identify synergy between the drug and the metabolite in prostatecancer cell lines and a complete reduction of alpha-synuclein accumulation in Parkinson's disease patient-specific dopamine neurons treated with the metabolite. These biological activities could be harnessed in the clinic or open new drug discovery opportunities in areas ofhigh unmet medical need. The study reported here highlights the importance of thoroughly characterizing the activity of significant drug metabolites to comprehensively understand drug response in the clinic and maximally exploiting our current drug arsenal in personalized and precision medicine.

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PO-040

Differential G-protein activation by cannabinoid ligands in *postmortem* human brain tissue.

Callado, L.F.[1,2,3], Bedia, I.[1], Vázquez-Durán, A.[1], Diez-Alarcia, R.[1,2,3], Urigüen, L.[1,2,3]

[1] *Department of Pharmacology, University of the Basque Country, UPV/EHU, Leioa, Bizkaia, Spain*
[2] *Centro de Investigación Biomédica en Red de Salud Mental CIBERSAM, Spain*
[3] *Instituto de Investigación Sanitaria Biobizkaia, Spain*

E-mail LF.callado@ehu.eus

The endocannabinoid system participates in the regulation of numerous physiological processes of the central nervous system (CNS). Moreover, different cannabinoid ligands have shown potential therapeutic effects, including antiepileptic, anti-inflammatory, analgesic and anticancer properties, highlighting the therapeutic potential of modulating this system.
Cannabinoid receptors (CB1/CB2) are members of the GPCR superfamily. They trigger the activation of several different intracellular signalling pathways depending on the ligand, the cellular and subcellular locations, and the presence of allosteric modulators. However, no study has compared G protein activation by different cannabinoid drugs in *postmortem* human brain tissue.
The objective of this work was to evaluate the functional activation of different Gα protein subunits by five structurally different cannabinoid ligands (THC, CBD, WIN 55,212-2, HU308 and HU210), in *postmortem* human brain membrane homogenates from prefrontal cortex and hippocampus. Additionally, the putative modulatory effect of CBD over WIN 55,212-2 signalling was also studied. [^{35}S]GTPγS binding assays coupled to immunoprecipitation with specific anti-Gα-protein antibodies were carried out to determine the agonist, antagonist or inverse agonist properties of the different cannabinoids over Gαi1-, Gαi2-, Gαi3-, Gαo-, Gαq/11-, Gαs-, Gαz- and Gα13-proteins activity. Furthermore, concentration response curves of WIN 55,212-2 induced Gα-protein stimulation were performed either in absence or in presence of 1 µM CBD.
The results showed that each ligand exhibits a different pattern of Gα subunit activation. In almost all cases, the effect was blocked in the presence of O2050, supporting the role of CB1/2 receptors on the observed activation. In general, signalling patterns in prefrontal cortex and hippocampus were similar. On the other hand, comparison of concentration-response curves for WIN 55,212-2 in the presence of CBD showed no significant effect of CBD over Gαo stimulation. Conversely, CBD hampered the activation of Gαi1 by WIN 55,212-2, increasing the EC_{50} values, and of Gαq/11, reducing the E_{max} values.
The present results demonstrate the functional selectivity of cannabinoid ligands in *postmortem* human brain. Although further studies are necessary for the characterization of the effect, CBD seems to act as a negative allosteric modulator of cannabinoid agonists at CB1/CB2 receptors. The results of this work can help understanding which specific signalling pathways are involved in the different pharmacological effects of cannabinoid ligands and facilitate their application in therapeutics.

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PO-041

Sex differences in the antidepressant-like responses induced by ketamine in adolescent rats: a dose-response study

Jordi Jornet-Plaza[1,2], **M. Julia García-Fuster**[1,2]

[1]*IUNICS, University of the Balearic Islands, Palma, Spain*
[2]*Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain*

E-mail: j.jornet@uib.es

In searching for novel pharmacological options to treat adolescent depression, ketamine, an N-methyl-D-aspartate receptor antagonist, recently approved as a fast-acting antidepressant for adult treatment-resistant patients, emerged as a great candidate to be explored. In this context, the present study was centered on evaluating the antidepressant-like potential of different doses of ketamine during adolescence in either naïve rats or previously exposed to early-life stress. To do so, whole litters of Sprague-Dawley rats were either exposed to maternal separation for 24 h (on post-natal, PND 9), or used as controls (naïve groups). During adolescence, male and female rats were treated (i.p.) with ketamine (1, 5 or 10 mg/kg) or vehicle (0.9% NaCl, 1 ml/kg) for 7 consecutive days (from PND, 33-39). Acute (30 min post injection on PND 33) or repeated (24 h post treatment on PND 40) antidepressant-like responses were measured in the forced- swim test (FST). Other behavioral tests were also performed after treatment to measure different dimensions of the potential antidepressant-like response: novelty suppressed feeding (NSF) and sucrose preference. So far, we have finished analyzing the data from the control naïve groups. The main behavioral results showed that ketamine induced an acute antidepressant-like effect observed as decreased immobility in the FST, which was dose- and sex-dependent, since only the dose of 10 mg/kg was efficacious, and exclusively in control female adolescence rats. These acute effects dissipated following the repeated treatment. Ketamine induced no significant changes in the NSF and SP tests for any of the experimental conditions tested. Overall, acute ketamine showed a promising dose-dependent antidepressant-like potential in adolescent female rats. This set of data is being completed by the analysis of the effects of ketamine on rats exposed to early-life stress. Moreover, we are also aiming at better characterizing the beneficial vs. negative effects following ketamine treatment during adolescence by evaluating the possible long-term addictive-like potential, as well as by figuring out the molecular underpinnings behind the observed sex-disparities.

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PO-042

Evaluating the antidepressant-like potential of cannabidiol in adult male and female rats: lack of hippocampal neurogenesis regulation

Gálvez-Melero, L., Ledesma-Corvi, S., Bis-Humbert, C., García-Fuster, M.J.

IUNICS, University of Balearic Islands, and IdISBa, Palma, Spain

E-mail: laura.galvez@uib.es

It has been demonstrated that cannabidiol displays certain antidepressant-like responses in rodents, which are dependent on the dose administered and on biological sex, since females seem more unresponsive and/or need higher dose regimens. Cannabidiol is known for its very complex pharmacological profile, since it interacts with multiple molecular targets. In this context, and given the accepted modulation of adult hippocampal neurogenesis by antidepressant drugs in general, and by cannabinoids in particular, it is hypothesized that its potential antidepressant-like responses might be mediated through this process. Our goal was therefore to characterize cannabidiol's effects in adult rats of both sexes while ascertaining whether there was a parallel regulation between its therapeutical response and hippocampal neurogenesis. To do so, following a 15 min pre-test session in the forced-swim test, where theanimals learnt that there was no escape, male and female Sprague-Dawley rats (i.p.) were treated with 3 pulses of cannabidiol (10 or 30 mg/kg) or vehicle (DMSO, 1 ml/kg) 23 h, 5 h and1 h before a 5-min forced-swim test session. Test sessions were videotaped to then scored the amount of time each rat spent immobile vs. active. A decrease in immobility paired with an increase in active behaviors (climbing or swimming) was considered a measure of an antidepressant-like response. Furthermore, we studied an early stage of adult hippocampal neurogenesis by immunohistochemistry, using Ki-67 as a marker of cell proliferation. The mainresults showed that both doses tested of cannabidiol exerted an antidepressant-like response (i.e., decreased immobility and increased climbing) in adult rats, but exclusively in male rats, reiterating the lack of efficacy in female rats. Moreover, cannabidiol did not modulate hippocampal cell proliferation in neither male nor female rats. Taken together, our findings revealed a sex-dependent response for the antidepressant-like effects of cannabidiol in rats. In addition, the early stage of hippocampal neurogenesis did not seem to be mediating the acute effects induced by the drug in male rats, since no parallel effects were observed. Future studies should aim at finding efficacious doses for female rats as well as the molecular mechanisms behind these sex-disparities.

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PO-043

Expression of TrkB, $5HT_{2A}$ and mGluR2 receptors in cell lineages derived from neural stem cells

Marco Taddei-Tardón, Bojan Kuridža and Juan F. López-Giménez

Instituto de Parasitología y Biomedicina "López Neyra" IPBLN-CSIC, Granada, España

jf.lopez.gimenez@csic.es

In recent years, psychedelics have gained popularity as potential therapeutical drugs in the treatment of mental disorders due to their capacity to induce neuroplasticity.

To increase the current comprehension of the molecular mechanisms underlying this neuroplasticity, we are studying the expression of serotonin $5HT_{2A}$ receptor (with whom psychedelics interact), metabotropic glutamate 2 receptor (mGluR2; which has been suggested to physically interact with $5HT_{2A}$) and TrkB receptor (whose activation has been suggested to be necessary for neuroplasticity to take place), using a cell culture model. In this model, proliferative neural stem cells (NSCs), obtained from fetal mouse cortical brain tissue, are differentiated into neurons and astrocytes in order to resemble the brain environment.

With the purpose of studying the expression of our receptors of interest we carry out immunocytochemistry assays of NSC cultures after 5 days of differentiation and Western blots of samples collected at the same stage. The 5-day differentiation period was established based on the visual detection of morphologically mature neurons, and subsequently validated by the expression of markers specific to this group, such as MAP2.

At this point in time, cultures are constituted essentially by astrocytes and neurons that possess complex dendritic arborization. Several antibody combinations are tested in order to establish the lineage where the receptors are expressed and if co-expression takes place (Table 1).

Table 1. Combination of antibodies used to establish the co-expression of receptors and their presence or absence in the different lineages that originate from NSC differentiation.

Antibodies	TrkB	$5HT_{2A}$	mGluR2	Nestin	Map2	GFAP
TrkB	n.d.	X	X	X	X	X
$5HT2_A$	n.d.	n.d.	X	X	X	X
mGluR2	n.d.	n.d.	n.d.	X	X	X

Our results suggest that TrkB is broadly expressed in both astrocytes and neurons, while mGluR2 and $5HT_{2A}$ are mainly expressed in neurons. Due to technical limitations, we could not test the co-expression of all three receptors in a single step, therefore we analyzed the co- expression of TrkB with mGluR2 and $5HT_{2A}$ separately.

Based on our results, we propose this cellular model as a useful tool to study pharmacological pathways targeting any of the aforementioned receptors, in both astrocytes and glutamatergic neurons.

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PO-044

A novel sEH inhibitor reduces neuroinflammation and improves mitochondrial dysfunction, rescuing cognitive impairment in 5xFAD mice.

Jarne-Ferrer, J.[1]; Griñán-Ferré, C.[1,2], Pérez, B.[3]; Jora, B.[4]; Codony, S.[4]; Vázquez, S.[4]; & Pallàs, M.[1,2].

[1]Department of Pharmacology and Therapeutic Chemistry, Faculty of Pharmacy and Food Sciences, Institut de Neurociències, Universitat de Barcelona, Avda. Joan XXIII, 27, 08028 Barcelona, Spain.
[2] Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.
[3]Department of Pharmacology, Therapeutics and Toxicology, Institute of Neuroscience, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.
[4]Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII, 27-31, E-08028, Barcelona, Spain.

E-mail: pallas@ub.edu

Neuroinflammation has been postulated as one of the leading causes of Alzheimer's Disease (AD). Then, targeting brain inflammation is a promising therapeutic strategy to cope with this neurodegenerative disease. In this regard, soluble epoxide hydrolase (sEH) enzyme promotes neuroinflammation, and is upregulated in the brains of AD patients. Taking this into account, its inhibition would be beneficial to stop neuroinflammation and promote a positive outcome in AD. Furthermore, UB-BJ-02 is a new and selective inhibitor of this enzyme, capable of crossing the blood-brain barrier.

In the present study, we tested this compound at a 5mg/Kg dose in 5-month-old 5xFAD mice, including males and females. Firstly, as we expected, behavioural tasks (NORT and OLT) demonstrated that UB-BJ-02 improves short- and long-term memory and spatial memories in the 5xFAD mice model. Secondly, we found a reduction of neuroinflammatory markers evaluated by gene expression such as *Il-1β, Tnf-α, Il-6* and *Trem2* in the brain of 5xFAD-treated mice and a significant decrease in the number of Aβ plaques. Finally, we also explored mitochondrial dysfunction, that it is also altered in AD pathological cascade. Here, we found a substantial improvement in several markers such as DRP1 , OPA1 and PGC1-α after sEH inhibition. This suggested that sEH modulates mitochondrial dynamics and, therefore, that part of the beneficial effects observed on cognition come from the reduction of these mitochondrial alterations.

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PO-045

Characterization of m6A and METTL3 in different AD mice models

Irisarri, A.[1]; Bellver-Sanchis, A.[1]; Valle-Garcia, D.[2]; Pallàs, M.[1,3]; & Griñán-Ferré, C.[1,3].

[1]*Department of Pharmacology and Therapeutic Chemistry, Institut de Neurociències-Universitat de Barcelona, Avda. Joan XXIII, 27. 08028 Barcelona, Spain.*

[2]*Institute of Biotechnology, National Autonomous University of Mexico (UNAM).*

[3]*Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.*

E-mail: christian.grinan@ub.edu

Alzheimer's Disease (AD) is a progressive brain disorder known to be the most common type of dementia in aging population, yet therapeutical curative approaches remain unmet. Epigenetics is one of the many factors that contribute to the development of the disease, and it is considered a potential therapeutical target on the search of novel pharmacological drugs. Particularly, N6-methyladenosine (m6A) RNA methylation has been shown to be one of the main dynamically reversible epigenetic modifications contributing to the progression of neural degeneration. This way, abnormal levels of m6A have been shown to be involved in the regulation of gene expression programs in various neurodegenerative diseases such as AD. Its methylation is catalyzed by the combined action of different groups of enzymes including, writers, or methylases, and erasers, or demethylases, and it is later recognized and interpreted by YTH domain family proteins known as readers. Among writers, methyltransferase-like 3 (METTL3) protein is described as a main contributor to the pathophysiology of AD.

Here, we characterized m6A levels in various AD mice models, which included 5xFAD and 3xTg-AD, (both transgenic for featuring severe AD pathology by the overexpression of toxic Aβ plaques) and SAMP8 (a senescence-accelerated mouse model used widely in aging research). Results provided evidence of a significant decrease of methylated RNA in each corresponding disease condition, consistent with the group's previous research on human AD brains. We also studied m6A regulatory enzymes gene expression obtained by qPCR and demonstrated a decreasing tendency of *Mettl3* in all of them, significantly different in SAMP8. We also observed a significant increase of *Fto* in 3xTg-AD and SAMP8, while interestingly 5xFAD showed a decreased expression. Furthermore, readers are responsible for the signalling of m6A changing levels were also decreased in all the models, suggesting a diminution of the methylation itself. Besides, we studied METTL3 protein levels and showed a significant decrease in SAMP8, although no changes were reported in the rest of the animal models. Therefore, our results suggest SAMP8 as a possible murine model for studying the effect of pharmacological METTL3 activation and promoting future research on m6A regulatory enzymes as novel targets for developing therapeutic drugs and treatments for AD.

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PO-046

Could intraoperative cerebral oxygen desaturation predict postoperative cognitive dysfunction in the elderly subjected to cardiac surgery.

Bellido-Estevez, I.; Blanco Reina, E.; Aldana-Díaz E.; Valverde-Junguito JL; Gomez-Luque A.
Department of Pharmacology. School of Medicine, IBIMA, Vithas Xanit International Hospital, Virgen de la Victoria University Hospital, Malaga, Spain,

E-mail: ibellido@uma.es

Background: Cognitive decline following cardiac surgery is associated with decreased quality of life, functional capacity, and the ability to perform activities of daily living. Cognitive decline presence in elderly will be associated with increased mortality, length of stay and hospital readmissions. Understanding that risk and protective factors for cognitive decline after cardiac surgery has critical clinical implications, including more precise targeting of preoperative and perioperative interventions and the development of a sensitive risk screening tool for these outcomes, the use of a prediction tool for cognitive decline after cardiac surgery setting could lead to earlier intervention opportunities, greater prognosis, and, in turn, better patient management. Intraoperative cerebral desaturation (rSO_2) measured by near-infrared spectroscopy cerebral/somatic oximeter (INVOS®) had prognostic relevance thus it reflected cerebral and systemic oxygen balance. Intraoperative rSO_2 and depth of anaesthesia are related to postoperative cognitive dysfunction in cardiac surgery.
We evaluated if intraoperative cerebral desaturation (rSO_2) is related to postoperative cognitive dysfunction in cardiac surgery in the elderly.
Materials and Methods: A prospective, before and after, longitudinal study in ASA class II-IV patients scheduled for cardiac surgery undergoing intravenous general anaesthesia with remifentanil plus propofol was done. Clinical and surgical parameters, cardiopulmonary function, rSO_2 measured by INVOS® and depth of anaesthesia measured by bispectral index (BIS) were continuously recorded and corrected throughout surgery. Standardized test measuring capacity of attention, language, verbal and visual memory, visual-spatial orientation, executive, psychomotor and motor capacity as well as independence in daily life and the perception of the patient of their psychological situation (WAIS III, Mini Mental Test, trail making test a/b y digit & symbol, WSM III list of words, digit span, executive function letters and numbers (L&N), Stroop test, STAIC, EPQ-R, Yesavage, QOLIE-31 and Barthel test) were used to assessed the cognitive function before and 7 days after surgery.
Results and Discussion: Patients (n=44, 77.3% male, aged 59.9±1.9 years old, 65.9% < 65 years vs. 34.1% ≥65 years), scheduled to coronary (36.4%), aortic valve replacement (18.2%), mitral valve replacement (13.6%), coronary plus valve replacement (13.6%) and others (18.2%) surgery, on pump 98.4% were enrolled. Reduction of rSO_2 higher than 15% at the end of the surgery compared with basal values were related with significantly lower values of concentration-auditive memory (WSM III) and concentration-visual memory (trail making test) and executive function (executive function L&N test) ($p< 0.05$), and with non-significant lower values of capacity of attention, language, visual-spatial orientation, and visual memory in patient over 65 years old, 7 days after surgery.
Conclusion: rSO_2 may help to predicts early postoperative cognitive dysfunction in the elderly subjected to cardiac surgery.

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PO-047

Heteromer-selective antagonists for the adenosine A_1-dopamine D_1 receptor heterotetramer

Llopart, N.[1,2], Casadó-Anguera, V.[1,2,3], Alonso-Carrasco, A.[1,2], Moreno, E.[1,2], Casadó, V.[1,2]

[1]*Nanomedicine and Molecular Neuropharmacology, Consolidated Research Group of the Generalitat of Catalonia, Barcelona*
[2]*Institute of Biomedicine of the University of Barcelona, Barcelona, Spain*
[3]*Universitat Pompeu Fabra, Barcelona, Spain.*

E-mail: nllopart@ub.edu

Spinal cord injury (SCI) is a neurologic condition that directly results in motor and sensory dysfunctions. A common hallmark in that pathology is a dysfunction of the dopaminergic and adenosinergic systems. Dopamine and adenosine receptors are involved in crucial physiological processes and, as other G-protein coupled receptors, form homo- and hetero-oligomers. That oligomers show pharmacological and functional properties different from those of the constituent monomers. Evidence has shown the existence of the adenosine A_1 receptor-dopamine D_1 receptor (A_1R-D_1R) heteromer, with high importance in motor control. Specifically, to solve the reduced motor activity present in SCI it is needed the blockade of A_1R.

In this study, we wanted to search for heteromer selective antagonists at the level of ligand binding and G protein activation which were able to block the inhibitory action of the endogenous adenosine through A_1R on the motor activity exerted by D_1R. We also wanted to analyse their ability to perform cross-antagonism to find a heteromer selective ligand that has a lower potency to perform cross-antagonism.

We determined the affinity by radioligand binding assays, and the potency and efficacy a library of 12 commercially available A_1R antagonists. Furthermore, we analysed the heteromer's fingerprint (cross-antagonism) related to intracellular signalling, such as intracellular cAMP levels. Statistical significance was determined through appropriate statistical methods such as t-tests, F-test for curve fitting, or repeated measures ANOVA.

We have identified the A_1R ligand, CGH2466 which may be a non-orthosteric ligand in both, the homomer and the heteromer. With CODA-RET assay, we have found that DPCPX in $G\alpha_{i1}$ protein subunit, and KW3902, PSB36, SLV320, DPCPX, caffeine, and theophylline in $G\alpha_{o1}$ protein subunit have a better potency for the heteromer. Whereas, CPT, SLV320 in $G\alpha_{i1}$ protein subunit, and PQ69, CPT, and XAC in $G\alpha_{o1}$ protein subunit have a higher potency for the homomer. There were no differences in efficacy between the homomer and the heteromer. We selected as heteromer selective ligands KW3902, PSB36, DPCPX, SLV320. With these antagonists, we performed an intracellular cAMP accumulation assay to analyse their ability to perform cross-antagonism on D_1R. All ligands were able to perform cross-antagonism having the higher potency SLV320, followed by DPCPX, PSB36, and KW39 with the lowest potency.

In the future, KW39 and PSB36 could be used as a base for a future design of new drugs to treat SCI and other motor deficits.

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PO-048

Effectiveness of Sugammadex in Postoperative Cognitive Function: A Translational Study design

Lopez de Coca, T.[1,3], Muedra, V.[1,2,3], Hernández-Rabaza V[1]., Rodilla V[1]., Villagrasa V[1,3].

[1]*Universidad CEU Cardenal Herrera. CEU Universities. Valencia, Spain*
[2]*University La Ribera Hospital, Valencia, Spain*
[3]*Catedra DeCo MICOF UCH*

E-mail: teresa.lopezperez@uchceu.es

A persistent belief suggests that elderly patients (>60 years) undergoing surgical procedures may experience accelerated cognitive impairment (CI) beyond the normal aging process, particularly in relation to surgery and general anesthesia. Cardiac surgery, for example, has been associated with postoperative CI. Considering the current increase in life expectancy and the growing number of geriatric patients undergoing cardiac surgery, this complication can significantly burden the healthcare system. Recent studies have proposed that sugammadex (SG), a modified gamma-cyclodextrin designed to counteract the effects of rocuronium, may enhance the recovery of consciousness after general anesthesia. The process of encapsulation by SG can improve the aqueous solubility of hydrophobic compounds, enabling easier administration, absorption, and delivery to their intended sites in the body. However, the role of SG in postoperative cognitive function remains poorly understood, especially in primary care settings.

The objective of this study was to evaluate the efficacy of SG compared to neostigmine (NG), used as a reversal agent for neuromuscular blockade, on postoperative cognitive function in surgical patients. A prospective, observational, single-center pilot study was conducted with two study groups: SG (n=14) and NG (n=7). Cognitive recovery was assessed at various time points using the Postoperative Quality Recovery Scale. In order to explore the potential relationship between hippocampal inflammation and the occurrence of CI, as well as the effects of SG, an experimental study was conducted using Wistar rats that underwent Morris water maze training before the intervention. Hippocampal inflammation and the impact of surgical intervention on the inflammatory response were evaluated using Western blot and immunohistochemistry techniques. The study received approval from the Ethics Committee under code 2018/VSC/PEA/0081.

Quantitative analysis revealed a more favorable overall cognitive recovery in patients treated with SG compared to NG (85.7% vs. 42.9%, p-value: 0.04). These findings align with clinical evidence demonstrating an increased awakening of consciousness in patients who received SG as a reversal agent. In the experimental study, results indicated a reduction in microglial activation in the surgery group treated with SG compared to the saline group (p-value: 0.003). These data suggest a potential effect of SG, as gamma-cyclodextrin, in forming inclusion complexes, including those with volatile molecules. By encapsulating these molecules within its cavity, SG effectively traps them and could reduce their release into the environment by modify the physicochemical properties of inflammation cytokines, such as solubility, stability, and bioavailability, thereby enhancing the sensory characteristics of guest molecules.

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PO-049

Dysfunctional management of serotonin by platelets as a biomarker for the diagnosis and treatment of Parkinson's Disease

Borges, R.[1]; Montenegro, P.[1]; Méndez, A.[1]; González-Brito, R.[1]; Lorenzo, J.N.[2]; Pueyo, M.[3]

[1]Pharmacology Unit, Faculty of Medicine. Universidad de La Laguna. [2]Service of Neurology Hospital Universitario La Candelaria.,[3]Service of Neurology, Hospital Universitario de Canarias. Tenerife. Spain.

E-mail: rborges@ull.edu.es

Since platelets use similar, if not the same, mechanisms to accumulate serotonin as dopaminergic neurons to store dopamine in their secretory vesicles; we wonder if a functional failure in the handling of serotonin would reflect what is happening in dopaminergic neurons and in others of aminergic lineage. This could be diagnostic and prognostic platform.

The presence of high cytosolic concentrations of dopamine and its metabolites in neurons has been associated with increased vulnerability associated with Parkinson's disease (PD). More than 99% of the amines are confined to secretory vesicles, making these structures crucial for keeping cytosolic dopamine low. Platelets have been used as cell models of various neurological diseases.

We have used freshly isolated blood platelets from 114 patients with PD, 168 control individuals and 42 patients with parkinsonism (iatrogenic origin, multi-systemic atrophy, dementia associated with Lewy bodies, progressive supranuclear palsy or parkinsonism of vascular origin). We have carried out a functional assay of serotonin handling in human platelets in which its basal content and its capacity for accumulation, secretion and spontaneous loss have been quantified.

We found a drastic decrease in serotonin content and uptake, as well as a decrease in thrombin-induced release in platelets from PD patients, but not in most cases of parkinsonism. Platelets from PD patients had impaired ability to retain serotonin in secretory vesicles.

These findings indicate a functional impairment of the secretory vesicles for amine handling in patients with PD. We will discuss its use of this technique as i) a biomarker, ii) its potential capacity for subclinical detection of PD and iii) how these tests can serve as a platform to screen disease-modifying drugs.

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INFLAMMATION

PO-050

Anti-inflammatory and anti-quorum sensing effect of a novel biotech ingredient for treatment of acne

Cañellas-Santos, M. Rosell-Vives, E. Montell, L. Fernandez-Campos, F.
Laboratory Reig Jofre, Av. de les Flors s/n, 08970 Sant Joan Despí, Barcelona, Spain

E-mail: mcanellas@reigjofre.com

Acne vulgaris is characterized by an imbalance of bacterial populations due to uncontrolled *Cutibacterium acnes* (*C. acnes*) proliferation as an opportunistic pathogen, inducing a dysbiosis in the skin microbiota. This proliferation is mediated through interbacterial communication mechanism (quorum sensing) which increases virulence factors such as biofilm formation. *Morinda citrifolia* (*M. citrifolia*) is a plant known for its anti-microbial activity. *Camellia sinensis* (*C. sinensis*) contains several compounds with described anti-inflammatory and antibacterial properties. The aim of this work was to investigate the anti-inflammatory properties of a mixture (1:1 ratio) of *M. citrifolia* and *C. sinensis* callus culture lysate developed for this purpose, as well as its potential antibiofilm activity and virulence factors modulation properties against *C. acnes*. *C. sinensis* and *M. citrifolia* callus culture lysate was developed in collaboration with Vytrus Biotech corporation (Terrassa, Spain) through a methodology based in *in vitro* cultures of plant stem cells. The biotech ingredient was afterwards elicited with different stressors to modulate secondary metabolite production with a relevant potential anti-inflammatory activity. *C. acnes* ATCC 6919, one of the most severe strains in acne patients (phylotype IA_1), was selected as the inflammatory stimulus. Each condition was tested in biological triplicates. The expression of IL-8, TNF-α, and CXCL1 was quantified by RT-PCR. NF-kB translocation was measured by immunofluorescence staining. The antibiofilm activity was carried out with mature biofilms. Lipase activity was determined in biofilm cultures using a fluorescent substrate. The quorum sensing activity was measured through a key signaling molecule, the Autoinducer 2 (AI-2), which was quantified employing *V. harveyi* strain BB170.
Keratinocyte HaCaT cells incubated with thermoinactivated *C. acnes* exhibited an increase on the production of the tested interleukin IL-8 (2.4-fold increase), TNF-a (2.3-fold increase), and CXCL1 (1.9-fold increase). Co-incubation with the callus lysate (0.5% w/w) significantly reduced the expression of all these pro-inflammatory cytokines ($p<0.001$): IL-8 exhibited an expression after treatment of 47.67 ± 8.96%, TNF-a, 36.00 ± 3.61%, and CXCL1, 60.00 ± 3.61%, which corresponds to a reduction of 52.33, 64.00, and 40.00%, respectively. The lysate was also able to inhibit the NF-kB translocation to the nucleus. The decline in CFU/mL on the biofilms exerted by the compound (10% w/w) was 3.54 ± 0.63 $\log_{10}$-units, while the logarithmic decrease on planktonic cells was 1.10 ± 0.11. The ingredient also inhibited the lipase activity by 66.17 ± 8.34% compared to the *C. acnes* control ($p<0.05$). The results showed that *C. acnes* increased AI-2 production considerably. The lysate was able to reduce these levels by 364.10 ± 157.80% ($p<0.05$).
Interestingly, instead of obtaining a high bactericidal effect, the product seemed to limit the proliferation of *C. acnes* and restrict the induction of virulence factors, suggesting an interesting role to reduce acne-related symptoms without eradication of *C. acnes*, which is, in fact, part of the natural skin microbiome. Furthermore, the present findings demonstrated a relevant anti-inflammatory action and a quorum-quenching activity of this novel ingredient, opening novel avenues in the treatment of acne for the development of new antimicrobial skin therapies based on biotech plant callus lysates.

PO-051

Dietary Oleacein attenuates Lupus Nephritis in Balb/C Pristane-Induced Mice.

Muñoz García R [1,3], Sánchez-Hidalgo M[1,3], Vázquez-Román MV[2], de Sotomayor MA[1], González-Rodríguez ML[1], Alcarranza M[1,3], Ortega-Vidal J[4], Alarcón-de-la-Lastra C[1,3]

1Faculty of Pharmacy, University of Seville, 41012 Seville, Spain. 2Faculty of Medicine, University of Seville, 41012 Seville, Spain.
3 Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla,41013 Seville, Spain
4 Faculty of Experimental Sciences,Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Jaén, 23071 Jaén, Spain
E-mail: rociomungar@gmail.com; rmgarcia@us.es

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterized by abnormal regulation of innate and adaptive immune response, impaired levels of T-helper cell (Th) cytokines, autoantibodies production and the deposition of immune complexes (1). Oleacein (OLA), one of the main extra virgin olive oil derived secoiridoid, has been shown to modulate oxidative and inflammatory responses in a variety of pathological conditions (2–4); nevertheless, its potential benefit in SLE is still unknown. The present work aimed to evaluatethe preventive effects of OLA dietary treatment in the development of lupus nephritis in a murine pristane-induced SLE model, evaluating the renal damage induced and exploring the signaling pathways involved. 12 weeks-old female BALB/c mice were injected with pristane and fed with OLA enriched diet (0.01 % (w/w)) for 24 weeks. General morphological and histopathological changes were assessed by hematoxylin-eosin, glomerular status and fibrotic changes were analyzed by periodic acid-Schiff (PAS) and Masson's trichrome (MT) respectively. Besides, the presence of immune complexes was evaluated by immunohistochemistry and immunofluorescence. Protein expression was evaluated by Western blotting. OLA enriched diet reestablished kidney architecture and decreased the deposition of immune complexes. These protective effects could be related to the modulation JAK3/STAT-3, Nf-κB and inflammasome signaling pathways. This work introduces OLA asa promising nutraceutical compound useful in SLE management. However, additional research is necessary to explore the beneficial effects of OLA mainly to evaluate these effects in clinical trials.

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PO-052

Photoprotective effects of a polyphenol-carotenoid combination in an *in vitro* model of human skin.

García-Gil S[1], Rodríguez-Luna A[1,2], Ávila Román J[1], Montero P[3], Fernández-Romero AM[4], González-Rodríguez ML[4], Gómez-Hurtado MA[5], Cortijo J[3], Motilva V[1], Talero E[1]

1. Department of Pharmacology, Faculty of Pharmacy, University of Seville, Spain
2. Department of Basic Health Sciences, Faculty of Health Sciences, Universidad Rey Juan Carlos, Spain.
3. Department of Pharmacology, Faculty of Medicine, University of Valencia, Spain.
4. Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Sevilla, Spain
5. Instituto Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, México

E-mail: sgarcia18@us.es

Sunlight exposure is one of the principal environmental risk factors for skin cancer. Ultraviolet (UV) radiation can produce redness, swelling and skin inflammation, as well as DNA damage[1]. Due to their antioxidant and anti-inflammatory properties, some natural products, such as polyphenols and carotenoids, are being studied as photoprotection boosters[2]. Currently, population is more aware with skin care and with the environment. For that reason, the search of new sunscreens based on natural products is growing. The present study aimed to evaluate the photoprotective potential of the combination of a polyphenol (MAG-48) and a carotenoid (F-48) formulated in a liposomal topical gel by using an *in vitro* model of human skin (Phenion® ST). The skin model was treated with the formulation for 4 h and then irradiated with a solar simulator (12 J/cm^2). Skin sections were stained with haematoxylin/eosin for histological analysis. Different pro-inflammatory markers (IL-6, IL-8, IL-1α, MMP-1, and MMP-9) were measured by ELISA. Gene expression was investigated using quantitative real-time PCR. Histological studies showed that the tissues pre-treated with the liposomal gel maintained the architecture of the skin layers and presented a lower number of sunburn cells, in comparison with the control irradiated tissues. Solar radiation exposure produced high levels of pro-inflammatory markers. However, pre-treatment of Phenion® ST with the formulation presented a significant decrease of pro-inflammatory cytokines as well as of MMP-1 and MMP-9 expression. In addition, PCR analysis reported that the collagen 1A1, involucrin and elastin gene expressions were notably increased after treatment with the combination. These results suggest that this polyphenol-carotenoid combination exerts photo-protective effects and could be a promising natural strategy in the prevention of solar skin damage as photo-aging and skin cancer.

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PO-053

Exploring JNK Pathway Influence on Psoriasis Dynamics in a Human Reconstructed Skin Model

Carvajal, V.[1,2], Pascual García, D.[1], Andrés Ejarque, R.M.[3], Ferrándiz, M.L.[1,4], Terencio, M.C.[1,4], Montesinos, M.C.[1,4]

[1]University of Valencia, Spain, [2]Universidad Miguel Hernández, Elche, Spain, [3]King's College., London, UK, [4]IDM, Valencia, Spain

E-mail: valentina.carvajal@goumh.umh.es

Psoriasis is a chronic inflammatory skin disorder characterized by dysregulated keratinocytes, amplified immune cell responses, and abnormal skin remodelling. The interplay among these cell types contributes to the disease pathogenesis (1). Previous studies have indicated a downregulation of JNK pathways in psoriatic fibroblasts under inflammatory conditions, accompanied by a pro-inflammatory activation of macrophages (1). In this study, we aim to induce a psoriatic-like response in a Reconstructed Human Skin model (RHS) and explore the effect of inhibiting the JNK pathway on normal skin morphology and psoriatic biomarkers. By assessing the impact of JNK pathway inhibition, we aim to enhance our understanding of its role in the possible modulation of psoriatic features.

RHS models (Labskin, Innovenn, York, UK) were set under two conditions: unstimulated, and stimulated with TNF-α/IL-22 (10µg/mL) to induce psoriasis. Each condition was treated under presence or absence of the JNK inhibitor SP660125 (10-50 µM) for 16 days. Skin morphology, psoriatic and skin barrier markers were assessed by immunohistochemistry and immunoassay.

Psoriatic plaques were formed following treatment under stimulated conditions. This result was supported by an increase in psoriatic biomarkers Human Beta-Defensin 2 (hBD2) (46.5 ±14.9 pg/mg protein *vs* 21.6±6.2 pg/mg protein), and SKALP-Elafin (12.5 ±2.3ng/mg protein *vs* 9.6 ±1.9ng/mg protein) under psoriatic stimuli compared to unstimulated condition, respectively. The treatment with SP660125 demonstrated a dose-dependent decrease in hBD2 in stimulated RHS suggesting a potential modulating role for this biomarker. However, the use of SP660125 at 50µM resulted in a significant increase in SKALP-Elafin levels in unstimulated RHS (14.5±3.4 ng/mg protein *vs* 9.6±1.9 ng/mg protein) implying a detrimental effect on skin homeostasis. Additionally, the chemokine IL-8 also exhibits a significant increase under psoriatic stimulation (161.9±26.8 pg/mg protein) respect to unstimulated (66.7±17.1 pg/mg protein). Under both conditions, treatment with SP660125 at 10µM produced a decrease, whereas there is no further effect with a higher concentration. These findings are controversial but may be attributed to the non-specific production of IL-8 by fibroblast and keratinocytes, highlighting the distinct roles of these cell types in this model.

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PO-054

Exploring the potential beneficial effect of plant sterols on intestinal inflammation: Focus on lipocalin 2 as a non-invasive biomarker in a murine colitis model

Makran M., Giner R.M., Cilla A., Garcia-Llatas G. & Recio M.C.

Faculty of Pharmacy, University of Valencia. Av. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

E-mail: maria.c.recio@uv.es

Plant sterols (PS) are natural compounds that have shown various health benefits, including potential anti-inflammatory properties, even at gastrointestinal level[1]. Lipocalin 2 (Lcn-2) is a protein secreted by intestinal epithelial cells related to immune response, serving as a non-invasive biomarker to assess the effectiveness of potential therapeutic interventions in reducing inflammation[2]. This study aims at investigating the anti-inflammatory effect of PS by measuring Lcn-2 secretion in a murine model of chronic ulcerative colitis. Female C57BL/6J mice ($n = 34$) were exposed to 1.5% (w/v) dextran sodium sulfate (DSS) in drinking water *ad libitum* for three 5-day periods, with 10-day rest intervals in between. The mice received daily PS dietary supplement (PS-DS, 35 mg PS/kg, group 1) or placebo (providing excipients that are present in the PS-DS, group 2) by oral gavage throughout the experiment. In addition, other groups received PS-DS (group 3) or placebo (group 4) for a period of 30 days prior to the first DSS exposure to determine whether the PS pre-treatment could have an effect in reducing inflammation. Fresh feces were collected before and after each DSS cycle and Lcn-2 fecal concentration was analysed by enzyme-linked immunoassay (ELISA)[2]. In the first cycle of DSS, PS-DS alleviates the increase of Lcn-2 fecal concentration compared to their respective control groups, both with the treatment (group 1: 37 to 7495 *vs*. group 2: 37 to 14231 pg/mg) and with the pre-treatment (group 3: 29 to 6196 *vs*. group 4: 27 to 9676 pg/mg). Although in the second and third cycles the increase of Lcn-2 concentration was similar in control and PS-DS groups, the absolute concentrations were significantly lower in the mice treated (group 1: 4665– 5001 to 11465–13537 *vs*. group 2: 6512–7253 to 18429–19109 pg/mg) and pre-treated with PS-DS (group 3: 2846–5454 to 10146–12016 *vs*. group 4: 4686–8986 to 15806–19586 pg/mg). The findings of the study indicated that the treatment with PS-DS resulted in a reduction in Lnc-2 fecal concentration in mice with chronic ulcerative colitis. This fact suggests that PS may have anti-inflammatory properties and could potentially modulate the inflammatory response in the colon. However, the pre-treatment with PS-DS does not appear to enhance the anti-inflammatory effect when compared to non-pre-treated mice, implying that the timing of the administration of PS does not significantly impact their ability to reduce inflammation in this context.

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PO-055

The absence of non-tissue-specific alkaline phosphatase in intestinal epithelial cells causes increased infiltration but reduced expression of inflammatory markers during experimental colitis

Tena-Garitaonaindia M[1], Ceacero-Heras D[1], Córdova S[1], Seguí A[1], Millán JL[2], Sánchez de Medina F[1], Martínez-Augustin O[1].

[1]*University of Granada, Granada, Spain*
[2]*Sanford Children's Health Research Center, La Jolla, EEUU*

mireiatena@ugr.es

Alkaline phosphatases (APs) catalyze the hydrolysis of phosphate groups from different substrates. Recently, APs have received attention due to their protective role in inflammation and their implication in intestinal barrier function. It has been described that there is an increased expression of the tissue-nonspecific AP (TNAP) isozyme during intestinal inflammation, not only due to the infiltration of immune cells, but also to the increased expression of TNAP in intestinal epithelial cells (IECs). However, the specific physiological role of TNAP in the intestinal epithelium cells during inflammation is not well known. We studied the role of TNAP in experimental colitis using epithelial-specific *Alpl* deficient mice ($Alpl_{flox/flox}$-Villin-CreERT2, referred to as $Alpl^{IEC-/-}$) employing dextran sodium sulfate (DSS) at 2.5 % for 7 days. Mice receiving DSS exhibited substantial loss of body weight between days 4 and 7, which was enhanced in $Alpl^{IEC-/-}$ mice. The disease activity index (DAI) score was increased early after DSS exposure in $Alpl^{IEC-/-}$ mice owing to early bleeding and sticky stools, although it was later normalized. No differences were observed between genotypes in colon shortening. Histopathological assessment of the colon revealed a higher leukocyte infiltration in DSS-treated $Alpl^{IEC-/-}$ compared to WT mice. However, $Alpl^{IEC-/-}$ mice exhibited an attenuated expression of various inflammatory response markers measured by qRT-PCR, including *S100a8*, *Il6* and *Tnf*. Colonic AP activity was greater in DSS-treated mice, with no differences between genotypes. Interestingly, $Alpl^{IEC-/-}$ mice displayed higher colonic expression of genes involved in mucosal barrier maintenance, *Muc4* and *Tff3*. This increased expression was observed both in baseline conditions and with DSS administration. Lack of *Alpl* in the intestinal epithelium also caused an increased expression of genes related to the signalling pathway of LPS (*Cd14* and *Lbp*) in the liver. Therefore, $Alpl^{IEC-/-}$ mice present greater colonic infiltration and histological damage but lower expression of proinflammatory markers in the intestine.

PO-056

Ménière disease and interleukin 17A

Gayo-Abeleira, I[1], Zaragozá-Arnáez, F[2], Villaescusa, L[1], Zaragozá, F[1], Zaragozá, C[1].

[1] *University of Alcalá, Madrid. Spain.*
[2] *University of Alfonso X, Madrid. Spain.*

E-mail: Zaragozá, C.

Background: Ménière disease (MD) is a long-lasting disorder of the inner ear characterized by episodes different episodes of vertigo lasting from 20 minutes to hours, low to middle frequencies sensorineural hearing loss, episodic vestibular symptoms, tinnitus, and aural fullness, associated with several comorbidities, such as migraine or autoimmune disorders (Girasoli L, 2018). Patients with MD have signs of endolymphatic hydrops as the endolymphatic hydrops will not explain all symptoms of MD. The aim of medical treatment for MD is to control symptoms of MD, reducing impact of vertigo (Li S, 2022). The conservative treatments are used whatever the hearing function, as destructive ones are preferentially used in patients with hearing loss (Nevoux J, 2018). First, conservative treatments include betahistine, acetazolamide, intratympanic corticosteroids and conservative surgery as endolymphatic sac surgery. On the other hand, destructive treatments involve intratympanic gentamicin and ablative techniques (Jacques M, 2018). Basal levels of certain proinflammatory cytokines could be increased in some MD patients (Frejo L, 2022).
Psoriasis is a chronic and inflammatory skin disease. The dysregulation of cytokines signaling is common in this pathology, generally leading to an increase in inflammation and joint damage (Amstrong W, 2020). A number of targeted biologic therapies inhibiting tumor necrosis factor alpha (TNF-α), interleukin (IL) 23 and IL-17A cytokine signaling are approved to treat this disease. Elevated expression of proinflammatory cytokine IL-17A is a future of the pathogenesis of the psoriasis disease cluster and represents a common therapeutic target across this indication. IL-17A induce keratinocyte mediated inflammation in psoriatic lesions. The monoclonal antibody secukinumab is approved by the European Commission for the treatment of patients with psoriasis. (Kolbinger F, 2022).
Objective: to correlate two autoimmune pathologies to define a common treatment.
Results: Recently, MD have been linked with autoimmune disorders, although a consistent target has not yet been found.We have observed that, in certain cases, there is comorbidity between psoriasis and MD. Since IL-17A is overexpressed in MD and there are monoclonal antibodies that block this cytokine, such as secukinumab, we have verified high positive results simultaneously for both diseases in case of comorbidity. These observations suggest starting the necessary clinical studies to corroborate it.
Conclusions: Following these observations, we propose a therapeutic repositioning of secukinumab, suggesting the use in MD´s treatment, for the unmet needs in this field.

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PO-057

Fibroblast-keratinocyte interplay in skin homeostasis

Riske A.[1], Carvajal V.[1,2], Valverde M.[1], Arasa J.[1], Noguera MA.[1], Terencio MC.[1,3], Montesinos MC.[1,3]

[1]University of Valencia, Spain, [2]Universidad Miguel Hernández, Elche, Spain, [3]IDM, Valencia, Spain

E-mail: Carmen.terencio@uv.es

Dermal fibroblasts are key players in maintaining skin homeostasis. They proliferate and migrate to efficiently heal cutaneous wounds, and they also display immunomodulatory effects through the release cytokines, prostanoids and other mediators. Thus, soluble factors released by dermal fibroblasts via the JNK pathway are required for efficient keratinocyte differentiation (1). We recently showed that a diminished JNK activity in psoriatic fibroblasts resulted in decreased release of PGE_2 and contributed to the persistence of skin inflammation (2). In the present study, we sought to determine the involvement of JNK signaling in fibroblast motility in an *in vitro* wound scratch assay and on keratinocyte proliferation. In addition, we analyzed the potential anti-inflammatory role of PGE_2 from fibroblast supernatants on keratinocyte response.

Human fibroblasts and keratinocytes were isolated from foreskins of adult healthy donors, after dispase II treatment and digestion with collagenase IA (dermis) or trypsin (epidermis). Fibroblasts were cultured in DMEM/F12 HAM medium supplemented with 10% FBS. Keratinocytes were grown in KGM media in a serum-free low-Ca^{2+}. Scratch wound assay was performed in fibroblasts seeded into a 12-well culture plate ($2x10^5$ cells/well) in the presence or absence of the JNK-inhibitor SP600125 (10-50µM). MTT assay was performed in fibroblasts and keratinocytes in 24-well culture plates (10^5 cells/well). PGE_2 levels were determined in supernatants of fibroblasts stimulated with IL-1β (2.5 g/ml) and added to keratinocytes to determine IL-8 release after stimulation with TNF α (10 ng/ml).

Results showed that basal JNK activity is required for fibroblast motility, since a low concentration of SP600125 (10 µM) already sufficed to significantly block cell migration (50% inhibition at 24h) without affecting cell viability. JNK inhibition caused a slight decrease of keratinocyte proliferation at 48h (16.5%). Interestingly, when TNFα-stimulated keratinocytes where treated with PGE_2 at the same concentration present in the supernatants of stimulated fibroblasts (30 ng/ml), the release of IL-8 was significantly reduced (317±21.9 pg/ml *vs* 663 ± 19.4 pg/ml in control cells).
These results are consistent with a role of dermal fibroblasts-derived PGE_2 in the regulation of the inflammatory response in keratinocytes. Since PGE_2 generation in fibroblasts is mediated by JNK pathway, further studies in fibroblast/keratinocytes co-cultures in the presence of a JNK inhibition will be performed.

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PO-058

Effect of sodium nitroprusside on the disruption of the brain endothelial barrier induced by inflammation

Nuria Seoane[1,2], Aitor Picos[1,2], Amalia Dolga[3,4], Martina Schmidt[3,4], Manuel Campos-Toimil[1,2], Dolores Viña[1,2]

1. Pharmacology of Chronic Diseases, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela, Spain.
2. Department of Pharmacology, Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, Spain.
3. Department of Molecular Pharmacology, Faculty of Science and Engineering, Groningen Research Institute of Pharmacy (GRIP), University of Groningen, Groningen, The Netherlands.
4. Groningen Research Institute for Asthma and COPD (GRIAC), University Medical Center of Groningen (UMCG), University of Groningen, Groningen, The Netherlands.

E-mail: mdolores.vina@usc.es

Inflammation plays a central role in the complex response of the organism against potentially damaging agents. However, an increased inflammatory response and pro-inflammatory cytokines release have been described to compromise the blood-brain barrier (BBB) integrity, leading to oxidative stress, accumulation of protein aggregates and neurodegeneration. Therefore, the maintenance of its integrity is crucial to prevent the development of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis.

Vascular endothelium secretes several vasoactive molecules that regulate endothelial functioning and vascular contractility. Among those, nitric oxide (NO) plays a central role on vascular homeostasis while exhibiting anti-inflammatory and antioxidant effects.

The aim of our study was to evaluate the effect that sodium nitroprusside (SNP), a NO donor, has against lipopolysaccharide (LPS)-induced inflammation in murine vascular endothelial cells (bEnd.3) used as a model of BBB.

Our results suggest that 24-hour exposure to inflammatory stress increase IL-6 expression through the JAK-STAT pathway, leading to increases in the cellular index, ROS production and barrier permeability. Under these conditions, SNP shows a protective activity against oxidative stress and inflammation. However, it fails to prevent barrier disruption. More studiesneed to be carried out to further elucidate the SNP mechanism behind these effects.

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PO-059

Antiinflamatory and antioxidant activity of carvone derivaties

Ordoñez, C [1,2,+], González, Y [3,+], Moreno, D [3], González, A[1], Morales A [1,2], Guerrero, E [1,2], Silva, L[4], Rodilla, J [4,*], Morán-Pinzón, J [1,2,*]

[1]Centro de Investigaciones Psicofarmacológicas. Universidad de Panamá,
[2]Departmento de Farmacología, Escuela de Medicina. Universidad de Panamá.
[3]Instituto de Investigaciones Científicas y Servicios de Alta Tecnología.
[4] Departamento de Química, Materiais Fibrosos e Tecnologias Ambientais-FibErTech, Universidade da Beira Interior, Rua Marquês d'Ávila e Bolama. Covilha, Portugal.

ciaraordonez001@gmail.com; guerrerodleon@gmail.com

Carvone is a monocyclic terpene, found in aromatic plants; it has shown versatility and functionality, as a starting material, in the synthesis of organic compounds with biological interest [1,2]. Carvones and their derivatives have shown antinociceptive, anticancer, antifungal, antibacterial and antioxidant properties, [2,3]. Because there is interest in the search for new pharmacological alternatives for the treatment of chronic inflammatory diseases, we have evaluated anti-inflammatory and antioxidant, activity of 5 carvone derivatives and their R- (-) and S- (+) standards, using an anti-inflammatory assay and TBARS reaction [4,5]. The anti-inflammatory effect of carvones was tested in RAW264.7 cells incubated at different concentrations (10-160 µM) and in the presence or absence of LPS, for evaluating the ability to inhibit TNFα and IL-6, preincubation of RAW264.7 with the carvones for 1 hour and the addition of LPS for 24 h significantly reduced the levels of TNF-α and IL-6 *($p < 0,001$)*, without affecting cell viability. TBARS assay showed, that 29-11 carvone exhibited the higher reduction of lipid peroxidation up to 66.3 % (160 µM), very similar to quercetin control. Our results demonstrate that tested carvone derivatives compounds CAR 22-5; CAR 23-6, CAR 26-12, CAR 26-14 y CAR 29-11, could inhibit the LPS-induced macrophage cellular response, and CAR 29-11 has powerful antioxidant effect; suggesting their potential application in oxidative stress and inflammation animal models.

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PAIN

PO-060

NSAIDs reverse inflammation but not pain in a model of rheumatoid arthritis in rats

Huerta, M.Á., Gómez-Navas, C., Artacho-Cordón, A., González-Cano, R., Cobos, E.J., Nieto, F.R.

Faculty of Medicine, University of Granada, Granada, 18016, Spain

E-mail: huerta@ugr.es

Background: Non-steroidal anti-inflammatory Drugs (NSAIDs) are widely used as analgesics in low to moderate painful conditions, including inflammatory arthritis such as rheumatoid arthritis (RA) [1,2]. However, their efficacy relieving arthritis-related pain is questioned and they do not benefit all patients, mainly patients with a higher neuropathic component [3].
Moreover, NSAIDs cause serious adverse events (e.g., gastrointestinal damage, renal problems, cardiovascular issues, etc.) [1,2]. Because of that, our goal was to evaluate the anti-inflammatory/analgesic properties and gastrointestinal (GI) effects of two well-known NSAIDs (naproxen and diclofenac) in a translational model of RA.

Material and methods: We used the collagen-induced arthritis (CIA) model of RA in female Wistar rats. NSAIDs were administered chronically with osmotic minipumps. Naproxen was used at doses of 20, 40 and 80 mg/kg/day and diclofenac at doses of 2.5, 5 and 7.5 mg/kg/day. Sensory hypersensitivity was assessed using von Frey (mechanical allodynia) and acetone tests (cold allodynia), and loss of function with the grip strength test. Inflammation in the hindpaws was assessed with an electronic caliper. GI status was evaluated by a macroscopic scale.

Results: Both diclofenac and naproxen reduced peripheral inflammation in a dose-dependent manner. On the contrary, they were ineffective in reducing mechanical allodynia, and only partially reduced cold allodynia, even at high doses. Interestingly, both drugs induced moderate, but not full, efficacy in grip strength deficits. The improvement in grip strength deficits might be explained by the reduction of inflammation by NSAIDs and the consequent increase of the capability to grip, but this might be limited by pain, as it is known to influence functionality and sensory hypersensitivity is not relieved with these drugs. The higher dose of each drug produced serious gastrointestinal damage (ulcerations), so the inefficacy in pain is not due to low dose.

Conclusions: NSAIDs had dose-dependent anti-inflammatory properties in the CIA model of RA, but they had a modest effect on function and were totally ineffective relieving sensory hypersensitivity. Higher doses produced gastrointestinal damage. These results have clinical relevance and support that NSAIDs in monotherapy are not enough for relieving arthritis- related pain and should be used in combination with other analgesics.

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PO-061

Molecular mechanisms that underly the low abuse liability of methadone and, specifically, of S-methadone

Casadó-Anguera, V.[1,2], Llopart, N.[1], Alonso-Carrasco, A.[1], Cai, N.S.[3], Casajuana-Martin, N.[4], Quiroz, C.[3], De Oliveira, P.A.[3], Belcher, A.M.[5], Moreno, E.[1], Pardo, L.[4], Ferré, S.[3], Casadó, V.[1]

[1]*University of Barcelona and Institute of Biomedicine of the University of Barcelona, Barcelona, Spain*
[2]*Universitat Pompeu Fabra, Barcelona, Spain*
[3]*National Institute on Drug Abuse, National Institutes of Health, Baltimore, USA*
[4]*Universitat Autònoma de Barcelona, Bellaterra, Spain*
[5]*University of Maryland, Baltimore, USA*

E-mail: veronica.casado@ub.edu

μ-opioid receptor (MOR) agonists are the most effective drugs to treat chronic pain. MORs belong to the superfamily of G protein-coupled receptors (GPCRs) and mediate both the analgesic and addictive effects of opioids. The neuropeptide galanin acts as a modulator of neurotransmission in the CNS and in the peripheral nervous system, acting on three GPCR subtypes (Gal1R, Gal2R and Gal3R). Gal1R is expressed in brain dopaminergic areas, including the ventral tegmental area (VTA) and nucleus accumbens (NAc). In these regions Gal1R physically and functionally interact with MOR, mediating antagonistic effects on opioid reward. Therefore, the main aim of this project was to search for non-addictive opioid drugs useful to treat chronic pain taking advantage of the distinct properties that MORs acquire when forming complexes with Gal1R. To achieve this objective we used different biochemical, pharmacological and functional techniques in transfected cells as well as in vivo assays in rodents. We have found significant differences between S-, R- and racemic methadone, morphine and other opioid compounds. These differences depend on the heteromerization of MOR with Gal1R, which generates a significant decrease in the potency and efficacy of methadone, especially of S-methadone, at activating its receptor when forming complexes with Gal1R. This enables us to explain the lower ability of methadone to activate the dopaminergic system compared to that of other opioids and predicts a dissociation of the analgesic versus euphoric effects of methadone, since it binds preferentially to peripheral MOR that does not form heteromers with Gal1R. Using several behavioral assays such as drug self-administration as well as tests to assess the analgesic properties of the studied compounds, we demonstrated that S-methadone induced analgesia with similar efficacy as R-methadone but was not self-administered, indicating low abuse liability. Therefore, our results suggest that, since MOR-Gal1R heteromers mediate the dopaminergic effects of opioids, opioids with low potency and/or efficacy for this heteromer may have low abuse liability but the same analgesic properties and could be used clinically as analgesics.

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PO-062

Role of TGF-β signalling and gender influence in a model of oxaliplatin-induced neuropathic pain.

Saguillo, A Y[1,2], Marques, J[1], Silva, E P[1,2], Tramullas, M[1]

[1]Facultad de Medicina, Universidad de Cantabria, 39011 Santander, Spain. [2]Instituto de Investigación Sanitaria Valdecilla (IDIVAL), 39011 Santander, Spain.

Email: andrea-ysabel.saguillo@alumnos.unican.es3

Chemotherapy-induced peripheral neuropathy (CIPN) is the most frequent complication of cancer treatment, affecting more than 60% of cancer patients. CIPN is a complex pain syndromethat includes sensory symptoms (numbness, paresthesia, spontaneous pain, hypersensitivity tomechanical and/or cold stimuli in hands and feet, etc.), autonomic, and motor dysfunction. CIPN manifestations are often highly refractory to current analgesics, impacting 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 (Tramullas et al., 2010). 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 (Tramullas et al., 2018). 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 to C57BL6 and BAMBI-KO male and female mice by an oxaliplatin cycle administration consisting of five intraperitoneal injections on alternate days. Mechanicalallodynia 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. However, the response intensity was significantly lower in BAMBI-KO mice compared to C57BL6 mice. The expression levels of miR-30c-5p were dysregulated in plasma, SDH, and DRG 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.

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NANOPHARMACOLOGY

PO-063

Functionalization of extracellular vesicles with senolytic drugs

Gonzalez-Moro, A[1]; Morales-Rodríguez de Lope M[1]; Cercas, E[1]; Rivera-Tenorio, A[1]; González-Camuñas, A[2]; Campos LA[2]; Castellanos M[2]; Somoza A[2], Sanchez-Ferrer CF[1,3]; Peiro, C[1,3]; de la Cuesta, F[1,3].

[1] *Universidad Autónoma de Madrid. Madrid, Spain.*
[2] *IMDEA Nanociencia. Madrid, Spain.*
[3] *Instituto de Investigación Sanitaria Hospital Universitario La Paz, IdiPAZ. Madrid, Spain.*

E-mail: fernando.delacuesta@uam.es

Cellular senescence is involved in the progression of age-associated diseases. Senolytic drugs are compounds that induce apoptosis of such senescent cells and two of the most used are quercetin (Q) and dasatinib (D). Several studies have demonstrated that mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) exert beneficial effects and most recently, plant-derived nanoparticles have arisen as an interesting alternative, due to their antiinflammatory and antioxidant properties. Furthermore, plant-derived EVs have lower regulatory and ethical issues for their clinical implementation.

Therefore, the main objective of our study is to develop novel advanced therapies using MSC-EVs and plant-derived EVs as vehicles for Q and D delivery.

MSC-EVs were isolated from immortalized adipose tissue MSCs and plant-derived EVs from 4 different sources (red cabbage, ginger, tea leaves and turmeric), by ultrafiltration and size exclusion chromatography (SEC). EVs were subsequently characterized by NTA, ExoView, western blot and TEM. NTA and light dispersion analyses were used to determine the optimal conditions for Q and D internalisation. Functionalization of EVs with the senolytics was evaluated by HPLC and MRM mass spectrometry. EV's uptake by HUVECs was imaged with CFSE or Cell Mask Deep Red staining. Cytotoxicity of the treatments in HUVECs was evaluated by MTT analysis. Besides, aptamers conjugated with cholesterol were synthesized inorder to evaluate their incorporation into both types of EVs, with the final goal of specifically delivering the treatment to the desired tissue.

Optimal concentration of the different agents for EVs functionalization were: DMSO 3%, Q 20µM and D 4 µM. Efficient functionalization of MSC-EVs with the senolytics was evidenced. EVs were efficiently internalized by HUVECs and cytotoxicity experiments demonstrated safety of the treatments at the concentrations assayed. Besides, cholesterol coupled aptamers showed substantial incorporation into MSC-EVs. Plant-derived EVs were efficiently isolated and tea leave EVs were selected for further proof-of-concept experiments, which showed theirability to incorporate Q and aptamers.

We have been able to encapsulate senolytics and incorporate cholesterol modified aptamers into MSC-EVs and plant-derived EVs. Initial cytotoxicity experiments show safety *in vitro*. These results set the basis for future experiments assessing efficacy of these novel EV-based therapeutic agents.

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PO-064

Nanoparticle-mediated siRNA transfection in glioblastoma cells derivedfrom patient organoids

Rodríguez-Clemente, I.[1,2], Buendía-Buendía, A. [1,2], Sztandera, K. [1,2], Pérez-Carrión, M.D.[1,2], Ceña, V.[1,2].

[1] CIBER, Instituto de Salud Carlos III, 28031 Madrid, Spain;[2] Unidad Asociada Neurodeath,Facultad de Medicina, Universidad de Castilla-La Mancha, 02006 Albacete, Spain

E-mail: irene.rclemente@uclm.es

Glioblastoma multiforme (GBM) is a malignant cerebral tumour with poor prognosis and high probability of recurrence. The only available treatment consists of surgery, radiation and temozolamide and survival is merely of 5% at 5 years from diagnostics. Thus, new treatments are needed. Small interference RNAs (siRNAs) are short (21 base pairs), double strand RNA molecules that target specific mRNAs to knockdown the encoded proteins. siRNAs require a vehicle (i.e., nanoparticles) to be protected from degradation and been transported into the target cells (1). Our objective is to use different nanoparticles to transport siRNA into GBM cells to knockdown proteins involved in proliferation and survival of GBM cells to increase the efficiency of temozolomide. In the present work, we have used cells isolated from organoids generated from GBM tumours obtained from patients (PDOs) which are 3D cultures that keepthe histopathological and genetic characteristics (2) of the original tumour and that can be grown in long-term cultures (3). We have explored the ability of different chemical families of nanoparticles (dendrimers, oligosaccharides and dihydropyridine-derived) to transport siRNA to these GBM cells to knockdown different proteins (p42-MAPK, Rheb, MGMT) involved in proliferation and/or survival of GBM cells isolated from PODs.

We have observed that selected nanoparticles that were previously tested for siRNA binding, and protection from RNAses-mediated degradation, and that did not show cell toxicity were able to transport fluorescent siRNA into the GBM cells and also to knockdown the target proteins to about 20 to 30 % of control levels for p42-MAPK, and to 5 to 10% in the case of Rheb and MGMT. Following these results in vitro, we labelled the nanoparticles with a fluorescence probe (Cy7.5) and studied its biodistribution in mice. We observed that they wereable to reach the brain, suggesting that they could be used as carriers of either antitumoral drugsor siRNA to target central nervous system diseases. In this work we show a combination of NP/siRNA complexes that transfect GBM tumoral cells coming from PDOs and knockdown target proteins, specifically p42 and Rheb,

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PO-065

Transferosomes loaded with cyanocobalamin effectively reduce oedema associated with atopic dermatitis in an *in vivo* assessment using a hypersensitivity mouse model

Antonio José Guillot[1], Miquel Martínez-Navarrete[1], Rosa Mª Giner[2], Mª Carmen Recio[2], Teresa Mª Garrigues[1], Ana Melero[1]

[1]Department de Farmàcia i Tecnologia Farmaceutica i Parasitologia. Facultat de Farmàcia. Universitat de València. Avda. Vicent Andrés Estellés, s/n. 46100, Burjassot, Spain.
[2]Department de Farmacologia. Facultat de Farmàcia. Universitat de València. Avda. Vicent Andrés Estellés, s/n. 46100, Burjassot, Spain.

E-mail: Rosa.m.giner@uv.es

Due to the prevalence of atopic dermatitis as a common chronic skin disease, alternative therapeutic options are needed to address the drawbacks associated with current first-line drugs such as immunosuppressive agents, corticosteroids, and calcineurin inhibitors. Utilizing the molecular mechanisms underlying atopic dermatitis, cyanocobalamin emerges as a potential therapeutic alternative. Specifically, its ability to scavenge nitric oxide, which plays a role in the development of atopic eczema and oedema, suggests cyanocobalamin's potential to counteract the effects of nitric oxide (1).

Three types of cyanocobalamin lipid vesicles were prepared: liposomes, transferosomes and ethosomes. They were characterized in terms of size, polydispersity index, zeta-potential, drug release and biocompatibility. As preliminary step, drug permeability through full-thickness porcine. *In vivo* performance of selected formulations was studied using a hypersensitivity mice model evaluating the ear thickness variation as evidence of oedema evolution.

Transferosomes and ethosomes presented favourable size and flexibility for improved transdermal drug absorption (size $\leq$ 200 nm and null size reduction after cold extrusion). Drug absorption increased using the ultraflexible vesicles compared to liposomes and aqueous solution. Jmax was 1.73 $\pm$ 0.77 and 1.51 $\pm$ 0.35 µg/cm2/h, respectively for transferosomes and ethosomes. Ear thickness variation in transferosome treated group was 52%, significantly lower compared to untreated controls (126%) and the rest of lipid vesicles.

Cyanocobalamin-loaded transferosomes and ethosomes increase the drug transdermal flux, but only transferosomes effectively diminished the skin oedema associated to atopic dermatitis.

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PO-066

Fucoidan/dendrimer-based nanoparticles: drug delivery nanosystems with intrinsic anti-angiogenic properties

Olim, F[1*], Neves, RN[1], Rodriguez-Clemente, I[2], Ceña, V[2], Tomás, H[1]

[1]*Centro de Química da Madeira, University of Madeira, Campus da Penteada, 9020-105 Funchal, Portugal*
[2]*Unidad Asociada Neurodeath, School of Medicine, University of Castilla-La Mancha, 02006 Albacete, Spain*

**E-mail: jose.olim@staff.uma.pt*

The formation of new blood vessels from pre-existing vascular structures constitutes the physiological process known as angiogenesis. Due to increased metabolic needs, tumours can secrete angiogenic factors that lead to the upscaled formation of new vessels, contributing to their ability to invade and metastasize in distant places[1]. That is the case of glioblastoma, a severe type of brain cancer characterized by high vascular density, for which strategies targeting angiogenesis may contribute to the patient's treatment[2].
Fucoidans are a class of sulfated polysaccharides mainly extracted from brown algae. They have been shown to exert various biological activities, including pro- or anti-angiogenic effects depending on their molecular structure[3]. After observing that non-hydrolyzed fucoidans exerted the best anti-angiogenic response when compared with the same molecules of lower molecular weight (the *in vitro* formation of endothelial tubular structures was lower), we decided to use these sulfated polysaccharides in combination with biodegradable dendrimers to prepare fucoidan/dendrimer nanoparticles. These systems were then characterized, their anti-angiogenic behaviour was evaluated, and the possibility of incorporating anticancer compounds in their structure, such as cisplatin, was studied. It was possible to observe that the prepared nanoparticles maintained the anti-angiogenic properties shown by the fucoidan counterparts. Additionally, cisplatin was also successfully included in the nanoparticles. Presently, further studies are being done to validate the possibility of using these nanosystems as bioactive nanoplatforms to treat glioblastoma.

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PO-067

Multivalent ligands as a paradigm of heteromer-selective ligands for GPCR

Casadó, V.[1,2], Llopart, N.[1,2], Alonso-Carrasco, A.[1,2], Pulido, D.[1,3], Royo, M.[1,3], Moreno, E.[1,2], Casadó-Anguera, V.[1,2,4]

[1] Nanomedicine and Molecular Neuropharmacology, Consolidated Research Group of the Generalitat of Catalonia, Barcelona
[2]Institute of Biomedicine of the University of Barcelona, Barcelona, Spain
[3]Institute for Advanced Chemistry of Catalonia-CSIC, Barcelona, Spain
[4]Universitat Pompeu Fabra, Barcelona, Spain

E-mail: vcasado@ub.edu

Currently, G protein-coupled receptors (GPCRs) constitute the most important superfamily of membrane proteins pharmacologically targeted in clinical practice. Interestingly, among all the drugs approved by the FDA, around 35% target GPCRs.[1,2] Until 2017, the FDA and the EMA had approved 475–704 drugs for 108–134 different GPCRs, mainly aminergic and opioid receptors.[1,2] Moreover, there are 321 drugs targeting GPCRs in clinical trials, 66 of which acting on GPCRs that are not currently a target of any approved drug.[1] One of the most promising drugs targeting GPCRs that will reach the market in the medium and long term are those derived from studies of GPCR oligomerization and the wide range of allosteric interactions that occur within the orthosteric and/or allosteric centers of the protomers that constitute the oligomers (dimers, tetramers o higher order oligomers). The allosteric modulations that emerge after receptor oligomerization can cause a modification of both orthosteric and allosteric centers, acquiring different properties, such as alterations in binding affinity and signal efficacy.[3] These modifications can benefit specific structures, the so-called heteromer selective drugs. Heteromer-selective compounds can be orthosteric (agonists and antagonists), allosteric, biased or bitopic ligands, but also antibodies and bivalent or multivalent molecules.[4] Here, we have designed and synthesized multivalent ligands for the adenosine A_{2A}–dopamine D_2 receptor (A_{2A}R- D_2R) heterotetramer constituted by four antagonist pharmacophores selective for each protomer linked by a polyethylene glycol-based linker with various lengths.[4,5] The simultaneous binding of these ligands to the different orthosteric sites within the heteromer was evaluated by radioligand competition-binding assays in the absence and presence of specific peptides that disrupt the formation of the heteromer, as well as by functional assays in living cells. This approach allowed us to identify a compound able to simultaneously bind with high affinity to the four different orthosteric sites of the A_{2A}R-D_2R heterotetrameric complex. Bivalent and multivalent ligands are a paradigm of heteromer-selective ligands. Moreover, thanks to the development of novel drug-delivery systems (biodegradable polymeric nanoparticles or virus-derived peptides carrying drugs), these compounds have emerged as promising therapeutic agents when receptor heteromers are involved in pathological states.[6,7]

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NATURAL PRODUCTS

PO-068

Neurotoxic Shellfish Poisoning: Brevetoxin 3 acute toxicity *in vivo*

Costas, C., Louzao, M. C., Raposo-García, S., Vale, C., Cifuentes, J. M., Vilariño, N., Vieytes, M.R., Botana, L. M.

Universidad de Santiago de Compostela, Lugo, Spain

E-mail: celia.costas.sanchez@usc.es

Brevetoxins (PbTxs) are polyether marine biotoxins synthetised mainly by the dinoflagellate *Karenia brevis*. They can accumulate in filter-feeding organisms like bivalve molluscs and after consumption of seafood contaminated with PbTxs, they can cause Neurotoxic Shellfish Poisoning (NSP). Neurologic symptomatology develops shortly afterwards, entailing paraesthesia, muscular weakness, ataxia, dysesthesia, vertigo, seizures and coma in most severe cases. No fatalities have been reported. NSP outbreaks are scarce and geographically restricted to areas where *Karenia brevis* blooms occur. PbTx3 has been determined to be the most toxic analogue and the one most commonly found in shellfish meat. However, little is known regarding their oral toxicity. Our aim was to assess acute neurotoxicity after oral administration in mice. Animals were given PbTx3 by oral gavage and functional tests performed the day prior, at 6 and 24 h (the end of the experiment). Besides, symptomatology was registered periodically. Functional tests allowed to evaluate changes in muscle strength, cold sensitivity and motor activity. As a result, we obtained that no deaths were recorded along this time, whereas neurological signs were observed in all tested doses with the exception of 10 µg/kg PbTx3. Loss of strength and changes in sensitivity were detected at 6 h with a recovery at 24 h indicating a potentially reversible effect of PbTx3.

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PO-069

HYDROXYTYROSOL (OHTYR) PHARMACOLOGICAL PROPERTIES IN A MODEL OF HYPERTENSION ASSOCIATED TO SLEEP APNEA

Melo Junior A[1], Correia, MJ[1], Mecha, E [2,3], Pimpão, A[1], Morello, J[1], Ventura, MR[2,3], Serra, T[2,3], Silva, S[2,3], Sequeira, CO[1], Monteiro, EC[1], Bronze, M[2,3,4], Pereira, SA[1]

[1]NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisboa, Portugal; [2]iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; [3]Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal; [4]Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal

E-mail:melo.junior@nms.unl.pt

Obstructive sleep apnea (OSA) is a common cause of resistant arterial hypertension (HTN), highlighting the need for novel drugs for its control [1,2]. We were pioneer in linking renal Aryl Hydrocarbon Receptor (AhR) activation [3] and cysteine redox dynamics [4] in OSA-HTN. To mimic OSA, we use an in vivo model of chronic intermittent hypoxia (CIH), which is a model of oxidative stress and inflammation [5]. In this work, we investigated the effect of hydroxytyrosol (OHTyr), the main phenolic compound in extra-virgin olive oil, in the prevention and reversion of HTN in our model.

This study was approved by NMS's Ethics Committee. We used 5 groups of male Wistar rats. Three groups were exposed to CIH (21% to 5% of O2, 5.6 cycles/h, 10.5 h/day, in their inactive period) for 21 days to establish HTN [6]. Still under CIH, one of the groups was then treated with OHTyr (15 mg/kg/day in vegetable oil by gavage) (CIH+OHTyr; n=6) for 14 days and other group treated with vegetable oil (CIH+vehicle; n=5) for 14 days. The third group under CIH conditions was not subjected to any treatment (CIH; n=5). The last two groups were maintained in normoxia (Nx), one treated with OHTyr (Nx+OHTyr; n=5) for 14 days, while the other group was not subjected to treatment (Nx; n=6). Systolic and diastolic blood pressure (SBP and DBP) were measured by radiotelemetry. Renal AhR activation (measuring Cyp1a1 expression – Western Blot), cysteine-related thiolome (HPLC) and untargeted metabolomics (UHPLC-MS) were performed.

OHTyr was able to revert the CIH-induced increase in SBP and DBP (93±13% and 59±23%, respectively). Moreover, OHTyr reverted the increase in AhR activation after 35 days of CIH (observed by decreased Cyp1a1 expression) and affected the cysteine-related thiolome. Metabolomics analysis showed that OHTyr decreased tryptophan metabolites formation (well-known AhR agonists), comparing to CIH.

OHTyr might benefit the treatment of HTN associated with OSA, both by blocking AhR activation and modulating antioxidant defences. However, the exact molecular mechanism by which OHTyr inhibit AhR activation and increase antioxidant defences must be further explored.

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PO-070

The probiotic Limosilactobacillus fermentum modulates the gut microbiota and produces anti-cancer metabolites to protect against experimental colorectal tumorigenesis

Molina-Tijeras, JA[1,2]; Ruiz-Malagón, AJ[1,2]; Hidalgo-García, L[1,2]; Rodríguez-Sojo, MJ[1,2]; García-García J[1,2]; Diez-Echave, P[1,2]; Vezza, T[1,2,3]; López-Escánez, L[1,2]; Rodríguez-Sánchez, MJ[1,2,3]; Pérez del Palacio, J[4]; Bañuelos, O[5]; Olivares, M[5]; Rodríguez-Cabezas, ME[1,2]; Rodríguez-Nogales, A[1,2]; Gálvez, J[1,2,]

[1]Department of Pharmacology, Center for Biomedical Research (CIBM), University of Granada, Granada, Spain. [2]Instituto de Investigación Biosanitaria de Granada (ibs. GRANADA), Granada, Spain. [3]Servicio de Digestivo, Hospital Universitario Virgen de las Nieves, Granada, Spain. [4]Fundación MEDINA, Granada, Spain. [5]Biosearch Life, Granada, Spain.

E-mail: jalbertomolina@ugr.es

Intestinal inflammation contributes to colorectal cancer (CRC) by activating oncogenes and inhibiting apoptosis. This study evaluates the effect of the immunomodulatory probiotic *Limosilactobacillus fermentum* CECT5716 in different experimental models of CRC, focusing on its impact on the microbiome.

The tumor-suppressive effect of *L. fermentum* was assessed in induced (azoxymethane/dextransulfate sodium (DSS)) and genetic ($Apc^{Min/+}$ mouse) murine models of CRC. CRC cell lines were also cultured with *L. fermentum* culture-supernatant (LFCS) to evaluate cell proliferation,apoptosis and cell cycle distribution. LFCS also was evaluated in CMT-93 allograft tumors in C57Bl/6 mice. Gut microbiota was assessed by 16S ribosomal DNA sequencing. Anti-tumor molecule produced from *L. fermentum* was characterized by liquid chromatography mass spectrometry (LC-MS/MS) and targeted mass spectrometry.

Oral gavage of *L. fermentum* significantly reduced tumor formation in both $Apc^{min/+}$ and azoxymethane/DSS mice, accompanied by decreased expression of markers involved in tumor proliferation, while increasing pro-apoptotic markers. In addition, a reduction in myeloid immune cell infiltration was observed, as well as a decrease in the expression of different pro-inflammatory cytokines (IL-1β, IL-6, IL-12 and IL-23). Culturing CRC cells with LFCS suppressed cell proliferation and colony formation. Furthermore, LFCS significantly promotedapoptosis in CRC cells, but not in normal colon epithelial cells. In CMT-93 allograft mice, LFCS intratumoral injection reduced subcutaneous tumor growth. Finally, *L. fermentum* modulated microbiota composition, which is altered due to the tumor process, by recovering microbial biodiversity.

L. fermentum CECT5716 decreases experimental tumor development, thus revealing an anti-proliferative and pro-apoptotic effect *in vitro* and *in vivo*. This beneficial effect is associated with its immunomodulatory properties. Thus, the probiotic *L. fermentum* could be considered acomplementary approach in the management of CRC in humans.

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PO-071

Systematic screening for selective anticancer activity of Andalusian plants of the Fabaceae family

Víctor Jiménez González[1]; Guillermo Benitez[2]; Miguel López Lázaro[1], José Manuel Calderón Montaño[1]

1.Department of Pharmacology, Faculty of Pharmacy, University of Seville
2.Department of Botany, Faculty of Pharmacy, University of Granada.

E-mail: vjimenez3@us.es

In recent decades some plants have provided useful anticancer drugs. For example, the researchers Monroe E. Wall and Mansukh C. Wani isolated campthotecin, and paclitaxel from Campthotera acuminata Decne. and Taxus brevifolia Nutt. respectively, following a systematic screening approach. Both drugs have been useful for themselves or for the synthesis of other anticancer drugs, such as topotecan and docetaxel. For this reason, we decided in 2013 to start a systematic screening of plants collected in Andalusia. In this work, we have screened the selective anticancer activity of 20 extracts from 20 plants of the Fabaceae family collected in Andalucía; Anthyllis vulneraria L., Argyrolobium zanonii (Turra) P.W. Ball subsp. Zanonii, Cercis siliquastrum L., Coronilla juncea L., Coronilla scorpioides (L.) Koch, Cytisus baeticus (Webb) Steud., Dorycnopsis gerardii (L.) Boiss., Erinacea anthyllis Link, Genista umbelata (L'Her.) Poir., Gleditsia triacanthos L., Hippocrepis multisiliquosa L., Lathyrus clymenum L., Lupinus angustifolius L., Parapiptadenia rigida (Benth.) Brenan, Prosopis chilensis (Molina) Stuntz, Robinia pseudoacacia L., Scorpiurus muricatus L., Sophora japonica L., Spartium junceum L., Tipuana tipu (Benth.) Kuntze, and Tripodion tetraphyllum (L.) Fourr.
Extracts were prepared with a mixture of ethanol/ethyl acetate/water (1:1:1), using an ultrasound water bath for one hour. After that, ethanol and ethyl acetate were evaporated using a rotary evaporator for 15 minutes. The remaining water present in the extract was eliminated by lyophilization for 72 hours. The extracts were preserved in amber bottles in a cold and dark room. Lung cancer cells A549 and non-malignant skin cells HaCaT were used to evaluate their selective anticancer activity and to compare it with that of the standard anticancer drugs cisplatin and gemcitabine. Cell viability was assessed with the resazurin assay. The highest selective anticancer activity was found for extracts of C. juncea L. and C. scorpioides L. (Koch), showing IC50 values 1,0 ± 0,4, and 167,1 ± 99,1, and selectivity indices, 59,6 and 108,2 respectively.

PO-072

Effect of β-sitosterol on cardiometabolic and renal alterations in an experimental model of Metabolic Syndrome by diet. Modulation of ACE2expression

Silvia Flaj Prados[1], Antonio González Ruiz[1,2], Esperanza Herradón Pliego[1,2], VisitaciónLópez-Miranda González[1,2]

[1] Area de Farmacología y Nutrición y Bromatología, Dpto. CC Básicas de la Salud. FacultadCC Salud, URJC. Alcorcón, Madrid. [2] Grupo de investigación de alto rendimiento en Farmacología Experimental de la Universidad Rey Juan Carlos, PHARMAKOM

E-mail: visitacion.lopezmiranda@urjc.es

Metabolic Syndrome (MS) is a set of cardiovascular risk factors represented by central obesity, dyslipidemia, abnormalities in glucose metabolism, and hypertension. Adherence to an unhealthy high fat, and hypercaloric diet is one of the main contributors to MS. This unhealthy diet increases angiotensin II and decreases ACE2 levels, contributing to the development of cardiovascular disease. β-sitosterol, a naturally occurring phytosterol, possesses antiobesogenicand anti-diabetic effects.

The aims of the study are: 1) to assess whether administration of β-sitosterol, can ameliorate cardiometabolic and renal alterations in an experimental model of MS by diet; 2) to analyze whether administration of β-sitosterol, modifies the altered ACE2 expression in the leftventricle, aorta, kidney, liver and pancreas caused by the development of MS by diet.

Adult male Wistar rats were divided into three experimental groups (n=8-10): a) Control, animals fed for 24 weeks with a normocaloric diet; b) MS, animals fed for 24 weeks with a high-fat and hypercaloric diet; c) MS+β-SIT, animals fed for 24 weeks with a high-fat and hypercaloric diet that during the last four weeks received daily 100 mg/kg of β-sitosterol (p.o).At the end of the experimental period, the animal's body weight, feeding behavior, anthropometric and biochemical parameters, blood pressure, and heart rate were assessed. In addition, basal cardiac function and vascular reactivity in the aorta were analyzed *in vitro*. ACE2 expression was determined in heart, aorta, liver and kidney by Western Blot.

The high-fat and hypercaloric diet causes MS in animals, with abdominal obesity, glucose metabolism alterations, and dyslipidemia. Besides, MS animals present cardiovascular autonomic dysfunction and a significant decrease of ACE2 expression in the heart. β-sitosteroldid not modify caloric intake, weight gain, or adiposity in MS animals. However, it caused a slight improvement in glucose metabolism and decreased plasma creatinine levels in MS animals. At the cardiovascular level, β-sitosterol improves left ventricular function and enhances endothelium-dependent relaxation in the aorta of MS animals. Treatment with β- sitosterol resulted in a significant increase in cardiac and renal ACE2 levels in MS animals.

These findings suggest that although β-sitosterol does not cause changes in feed behaviour and anthropometric parameters, it is able to partially improve cardiometabolic and renal alterationsin MS, enhancing ACE2 expression in key tissues in these complications. Further studies are needed to confirm the possible usefulness of this phytosterol in the treatment of these complications.

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PO-073

Use of natural products in pets by veterinarians

Romero, B, Sahagún, AM, López, C, Diez, MJ, Fernández, MN, Díez, R, Susperregui, J.

Pharmacology. IBIOMED. University of León, Leon, Spain.

E-mail: rdielz@unileon.es

Traditional veterinary medicine or ethnoveterinary medicine is a scientific discipline that focuses on people's knowledge about animal diseases, practices and remedies for their treatment and prevention (1). Nowadays, it is an important scientific field that is of growing interest, especially in Western Europe (2-4). In Spain, it is still considered a field to be explored as an alternative to improve animal health, framed within the "One Health" approach, which considers both human and animal health and the environment to be interconnected (5). Information on treatment with natural products is common in large production animals, but is limited in small ones. Thus, this study aims to analyse the current state of this field in Spain, trying to record the use of natural products well as the opinion, attitudes, and degree of acceptance among small animal practitioners.

An observational and descriptive cross-sectional study was carried out among small animal veterinarians in Spain. For this purpose, an online survey was conducted from November 2020 to February 2021. Data collection, processing and storage was carried out in accordance with Spanish regulations. Data obtained were processed and analysed using Microsoft Excel and SPSS Statistics v. 26.

A total of 200 valid responses were received. 85.5% were women, half of the practitioners were under 34 years and the majority (88%) worked in veterinary clinics. In terms of the animals cared, almost all respondents (99%) offer their services to dogs, 96% to cats and 36% to exotic animals. Only 3% have not heard of the use of herbal medicine or natural products in small animals.

A high proportion of practitioners have used this therapy (80%), mainly in musculoskeletal (67%), dermatological (55%) and gastrointestinal (51%) health problems. *Cannabis sativa* was the most commonly used as natural product (69%), followed by *Aloe vera* (64%) and *Thymus vulgaris* (48%). Tablets (82 %), syrup (65%) and ointment/cream (55%) prevail in terms of dosage form.

A widespread use of medicinal plants has been demonstrated, and most veterinarians also showed a positive attitude towards natural products.

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PO-074

Effect of *Sideritis hyssopifolia* on apoptosis in human prostate cancer PC-3 cells

Huerga, V, Sierra, M, García, JJ, Diez, MJ, Sahagún, AM, López, C, Fernández, MN, Díez, R.

Pharmacology. IBIOMED. University of León, Leon, Spain

E-mail: clopcd@unileon.es

There is a resurgence in discovering medicinal plants as a potential source of new drugs. *Sideritis hyssopifolia* shows potential therapeutic effects due to its high antioxidant activity, therefore it may be a possible candidate as chemopreventive for prostate cancer. This cancer is the most common diagnosed in men in the worldwide. The aim of the study was to determine the effects of the ether, methanol and chloroform extracts obtained from the aerial parts of this plant on apoptosis in PC-3 cells of human prostate cancer. For that, apoptosis was measured by using a specific kit (FITC-Annexin V Apoptosis Kit) following the manufacturer's instructions. Concisely, PC-3 cells were seeded (1 x 10^5 cells/well) in 2 mL of culture medium. After 24 h, the cells were treated with the extracts and incubated at 37 ºC for 72 h. Therefore, cells harvested by trypsinization were collected and washed twice with ice-cold PBS. After centrifugation, cell pellets were resuspended in 100 µL of binding buffer, and 5 µL of FITC-Annexin V and 1 µL of propidium iodide (1 mg/mL) were added. After 15 minutes of incubation, 400 µL of binding buffer were added and cells were analyzed by flow cytometry using Summit 4.3 software. Data were processed using Flowing Software 2.5.1. The results obtained indicated that in the untreated cells (control group), the majority of cells were viable (84.68 ± 1.91 %). And, in the groups that were treated with extracts, the percentage of viable cells were: 11.21 ± 2.15; 6.42 ± 3.41 and 9.60 ± 2.28 % (ether, methanol and chloroform extracts, respectively). Also, the highest number of cells in early apoptosis was found for ether extract (20.90 ± 5.90 %) and in late apoptosis for methanol extract (53.65 ± 8.05 %). In conclusion, all extracts assayed induced apoptosis of PC-3 cells, being the better result for methanol extract.

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PO-075

Colchlospermum angolense: antioxidant activity and toxicity profile

Morales, A.[1,2], Samba, N. [3,4], Morán-Pinzón, J.[1,2], Silva L. [2], Mero A.[1,2], Díaz, M. [2], Sánchez, H. [2], Rodilla, J. [3*], Guerrero, E. [1,2,*]

[1]Departamento de Farmacología, Facultad de Medicina Universidad de Panamá, Panamá. [2]Centro de Investigaciones Psicofarmacológicas, Universidad de Panamá, Panamá. [3]Departamento de Química e Unidade FibEnTech, Universidade da Beira Interior Covilhã, Portugal. [4]Departamento de Analises Clinicos Kimpa Vita, Uige 77, Angola.

E-mail: moba245@gmail.com; guerrerodleon@gmail.com

Cochlospermum angolense is an African tree native to Angola (West Africa), known as Borututu. According to some reports, the therapeutic purposes of species of the genus *Cochlospermum* (mainly hepatoprotective effects) can be explained by the presence of phytochemicals especially phenolic and polyphenolic compounds, with gallic and ellagic acids and their derivatives as the main compounds. Therefore, we have decided to determine the antioxidant activity and toxicity profile of extracts obtained using solvents with different polarity (hexane, acetone, and ethanol) and different parts of the plant (leaves, bark and roots) *C. angolense*. To determine the antioxidant activity against DPPH[1], nitric oxide (NO•)[3] and superoxide anion ($\bullet O_2^-$)[2] radicals, as well as the inhibition of lipid peroxidation, different concentrations of extracts were used. The toxicity was evaluated through the *Artemia salina* model[2].

For leaf and bark extracts obtained with acetone and ethanol, we recorded between 70 and 80% inhibition of the DPPH radical. These results were very similar to those obtained with the Quercetin standard (77.9 ± 4.4 %). Against the NO• radical, the leaf-ethanol, bark-acetone and bark-hexane extracts developed the highest inhibitory activity (63.3± 1.7, 65.9 ± 2.1 and 61.1± 0.8 %, respectively). The activity obtained for quercetin against NO• was slightly higher (74.8± 3.5%). Bark-acetate and bark-ethanol extracts obtained from *C. angolense* showed an inhibitory activity of radical $\bullet O_2^-$, which was not significantly different from the quercetin standard. (54.6±3.0, 55.8±0.9 and 53.0±0.8 %, respectively). For the remaining extracts evaluated, the inhibitory activity developed against the $\bullet O_2^-$ radical was lower compared to that of the quercetin standard. TBARS formation was significantly inhibited by leaf-acetone and leaf-ethanol extracts (93.9± 1.1 and 92.8 ± 1.3%, respectively), and a similar effect was observed with curcumin (97.02 ± 0.6 %). A high lipid peroxidation inhibitory activity was also obtained with the bark-acetone and bark-ethanol extracts, which showed an inhibitory capacity higher than 90%.

When evaluating the toxicity of *C. angolense* extracts in the *Artemia salina* model, we observed that at the concentration tested (1000 µg/ml) the extracts that showed the highest % lethality were those obtained from the bark (Hexane= 100%; Acetone= 62.3%; Ethanol= 35.6%).

In conclusion, *C. angolense* is a plant of pharmacological interest in many regions of Africa, possessing antioxidant properties and a toxicity profile that may vary depending on the part used.

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PO-076

Intestinal anti-inflammatory effects of *Salvia verbenaca* extract in the TNBSmodel of rat colitis

Vezza, T[1,2,3]; Algieri, F[1]; Molina-Tijeras, JA[1]; Rodríguez-Nogales, A[1,2]; Garrido-Mesa, J[1]; Rodríguez-Cabezas, ME[1,2]; Cádiz-Gurrea, ML[4]; Segura-Carretero A[4]; Pérez del Palacio, J[5];González-Tejero MR[6]; Galvez J[1,2,7]

[1]Department of Pharmacology, Center for Biomedical Research (CIBM), University of Granada, Granada, Spain. [2]Instituto de Investigación Biosanitaria de Granada (ibs. GRANADA), Granada, Spain. [3]Servicio de Digestivo, Hospital Universitario Virgen de las Nieves, Granada, Spain. [4]Department of Analytical Chemistry, Faculty of Science, University of Granada, Granada, Spain. [5]Fundación MEDINA, Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Granada, Spain. [6]Department of Botany, Universityof Granada, 18071 Granada, Spain. [7]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Instituto Salud Carlos III, Madrid, Spain.

E-mail:teresavezza@hotmail.it

Nowadays, there is an increasing interest in complementary medicine, including herbal remedies, in the treatment of inflammatory bowel diseases. The aim of this study was to evaluate the intestinal anti-inflammatory properties of a hydroalcoholic extract of *S. verbenaca*in the trinitrobenzenesulphonic acid (TNBS) model of rat colitis, a well characterised model with some resemblance to human IBD. Female Wistar rats were assigned to four groups: non-colitic, control colitic and colitictreated groups with *S. verbenaca* extract (10 and 25 mg/kg/day) or dexamethasone (1.2mg/kg/day), starting the same day of TNBS colitis induction. Rats were sacrificed one week after. Colonic damage was assessed macroscopically and biochemically. Several markers of pro-inflammatory status and intestinal epithelial integrity were evaluated by qPCR. *In vitro* immunomodulatory properties of different concentrations (0.1-100 µg/ml) of *S. verbenaca* extract were determined in LPS-stimulated CMT-93 cells by evaluating the production and/or expression of different cytokines involved in the intestinal inflammation.

S. verbenaca showed an intestinal anti-inflammatory effect, as evidenced by reduced colonic damage and weight/length ratio. The extract also decreased colonic myeloperoxidase activity and increased glutathione content. *S. verbenaca* extract reduced the colonic expression of the pro-inflammatory cytokines *Il-1β*, *Il-6*, *Il-12a* and *Il-23* and the adhesion molecule *Icam-1*, as well as of the chemokine *Mcp-1*. *S. verbenaca* extract was also able to significantly up- regulate the expression of the markers of intestine epithelial integrity: *villin* and the mucin *Muc- 2* and *Muc-3*. Moreover, it displayed immunomodulatory properties *in vitro* since it decreased *Il-6* and *Tnf-α* production and expression in LPS-stimulated epithelial cells.

S. extract showed intestinal anti-inflammatory activity in the TNBS-induced colitis. This beneficial effect can be related to its antioxidant properties and the downregulation of the immune response, which can improve the intestine epithelial barrier.

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TEACHING PHARMACOLOGY

PO-077

The (mis)use of scientific discourse to legitimize drug promotion in the opioid crisis in the US. An analysis for teaching purposes using TV series Dopesick

Irene Cambra-Badii[1,2], Joel Piqué[2,3] and Josep E Baños[2]

[1]Chair of Bioethics, Universitat de Vic – Universitat Central de Catalunya [2] School of Medicine, Universitat de Vic – Universitat Central de Catalunya[3] Observatory of Humanities, Hospital d'Olot
E-mail: josepeladi.banos@uvic.cat

Opioid crisis refers to a significant public health issue characterized by the widespread misuse and addiction to opioid drugs that has been occurring in the United States since the 1990s. It was related with the death of more than 600.000 individuals since 1999 [1]. Its beginnings are associated with the marketing of a new opioid drug Oxycontin® in 1996, which contained oxycodone. Purdue Pharma promoted it as having a small addicting potential. This marketing strategy was followed by a high use of the drug for the treatment of mild and moderate pain, resulting in skyrocketed sales. The consequence was a massive increase in addicted patients, that finally triggered a restriction policy in medical prescriptions. Many turned to illegal drugssuch as fentanyl and heroin, and the subsequent abuse triggered a huge increase of drug overdose deaths. The reasons that could explain the multiple dimensions and responsibilities ofthese events were deeply analyzed in some books and other media. In this regard, *Dopesick* (2021) is the first TV series whose plot deals exclusively with the opioid crisis in the United States, and is based in non-fiction book [2]. We analyzed how the TV series *Dopesick* portraysthe opioid crisis in the US, and explored if this representation is consistent with the scientific literature on the subject. We conducted a qualitative analysis of the three main narrative threads:the U.S. Attorney's and the DEA's investigation of the crisis, Purdue Pharma's role inpromoting opioids, and the stories of OxyContin addicts. We also analyzed how the pharmaceutical company used scientific facts in Oxycontin® promotion. Our analysis found that although *Dopesick* attempts to portray multiple dimensions of the opioid crisis, its narrative oversimplifies the story in attributing the cause of the problem almost exclusively to Purdue Pharma, while downplaying other factors that contributed to the opioid crisis. Thus, the storytelling in this TV series tends to offer simple explanations for a complex problem for which simple solutions are likely to be inadequate. As the promotion of the use of Oxycontin® was based on certain scientific facts, we conducted an analysis to answer how the scientific discourse was incorporated in *Dopesick*'s narrative to justify the use of this drug and its consequences. We identified four scientific assumptions that were misused in the promotion ofthe drug: the low drug addictive potential of opioid drugs; the importance of detecting pain (thefifth clinical sign); the pseudoaddiction and the concept of breakthrough pain. We suggest that Dopesick has pedagogical value in showing how opioid crisis appeared and to discuss the reasons that are linked to such tragedy. We propose that the TV series can be useful in universitypedagogical activities through methodologies such as cinemeducation.

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PO-078

An innovative teaching approach through the study of relatives as patients

Moreno, L, Lopez de Coca, T, García C, García-Lluch G.

Pharmacy Department. Universidad CEU Cardenal Herrera. Valencia, Spain

E-mail: lmoreno@uchceu.es

In today's evolving educational landscape, traditional teaching methods are giving way to innovative and sustainable curricula that prioritize students' learning experiences and inform decision-making processes. As part of this shift, the development of creative learning materials has emerged as a valuable tool to foster student engagement and contribute to the achievement of the Sustainable Development Goals (SGDs). By integrating methods such as case-based learning into university programs, institutions can effectively incorporate these goals into their educational frameworks.
In line with these principles, we undertook an activity involving 236 students pursuing a pharmacy degree. The objective was to provide them with hands-on experience by conducting personal interviews with family members. During these interviews, students applied pharmacotherapeutic follow-up techniques, screened for cognitive impairment, assessed cardiovascular risk, and calculated anticholinergic burden. To ensure ethical considerations, every participating patient-family member provided signed informed consent.
This activity yielded valuable didactic material in the form of 236 clinical cases, enabling students to enhance their communication skills while applying knowledge, reflecting on their experiences, and demonstrating synthesis and judgment abilities. The evaluation of these skills was conducted through various means, including assessment of each clinical case, role-play demonstrations, booklet evaluations, and traditional exams. Notably, students who achieved an excellent score of 65% or higher received digital badges as a communication competency-based accreditation.
Conclusions: Incorporating relatives as patients and utilizing digital credentials can play a crucial role in enhancing students' motivation to learn. By engaging with real-life scenarios and earning tangible credentials, students are likely to become more enthusiastic about their studies and develop a deeper understanding of the subject matter. These innovative approaches to education have the potential to revolutionize traditional teaching methods and empower students to become well-rounded professionals equipped with the necessary skills to address complex challenges in their respective fields.

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PO-079

Demonstrating the placebo effect to pharmacology students

González-Cano, R., Artacho-Cordón, A., Tejada, M.A, Huerta, M.Á., Baeyens, J.M., Cobos, E.J., Nieto, F.R.

Faculty of Medicine and Institute of Neuroscience, Biomedical Research Center, University of Granada, Granada, 18016, Spain
Biosanitary Research Institute ibs.Granada, 18012 Granada, Spain

E-mail: fnieto@ugr.es

The placebo effect contributes to the global effect of medicines. Drug effects during clinical development are usually compared with placebo through randomized double-blind placebo-controlled trials (RCTs). The use of placebos makes possible to discern which part of the observed effect of a medication is due to its pharmacodynamic effectiveness (specific effects) and which part is due to the placebo effect (unspecific effects), generally caused by the expectations of clinical improvement of the patients [1].
We present our experience with a practical activity, whose goal is to demonstrate placebo analgesia to university students, by using a local anaesthetic (EMLA®) cream and a placebo cream. As secondary goals, the mechanism of action of local anaesthetic drugs is reviewed, and students acquire some practical skills in sensory evaluation.
At the beginning of the class, the activity is explained to the students (omitting that there is a placebo cream), and they are informed that it has been approved by the local ethic committee (648/CEIH/2018). An information sheet and an informed consent are provided to the students that they must sign if they like to participate, being a voluntary activity. Students are randomly distributed in three groups in a single-blinded manner, balanced in number and sex. The experience consists of evaluating the analgesic/anaesthetic activity of "two different anaesthetic creams" (students do not know that one of them is a placebo), and of a known control cream, which will be applied to the index finger of one of the students' hands. After application, it is necessary to let the creams act for 1 hour, a time that is used, among other things, to review the pharmacological properties of local anaesthetics (to maximize the expectations of the efficacy of the analgesic/anaesthetic effect). Then, the students carry out different sensory evaluations on the finger where the cream was applied (the contralateral untreated index finger is also evaluated as a control): response to thermal stimuli, recording the latency to finger withdrawal induced by cold (0ºC) and hot (42ºC, 46ºC, and 50ºC) stimuli; response to a pinprick stimulus (Neuropen®) and recording of perceived pain using a visual analog scale (VAS); determination of the tactile threshold (von Frey filaments); and discrimination between two points (Diskcriminator®). The results of the different sensory tests were recorded and later graphed and analyzed (ANOVA) in a second session with the students. Placebo analgesia is usually evident in painful stimuli. The effect of the anaesthetic cream is always greater than that of the placebo cream and also more evident in painful stimuli. It is then revealed that one of the creams was a placebo and a seminar on the mechanisms of the placebo effect is given, including its importance in clinical trials.

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PO-080

A multidisciplinary approach towards identification of pharmacokinetics core concepts for undergraduate medical students

Teixeira-Santos, L; Monteiro, T; Sousa, C; Melo, A; Morello, J; Monteiro, EC; Pereira, SA

NOVA Medical School | Faculdade de Ciências Médicas (NMS|FCM), Lisboa, Portugal.
CCAL – Centro Clínico Académico de Lisboa, Lisboa, Portugal.

E-mail: luisa.santos@nms.unl.pt

Identification of core concepts in Pharmacokinetics (PK) for undergraduate medical education is relevant and challenging due to knowledge overload, curricular integration and associated shorter time dedicated to fundamental disciplines in the medical course [1]. Moreover, PK is in general considered more difficult than pharmacodynamics by students [2] and the level of detail in PK concepts varies among the recommended bibliography. We aimed to identify and rank by relative relevance PK concepts for undergraduate medical students, using a multidisciplinary approach.

This project was approved by the Ethics Committee of NOVA Medical School. A questionnaire containing a PK concepts list was firstly developed. The final questionnaire contained 110 concepts and asked participants to rate their relative relevance according to a *Likert* scale (1-6 points). Potential participants were invited to answer it via e-mail and LinkedIn®. Two groups were formed and analysed: Pharmacology educators in Medical Schools (Pharmacologists) and Portuguese Clinicians not involved in Pharmacology teaching (Non-Pharmacologists), who were Specialist Interns or Specialists in Internal Medicine, General and Family Medicine, Intensive Medicine, Nephrology and Gastroenterology. The concepts that obtained a score equal or above 5.0 were considered core. Principal component analysis (PCA) was used to investigate differences among groups on the answers to the questionnaire.

A total of 170 participants voluntarily answered to the questionnaire. The majority was non-pharmacologists (68%), and gender parity was achieved in both groups. Among Pharmacologists, 60% were graduated in medicine, 76% had more than 10 years of teaching experience in pharmacology (median 17 years). The majority were from Portuguese medical schools (47%), but it included representatives from 21 different countries. Among Non-Pharmacologists, 66% were Medical Specialists and there were 21-26% of Clinicians in each Medical Specialty, except for Gastroenterology (10%).

A total of 58 concepts were considered "core" by the Pharmacologists and/or by at least one of the Medical Specialties, and 27 concepts were considered "core" exclusively by the Pharmacologists. Only 20 concepts were unanimously classified as "core" by the Pharmacologists and all Medical Specialty. Moreover, 11 concepts not selected by the Pharmacologists were considered "core" by at least one Medical Specialty (Gastroenterology–10, Intensive Medicine–4, Internal Medicine–4, Nephrology–2; General and Family Medicine – 2). Differences in the responses were observed in PCA models built with Pharmacologists and non-Pharmacologists and with Pharmacologists and each of the other Medical Specialties.

In conclusion, our results show that the construction of a PK concepts inventory may benefit from multidisciplinary teams.

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PO-081

When students teach other students: A mixed-methods approach

Sahagun, AM, Lopez, C, Rodriguez, JM, de la Puente, R, Fernandez, N, Diez, R, Diez, MJ

Pharmacology. IBIOMED. University of Leon, Leon, Spain

E-mail: amsahp@unileon.es

Active and self-directed learning has been enhanced in Health Sciences curricula. A novel approach was trialed to successfully work in groups in the subject Veterinary Pharmacology (Degree in Veterinary Medicine) using two combined methodologies (poster and videos) in academic course 2022-23. A total of 113 students participated in the activity. They had to elaborate a poster and a video on a certain topic related with Pharmacology of Autonomic Nervous System and Autacoids. Four sessions throughout the semester were carried out to design and create both materials, working in groups of 4-5 people. They prepared 26 posters and the same number of videos, explaining the main features on the assigned topic. Posters were exhibited, explained and discussed in 2 sessions, and videos were made available for the rest of the students to be viewed. At the end of both activities, 99 students (87.6% of participants) evaluated and scored the work of the rest of students in both activities with a 5-point Likert-scale (1: very low; 5: excellent), and two groups were awarded as winners. Teachers also participated in the assessment. The students were able to produce acceptable quality posters and videos, with reasonable effort. A satisfaction survey was made available to students in order to know their opinion about both activities. All of them were highly satisfied with the strategy carried out in the subject. The results obtained support the hypothesis that these activities may become an effective tool to extend students' communication abilities, specially for academic purposes and client communication.

PO-082

Consolidation of theoretical knowledge in a practical-professional context: Experiences from teaching Pharmacology in the School of Medicine.

Yáñez-Gómez, F [1,2], García-Fuster, M.J. [1,2]

[1] *School of Medicine, University of the Balearic Islands, Palma, Spain*
[2] *IUNICS and IdISBa, Palma, Spain.*
E-mail: f.yanez@uib.es

Introduction: In recent years, new learning strategies are being implemented in the classroom. In this context, the possibility of combining theoretical knowledge with clinical practice to resolve a real situation is presented as a great opportunity to help students assimilate the contents, as well as to deepen their interest and motivation in the study of Pharmacology.
Materials and Methods: After the theorical explanation of a pharmacology related topic (i.e., cardiovascular system) in a Master Class Session, students were challenged to solve a clinical case in a Seminar Session (smaller groups of 20 students or less). We provided students with several pharmacological bibliographic reviews covering the main pharmacological groups involved in the treatment of heart failure. These reviews revealed the main advantages and/or disadvantages of each one of the pharmacological groups of study, providing vital aspects for selecting the most appropriate treatment course for the patient. During the Seminar, smaller groups of 2-3 students were assigned with one of the reviews covering a particular treatment option. Finally, one student from each group explained the benefits and/or risks of using their pharmacological option for the possible treatment of the supposed patient. After all groups showed their results, the whole class as a group reached an informed consensus on which one should be the best treatment option for the clinical case, and according to the particular characteristics of the patient presented. Students' grades and a satisfaction survey were used as indicators of the knowledge acquired.
Results and Discussion: During the last two academic years (2021-22 and 2022-23), 114 students were evaluated using this learning method to study Pharmacology that combined theoretical knowledge with daily practice though invented clinical cases. In summary, 48% of the students obtained grades between 7 and 8, while 43% reached values between 9-10. Only the remaining 10% got lower scores than 7 out of 10 points. All students rated the session as really stimulating and as an excellent way to consolidate the learning experience.
Conclusions: In view of the results obtained, this type of seminar sessions presents itself as a valuable evaluation tool that rewards the involvement and motivation of the students while also improves their learning. All these aspects will be of great relevance in their daily professional practice since it will help them to link their prior theoretical knowledge with future clinical cases. Decision-making based on theoretical knowledge, as well as reaching a consensus with colleagues regarding the best therapeutic treatment, will be very common aspects of their professional practice. In conclusion, this learning strategy has proven to be excellent in the context of teaching Pharmacology to Medical students and will also be implemented in other subjects.

PO-083

MEDIQ Anaesthesia Simulator facilitates the learning of anesthetics drugs in pre-graduate Medicine Degree students.

Bellido-Estevez, I.; Blanco Reina, E.; Guerrero Orriach, JL.; Raigon-Ponferrada A.; Barroso Gonzalez, A.; Bellido Estevez, MV.; Gomez-Luque A.

Department of Pharmacology. School of Medicine, IBIMA, Virgen de la Victoria University Hospital, Regional University Hospital, Malaga, Spain.

E-mail: ibellido@uma.es

Background. Simulations as a learning tool have been used for many years. From the use of mannequins (for example to practice artificial respiration and basic resuscitation, the administration of medications by respiratory, intravenous, intramuscular, subcutaneous, intraspinal, transesophageal catheterization...), excel sheets and basic software programs that simulate pharmacokinetic and pharmacodynamic problems and possible development of pharmacological effects, to complex software programs in which the student can interact in real time, with different types of patients and using procedures and control elements similar to those that students will use later in their professional life. MEDIQ Anaesthesia Simulator (designed by Abraxas AB, Ekerö, Sweden©) is a low-cost training tool used to teach anaesthesia to medical professionals. It is primarily used by anaesthetic trainees, but can also be beneficial to surgeons in training, nurses in anaesthesia and intensive care, paramedics, and medical students. The simulator is designed to simulate the same environment as aviation flight simulators, making it an effective tool for anaesthesia and other topics training.
We have evaluated the effectiveness of the use of the simulation program MEDIQ Anaesthesia Simulator in the learning of analgesic and anaesthetic drugs in undergraduate medicine degree students.
Material and methods. We have carried out a prospective controlled study with pre-graduated volunteer students from the Anaesthesia, Recovery Care and Medicine of Pain course. The students were randomized into two groups (N= 90 per group), MEDIQ-A, with unlimited access to the MEDIQ Anaesthesia Simulator Program, and control, without access to the program. Both received identical class aids on analgesic and anaesthetic medications and hands-on hours in the operating room. To determine the effectiveness of MEDIQ, we compared the results of both groups through a specific virtual test of their knowledge about analgesic and anaesthetic drugs and about control parameters of anesthetized patients carried out one week after finishing access to MEDIQ, the class aids and operating room practices and through the score obtained by both groups in the final exam of the subject.
Results. Students of MEDIQ-A group correctly answered more questions both in virtual evaluation (+24.9%) and final face to face evaluation of the course (+22.6%) compared to control group. Percentage of students of MEDIQ-A group satisfied with the activity 98.9%.
Conclusions. MEDIQ Anaesthesia Simulator Program enhances the learning of analgesic and anaesthetic drugs and control parameters of anesthetized patients by undergraduate medicine degree students.

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PO-084

Pharmacy students developed an innovative action through collaborative learning in high schools

Inés Moragrega[1], Mª Amparo Blázquez[2], Nuria Cabedo[2], Isabel Andújar[2], Mª Dolores Ibáñez[2], Rosa Mª Giner[2]

[1]Department de Psicobiologia. Facultat de Psicología. Universitat de València. Avda. Blasco Ibáñez 21. 46010, Valencia, Spain.
[2]Department de Farmacologia. Facultat de Farmàcia. Universitat de València. Avda. Vicent Andrés Estellés, s/n. 46100, Burjassot, Spain.

E-mail: Rosa.m.giner@uv.es

The present project continues an innovative and collaborative activity that started five academic years ago (1) in which Pharmacognosy students of the Degree in Pharmacy at the University of Valencia (UV) developed an educational project addressed to high schools. The aim of this initiative is to inform high school students about the problems associated with the consumption of substances of abuse and to prevent such consumption in both these students and university students. University students developed a collaborative work using methodologies such as flipped classroom, gamification, and educational pills. These allowed them to play leadership roles and to develop various skills for the transmission of previously acquired knowledge on abuse substances and increased their motivation and interest in the subject because of their interaction with students from other educational levels. With this activity, university students reach a greater understanding of addictive substances, since they must search for the information in trustworthy sources, analyse it, and prepare a suitable presentation for high school students that is both accurate and adapted to their level of knowledge. This process is supervised by the Pharmacognosy teachers to assure the accuracy of the information. To evaluate the activity, two questionnaires were designed *ad hoc*, one at the beginning of the activity about socio-demographics, risk perception, and patterns of consumption; the second one post-activity, with questions regarding satisfaction and usefulness. Both were assessed on a five-point Likert scale and data was processed using SPSS 28.0.
Regarding the pattern of consumption in university students, no significant changes were observed after the activity, mainly since these students showed a "healthy profile" with occasional tobacco use but frequent alcohol consumption, in contrast to high school students, who had a more variable pattern depending on their age and the geographical location of the school. The activity was rated satisfactorily by all the students, showing their willingness to participate in similar initiatives. The effectiveness of the transmission of knowledge was achieved by students in both educational levels: in high school because their "almost peers" were the ones acting as teachers had a positive impact; in the case of university students, this teaching role encouraged them to delve deeper into the subject. Moreover, we expect a favourable influence in the prevention of abuse substance consumption.

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PO-085

From face-to-face to remote learning in the Degree in Pharmacy

Posadas, I. & Perez-Carrión, M.D.

University of Castilla-La Mancha, Albacete, Spain

E-mail: inmaculada.posadas@uclm.es

The COVID-19 pandemic emerged at the beginning of 2020 year and forced us to modify the teaching-learning system at the university. In that situation of lock-down and social restrictions, the information technologies (IT) acquired a fundamental role and became a basic tool to continue with the teaching system at the university. Suddenly, the face-to-face teaching model was replaced by an exclusively online approach, which was gradually modified according to the social situation and allowed to the students came back to the classes but in small groups. There are no doubts about the importance of the digital transformation in the university teaching model. It is an easy, flexible, and accessible way of teaching. However, what is the impression of students about the digital transformation? How has the pandemic impacted in the university education from the point of view of students? To answer these questions, we have carried out a pilot study with students of the Degree in Pharmacy at the University of Castilla-La Mancha, who have experienced the evolution of the teaching system from an exclusive virtual situation to a face-to-face model. In this study we have designed a survey to evaluate the impression and opinion of the students along 3 academic years with 3 different teaching systems. In addition to answering these questions, this study aims to make students reflect about advantages and disadvantages of each educational situation.

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Acknowledgements: Students of the Degree in Pharmacy at the Faculty of Pharmacy (University of Castilla-La Mancha).

PO-086

Digitalization in the Degree in Pharmacy

Pérez-Carrión, MD & Posadas, I

University of Castilla-La Mancha, Albacete, Spain

E-mail: mariad.perez@uclm.es

In March 2020, with the explosion of the COVID-19 pandemic, the university system had to overcome an unexpected huge challenge: the digital transformation of the learning- teaching process. In that situation of social restrictions, the information technologies (IT) acquired a fundamental role and became a basic tool to continue with the teaching system at the university. In a short period of time, the classical face-to-face teaching model was replacedby a new methodological approach based on a digital world. With the control of the pandemic,the students came back to the classes, first in small groups, that grew until reaching the currentsituation, in which we have recovered the classical teaching scenario designed before pandemic.However, although the digital transformation has been considered a revolutionary methodology, poorly explored in the past, we wondered about the opinion of the teaching staffwhen comparing digital and classical teaching system at the university. To answer this and otherquestions, we have carried out a pilot study with professors of the Degree in Pharmacy at the University of Castilla-La Mancha, who have experienced the evolution of the teaching systemfrom a virtual situation to the classical face-to-face model, with an intermediate period of teaching in reduced groups. In this study we have designed a survey to evaluate the impressionand opinion of the teaching staff along 3 academic years with 3 different teaching systems, in order to investigate the advantages and disadvantages of each educational situation.

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Acknowledgements
Professors at the Faculty of Pharmacy (University of Castilla-La Mancha)

PO-087

Design and dissemination of an awareness campaign for the rational use of antibiotics promoted by Pharmacy students

Marina Sánchez Hidalgo*, María del Carmen Monedero, Isabel Villegas, Virginia Motilva, Elena Talero

University of Seville, Seville, Spain

E-mail: hidalgosanz@us.es*

The fight against antibiotic resistance is a high priority for the World Health Organization (WHO), as it is today one of the most serious threats to global health, food security and development. The misuse of antibiotics in humans and animals is contributing to accelerate this process, and an increasing number of infections are becoming more difficult, and sometimes impossible, to treat as antibiotics are losing efficiency. In an attempt to raise awareness in society of the risks associated with the misuse of antibiotics and to promote their prudent use, a multifaceted campaign targeting Pharmacy students, Community Pharmacies and general population was designed and implemented in the Faculty of Pharmacy at University of Seville within the framework of "European Day for the prudent use of antibiotics" and "World Antibiotic Awareness Week". Innovative methodologies such as Service-Learning and Information and Communication Technologies were applied. A total of 60 tutored volunteers students of Pharmacology and Pharmacotherapy III and Clinical Pharmacy from Degree in Pharmacy (distributed in groups of 5) designed public health campaigns to fight antimicrobial resistance and promote prudent use of antibiotics, holding interactive educative triptychs, quiz, card games and video educating the public in pharmacies and other health and university establishments (https://linktr.ee/farmabioticas). Likewise, RadiUS and digital media were used for campaign diffusion, through social networks (@farmabioticas in Facebook, twitter, TikTok, Youtube, LinkedIN and Instagram) reaching a greater visibility and repercussion. In addition, the material elaborated by the students was distributed in the race for the prudent use of antibiotics organized by the University of Seville. The students rated very positively the slogan, design, structure, material and dissemination of the campaign through the an anonymous survey. This innovative learning experience has enabled to the students to develop transversal knowledge and skills in terms of critical and creative thinking, creativity, interpersonal/socio-emotional and citizen-oriented skills and learning to learn in a professional context.

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PO-088

Teaching of Pharmacology in the Nursing Degree based on the service-learning (ApS) methodology

Pérez-Baena, M.J.; García-Barrado, MJ.; Herráez-Aguilar, E.; Sancho-Sánchez, C.; Holgado-Madruga, M.

Facultad de Medicina, Universidad de Salamanca, Salamanca, España

E-mail: barrado@usal.es

Service-learning (ApS) is a methodology that combines the acquisition of knowledge with social benefit that has been implemented in the Higher Education in recent years. Basically, it presents an educational approach oriented towards the development of activities or programs in which university students, through active participation in service experiences aimed at generating benefits for the community, manage to acquire knowledge that can be integrated into their curriculum, and therefore, promote civic commitment, empathy and strengthen meaningful learning and comprehensive development of students (1). At the same time, they can be included within the sustainable development objective of the 2030 agenda "health education to promote a healthy life and well-being" (2).

At the University of Salamanca, we have implemented a pilot project in the Nursing Degree program for the compulsory course of General Pharmacology. The learning topic used was "Appropriate use of medications," and the service consisted of holding an informative talk-class with children from 9 to 12 years old (4th, 5th, and 6th primary school) from the Campo Charro public school in Salamanca. Throughout the entire process, we had the authorization of the school authorities. The activity was carried out with 84 students in their second year of the Nursing degree, divided into 6 groups of 14-15 students each, who took responsibility for preparing the topic adapted to a primary school level.

In this experience, its impact on student learning results was assessed through a post-activity-service test that was compared with the results obtained before this activity. Of the 4 questions of the specific area evaluated, two of them showed significant differences between the pre-test and the post-test, with a significance of p=0.008 and p=0.0005, respectively, according to the McNemar test. The mean score of the nursing students improved significantly after the intervention according to the Mann-Whitney U-test (p=0.0009). However, as for the service activity with the child population, the results obtained did not show significant differences. The evaluation of these results is influenced by the chosen population, as the children in this school come from a healthcare-oriented family background, and the management of the presented information did not meet our expectations. On the other hand, the nursing students showed a high interest in acquiring values, empathy, and protection of minors. The level of satisfaction of this activity by both, children and university students, was very high, as reflected in a survey.

This work carried out informs us of the usefulness of including teaching methodologies such as ApS to promote the learning of Pharmacology contents, the acquisition of values, and specific and transversal competences among undergraduate students. On the other hand, it encourages us to elaborate proposals in a more selective way in which the use of the beneficiaries of this ApS involves the most needy sectors of society.

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PO-089

Pharmacology I in the Degree in Pharmacy and the Double Degree in Human Nutrition and Dietetics: How the students study and their opinion

Montesinos, M.C.[1,2], Ferrándiz, M.L.[1,2], Andújar, I.[1], Apostolova, N.[1]

[1]*Faculty of Pharmacy, University of Valencia, Spain*
[2]*IDM, Valencia, Spain*

E-mail: Luisa.Ferrandiz@uv.es

Pharmacology I is a 6 ECTS subject that is taught in the third year of the Degree in Pharmacy and the Double Degree in Pharmacy and Human Nutrition and Dietetics at the University of Valencia. The students are divided into 4 groups: B and D, taught in Spanish; C, in Valencian; and ARA, in English. Face-to-face activities include theoretical classes (40h), practicum (15h), seminars (4h) and group tutorials (2h). The objective of this work was to analyze how the students have studied the subject, and their opinion on the usefulness of the different programmed activities and the materials provided in the Virtual Classroom.

An anonymous and voluntary survey of 16 questions was carried out. All enrollees, except for exchange students (Erasmus), were included (regardless of how many times they have enrolled in the subject). The first question had 4 options to choose from, while the other 15 were Likert scale questions with five points: "Strongly disagree", "Disagree", "Neutral", "Agree" and "Strongly agree".

A total of 149 students (71.6% of those enrolled in the subject) took the survey. Regarding the study material of choice, most of the students selected the slides provided through the Virtual Classroom, either exclusively or in combination with Internet searches on their own (87.9%), while only 12.1% used textbooks, either library hardcopies or e-books available through the Virtual Classroom, in addition to the slides. Most would prefer to have all the slides available at the beginning of the course (86.7%), and preferably slides with a lot of text (71.3%). 76.8% of the students answered “Agree” or “Strongly agree” to the statement "Attendance to theory classes has been very useful to better understand the subject", and 71.6% acknowledged that they used slides from previous years. Strikingly, only 31.1% claimed to have kept the subject up to date. When asked if it is appropriate that a significant percentage of the final grade is from continuous assessment (seminars, tutorials and questionnaires), 78.1% stated that they "Agree" or "Strongly agree". Regarding the usefulness of the seminars, tutorials or practicum for the better learning of Pharmacology I, 57.8%, 81.3% and 86.7% answered "Agree" or "Strongly agree", respectively for the three activities, indicating certain differences in how they are perceived. Finally, 88.2% of those surveyed believe that "Pharmacology I has awakened a lot of interest, more than other subjects so far".

This survey has allowed us to better understand the students’ opinions and the results will help us to improve the organization of the activities and the materials provided for the subject Pharmacology I in following years.

PO-090

Promotion of responsible medicine consumption: service learning activity for health sciences students

García Cabanes, C.[1]; Sánchez Castillo, C.[1]; Fernández Sánchez, L.[1]; Formigós Bolea, J. A.[1]; López Rodríguez, D.[2]; Kutsyr Kolesnyk, O.[1]; Martínez-Gil, N.[1]; Sánchez Sáez, X.[1]; Noailles Gil, A.[1]; Maneu Flores, V.[1]

[1] Universidad de Alicante (España). [2] Universitat Politècnica de València (España)

E-mail: Cristina García Cabanes (tinilla@ua.es)

Service-learning makes learning available to students and instills social values along with service performance (Blesa et al. 2019). With experiences aimed at university students, and designed for its application in various community groups, service-learning connects students with the real world, stimulates them, makes them aware of the relevance of their future professional contribution and increases the skills, aptitudes, and formative results of students through the provision of a service to the community. Service-learning experiences seek to improve the quality of life or social inclusion of the community group to whom the service is directed (Furco, 1996; Martínez, 2008; Puig Rovira, Bosch and Batlle, 2007).

Our goal was to develop a campaign to promote responsible consumption of medicines among students studying Nursing, Human Nutrition and Dietetics, and Optics and Optometry at the University of Alicante.

A poster contest on responsible medicine consumption, prepared by undergraduate health sciences students, was proposed. The posters were displayed on a web page designed for this purpose and voted on by students in training cycles and university students.

40 posters were presented, of which 25 were selected for the contest. 392 votes were received. The students who carried out the activity stated that it had influenced them to be more aware of the social application of their studies. The young people who were targeted at the activity valued it positively and stated that they had increased their health knowledge. We believe that this type of activity is positive, both for undergraduate students and for the social group to which it is addressed.

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KEY WORDS: service- learning , health promotion, social commitment, poster contest.

OTHERS

PO-091

Influence of polypharmacy and drugs prescribed for cardiovascular risk reduction on cognitive function: a cross sectional study.

Cristina Garcia[1,3], Luis A. Martínez[2,3], Lucrecia Moreno[1] and Mónica Alacreu[1,4].

(1)-Cátedra DeCo MICOF-CEU UCH, Universidad Cardenal Herrera-CEU, 46115 Valencia, (2)-Department of Medical Sciences, Pharmacy School, Universidad de Castilla La Mancha 02071 Albacete, (3)-Community pharmacist, 02161 Tiriez - Albacete, (4)-Embedded Systems and Artificial Intelligence Group, Universidad Cardenal Herrera-CEU, 46115 Valencia.

E-mail: cggarcia@redfarma.org

BACKGROUND

Dementias are driven by a variety of risk and protective factors, many of which are closely linked to cardiovascular risk (CVR). The association between the risk of dementia and pharmaceuticals (e.g., lowering CVR drugs) is also a subject of growing interest. Thus, from the perspective of cognitive impairment (CI) prevention, an appropriate selection of the drugs used to treat these prevalent pathologies may be relevant. Our objective was to assess the pharmacotherapy as a screening method aimed at designing pharmacological interventions in patients at risk of CI.

MATERIAL AND METHODS

A cross-sectional study in community dwellers over 50 years of age with no previous diagnosis of dementia was carried out. An *ad-hoc* questionnaire (DeCo-Booklet)[1] comprising 31 variables and 23 validated tests was administered in a personal interview. CI risk was assessed using the Memory Impairment Screen, the Short Portable Mental State Questionnaire and the Semantic Verbal Fluency tests. Chronic pharmacological treatments were reviewed, encoded according to the anatomical therapeutic chemical classification, and stored in a database for analysis.

RESULTS

The mean time required to interview and process data was 120 min per patient. The participants (n = 350) were aged 73.1 ± 11, mainly female (63.4%) and polymedicated (57.1%). The estimated prevalence of CI risk was 21.1%. Antihypertensives (60.4%), antidiabetics (24.0%), antiplatelet therapy (21.7%) and benzodiazepines (19.3%) were the most prevalent treatments. CI risk was found to be statistically associated (p-value <0.05) with polymedication, use of benzodiazepines, acetylsalicylic acid or vitamin K antagonists and metformin (monotherapy).

CONCLUSIONS

Assessment of the potential influence of the chronic intake of some pharmaceuticals on CI risk through randomized controlled trials appear necessary.

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PO-092

Nrf2 activators as promising therapeutic agents for attenuating cellular senescence in IPF

Roger I[1,2,3], Montero P[2,3], Milara J [1,2,4], Sanz C[2], Cortijo J [1,2].

1. Biomedical Research Networking Centre on Respiratory Diseases (CIBERES), Health Institute Carlos III, 28029 Madrid, Spain
2. Department of Pharmacology, Faculty of Medicine, University of Valencia, 46010 Valencia, Spain;
3. Faculty of Health Sciences, Universidad Europea de Valencia, 46010 Valencia, Spain.
4. Pharmacy Unit, University General Hospital Consortium, 46014 Valencia, Spain

E-mail: Irola3@gmail.com

Cellular senescence, a fundamental process involved in the progression of Idiopathic Pulmonary Fibrosis (IPF), is widely acknowledged. Senescence refers to a natural biological state where cells undergo permanent growth arrest, but the accumulation of these senescent cells over time can disrupt tissue function and contribute to IPF development. An imbalance between the production of reactive oxygen species (ROS) and the body's ability to counteract them, known as oxidative stress, significantly contributes to cellular senescence and the onset of IPF. Nrf2, a transcription factor responsible for regulating antioxidant genes, plays a pivotal role in safeguarding cells against oxidative stress and promoting cellular well-being. Gaining a comprehensive understanding of the mechanisms underlying cellular senescence holds the potential for novel IPF treatments, such as the utilization of Nrf2 activators. The aim of this project is to analyze the role of Nrf2 activators in bleomycin-induced senescent processes in fibroblasts from healthy donors and IPF patients. Lung parenchyma tissue were obtained from control subjects (n=33) and IPF patients (n=18) to study the expression of senescence markers. In addition, the effect of Nrf2 activators on bleomycin-induced senescence markers was evaluated in primary fibroblasts isolated from control subjects (n=2) and IPF patients (n=2). Senescence markers such as p16, p21 and telomere shorting are overexpressed in lung parenchyma tissue from IPF patients. Treatment with Nrf2 activators has been observed to lead to a reduction in mitochondrial ROS and GSH levels induced by bleomycin. In addition, a XL National Meeting. Spanish Society of Pharmacology. Toledo, September 6-8, 2023 decrease in bleomycin-induced senescent markers has been observed, resulting in attenuation of the senescence-associated secretory phenotype. On the other hand, these activators also prevent cell growth arrest, increased cell proliferation and act as a preventive measure against DNA damage. Nrf2 activators have shown to be effective in revert numerous senescence hallmarks induced by bleomycin in vitro.

PO-093

Tacrolimus intrapatient variability as a biomarker in solid organ transplantation

Nogueiras-Álvarez, R [*1,2]; **García Saiz, MM** [3]

** MD. Clinical pharmacologist [1]PhD candidate, Cantabria University, Santander, Spain. [2]Bioaraba Health Research Institute. Vitoria-Gasteiz, Spain. [3]Clinical Pharmacology Service, Marqués de Valdecilla University Hospital, Santander, Spain.*

E-mail: ritanogueirasalvarez@gmail.com; rita.nogueiras@alumnos.unican.es

Maintenance immunosuppressive therapy after solid organ transplantation (SOT), regardless of the type, involves a triple combination of drugs that include corticosteroids, a calcineurin inhibitor and an anti-metabolite drug [1]. Among the calcineurin inhibitors, tacrolimus is the most widely used today, as it has been shown to produce a lower incidence of rejection and improved long-term outcomes when compared to cyclosporine [2]. Tacrolimus intra-patient variability (Tac-IPV) has been postulated as a helpful biomarker for assessing graft outcome in different types of SOT [3] [4].
We conducted a search for published articles on the relationship between Tac-IPV and the evolution of different types of SOT from inception to May 2023 using the database PubMed.
The publications found referred to renal, hepatic, cardiac and lung transplantation.
When it comes to assessing Tac-IPV, there are differences in the methods employed in the different studies. The measures used to quantify Tac-IPV include standard deviation (10 publications), coefficient of variation (33 publications found), mean absolute deviation (6 publications) and time in therapeutic range (9 publications).
Tac-IPV is a biomarker that has proven to be useful when evaluating graft outcomes in different types of transplantation. Differences regarding the number of available studies exist depending on the type of transplantation, being more frequent the evidence in renal transplantation (n=30). There is no standardised way of measuring Tac-IPV, nor well-defined criteria on how to select tacrolimus levels for its calculation. A more in-depth evaluation to define the optimal approach to Tac-IPV quantification is required.

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PO-094

A novel positive allosteric modulator of the GABA A receptor prevents lipotoxicity in hepatocytes

Elisabeth Rohbeck[1,2], Corinna Niersmann[1,2], Alejandra Romero[1], Bengt-Frederik Belgardt[3], Karl Köhrer[4], Thorsten Wachtmeister[4], Michael Roden[1,5,6], Jürgen Eckel[1,2] Tania Romacho[1,7]

[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany.
[2] CureDiab Metabolic Research, , Düsseldorf, Germany.3
[3]Institute for Vascular and Islet Cell Biology, German Diabetes Center at Heinrich Heine University, Leibniz Center for Diabetes Research, Düsseldorf, Germany.
[4]Biological and Medical Research Centre (BMFZ), Medical Faculty, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany
[5]German Center for Diabetes Research (DZD), Partner Düsseldorf, München-Neuherberg, Germany.
[6]Department of Endocrinology and Diabetology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany.
[7]Department of Nursing, Physiotherapy and Medicine, Faculty of Health Sciences, University of Almería, Almería, Spain.
E-mail: tromacho@ual.es

Background. Besides the high prevalence of NAFLD, there is no specific pharmacological treatment. The neurotransmitter GABA has been previously proposed as hepatoprotective against toxicity. Therefore we wanted to explore if HK4, a novel positive allosteric modulator (PAM) of the GABA A receptor, could protect human hepatocytes against lipotoxicity-induced injury. HK4 is a thioacrylamide-derivative which cannot cross blood brain barrier.
Material and methods Patch clamping in HEK-293 cells. Calcium influx was measured in INS-1E. HepG2 cells were exposed to palmitate alone or after prior exposure to HK4. After the corresponding incubation time, protein expression was assessed by Western blot. With a Cytation 5 image analyser apoptosis was measured by TUNEL with immunofluorescence and caspase activity by luminiscence and calcium influx with a calcium probe. RNA sequencing was done on the NextSeq550 system. Differentially expressed genes were identified and subjected to the DAVID database and Ingenuity Pathway Analysis.
Results We proved HK4 as a selective PAM of the $GABA_A$ receptor with patch clamp and calcium influx. The expression of several main $GABA_A$ receptor subunits (α 1 , α 3 , α 5 , β 2/3 and γ 2) was demonstrated by Western blotting of HepG2 cells. HK4 prevented palmitate-induced apoptosis was demonstrated by reduced caspase 3/7 activity and TUNEL both in HepG2 and human primary hepatocytes. This anti-apoptotic effect was mediated through PARP-1, NF-κB and STAT3. In line, enriched pathways analysis of gene expression affected by HK4, pointed towards targeting mitochondrial respiration, protein ubiquitination, apoptosis and cell cycle. We identified TP53, KDM5B, DDX5, CAB39 L and SYVN as key upstream regulators.
Conclusion HK4 reduces lipotoxic-induced apoptosis in hepatocytes by preventing inflammation, DNA damage and ER stress. Therefore, we propose that HK4 may arise as an innovative pharmacological tool to treat/prevent NASH as a first-in-class drug.

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PO-095

Preclinical Models of *Helicobacter pylori* Infection in Rats Using SS1 Strain

Alcarranza, M.[1,2], Villegas, I.[1,2], Alarcón-de-la-Lastra, C.[1,2]

[1]*Faculty of Pharmacy, University of Seville, 41012 Seville, Spain.*
[2]*Instituto de Biomedicina de Sevilla, IBiS (Universidad de Sevilla, HUVR, Junta de Andalucía, CSIC), Seville, Spain.*

E-mail: villegas@us.es

Helicobacter pylori is a microaerophilic, gram-negative bacteria which colonises the gastric mucosa. It has been identified as an essential risk factor for several diseases, including extragastric illnesses[1,2]. The incomplete eradication of *H. pylori* may be due to the short residence time of antibiotics in the stomach, leading to insufficient concentrations in the gastric mucosal layers where the pathogen is found. To increase the residence time of the drug and enhance its penetration through the mucosal layers, innovative formulations must be developed. This requires *in vivo* models of *H. pylori* infection to evaluate and provide results that can be applied to humans[3]. In the current study, an attempt was made to develop a preclinical model of *H. pylori* infection in rats, based on a murine model developed by Navabi et al[4]. 32 Sprague-Dawleys and 32 Wistar rats (5 and 4 weeks old respectively, male, specific pathogen-free in *H. pylori*) were employed in three models of *H. pylori* infection, using the Sydney Strain 1 (SS1) bacterial strain instead of wild-type bacterial strains from human biopsies. Depending on the model, single or multiple inoculations per day for three consecutive days of a bacterial suspension at the concentration of 1 x 10^9 CFU/mL were performed. In two of the models conducted, animals were pre-sensitised in the stomach by intragastric administration of naproxen sodium (30 mg/kg animal weight) once a day for three consecutive days. Infection was verified by urease, catalase and oxidase tests on isolated colonies of the microorganism, compared to an uninfected control group. According to the model described by Werawatganon[5], the percentage of infection achieved in the present study was 20% compared to 69.8%-83.0% obtained by this author. The same result was obtained by carrying out a second model, introducing prior sensibilisation, sacrificing the rats in both models two weeks after the administration of the last bacterial inoculum. The third model was derived from the study by Thombre and Gide[6], using Wistar rats, obtaining an infection rate of 10% compared to the 100% reported by the authors. Considering the results obtained in the present investigation, Sprague-Dawleys showed a higher infection success rate compared to Wistar rats, using the SS1 bacterial strain of *H. pylori*. Notwithstanding, the infection obtained by inoculating the SS1 strain is not efficient to generate a high infection rate that could be used as a basis for a model to test molecules with antibacterial activity against this microorganism in comparison to wild-type strains obtained from biopsies of patients affected with this pathogen.

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PO-096

Compartmental pharmacokinetics of menbutone after intravenous and intramuscular administration to sheep

Sahagun, AM, Diez, R, Rodríguez, JM, Lopez, C, Romero, B, Fernandez, N, Diez, MJ

Pharmacology. IBIOMED. University of Leon, Leon, Spain

E-mail: amsahp@unileon.es

Menbutone is a choleretic drug commonly used in veterinary medicine. The objective of this study was to characterize the compartmental pharmacokinetics for menbutone in sheep. 6 healthy non-lactating animals were used. The drug was dosed at 10 mg/kg and administered by both intravenous (IV) and intramuscular (IM) routes, with a 14-day wash-out period between administrations. Blood samples were collected over 24 h. Drug plasma concentrations were measured by liquid chromatography with UV detection, and standard compartmental models were used to fit data (Phoenix WinNonLin 8.3). After IV administration, menbutone followed a two-compartment open model, with a mean total clearance (Cl) of 70.5 ± 7.7 $mL \cdot h^{-1} \cdot kg^{-1}$, a half-life associated with the terminal β phase ($t_{½\beta}$) of 2.56 ± 0.72 h, a volume of distribution at steady-state (V_{ss}) of 186.4 ± 51.1 $mL \cdot kg^{-1}$, a mean time of residence (MRT) of 2.67 ± 0.86 h, and an area under the curve (AUC) of 143.3 ± 15.8 $\mu g \cdot h \cdot mL^{-1}$. When the drug was injected intramuscularly, data best fitted a one-compartment open model. Mean peak plasma concentration (C_{max}) was 17.5 ± 1.8 $\mu g \cdot mL^{-1}$, the time to reach C_{max} (t_{max}) 2.71 ± 0.60 h, the elimination half-life ($t_{1/2ke}$) was 3.97 ± 0.69 h, and the AUC 162.4 ± 34.8 $\mu g \cdot h \cdot mL^{-1}$. Thus, after IV administration menbutone shows a fast elimination from the body, and after IM injection absorption is complete.

PO-097

Evaluation of the cardioprotective, vascular and antioxidant activity of Trimetazidine.

Mero-Ríos, A ; Morán-Pinzón, J; Hernandez, X; Morales, A ; Sánchez, H ; Díaz, M ; Guerrero de León, E.

1. Universidad de Panamá, Panama city, Panama

E-mail: aldahirmero_20@hotmail.com

Trimetazidine (TMZ) is a piperazine-derived agent with anti-anginal properties that reduces the rate of free fatty acid oxidation by inhibiting long-chain mitochondrial 3-ketoacyl CoA thiolase, stimulating the heart to utilize glucose oxidation for energy supply, and optimizing energy metabolism (Kantor et al., 2000). Our aim was to describe the pharmacological actions of TMZ that have not been previously described, such as its cardioprotective action in ischemia-reperfusion, vascular, and antioxidant models. For cardioprotective activity, two concentrations of TMZ (25 and 100 µM) were evaluated in the Langendorff isolated heart method where ischemia is induced by oxygen suppression for subsequent reperfusion to obtain the normalized values of ventricular developed pressure, the double product, the Max dP/dt and the Min dP/dt, where 100% of the effect corresponds to the value of the control group. Vascular activity was evaluated using a myograph and rat aortic rings, and DRC to TMZ was performed, followed by DRC to Acetylcholine/Noradrenaline in the presence of TMZ (25 and 100 µM). Finally, the antioxidant activity was evaluated by the free radical trapping capacity: DPPH and nitric oxide, and the percentage of inhibition was calculated. TMZ perfusion (25 µM) demonstrated efficacy in decreasing I/R injury in the isolated heart model, as the treatment improved ventricular pressure development (84.88 ± 9.01 %). This beneficial effect was also observed in Max dP/dt and Min dP/dt by reaching values equivalent to 90.93 ± 8.50 % and 81.20 ± 10.01 %, respectively. respectively. The effects of TMZ 100 µM perfusion were less than 65% for all cardiac parameters evaluated. Additionally, DRC to TMZ generated a maximum relaxation of 37.54 ± 6.29 % and a calculated EC50 of 1.49 x10-3 M compared to the standard acetylcholine, which reached a maximum relaxation of 97.05 ± 1.83% and an EC50 of 2.53 x10-6 M. When TMZ 100 µM was incubated in the Noradrenaline DRC, the Emax was assayed (Emax= 56.53 ± 0.78%), as well as the EC50 (1.10 x10-6 M); this effect was not presented with TMZ 25 µM. In acetylcholine DRC, incubation with TMZ at 100 and 25 µM decreased the relaxation capacity of acetylcholine (54.78 ± 15.98 %). and 87.97 ± 10.35 %, respectively). Finally, the trapping capacity of TMZ 100 µM reached a DPPH inhibition percentage of 32 ± 2.97 % vs Control 62.7± 1.4 %. For NO, the value was 34.3 ± 2.3 % vs Control 24.5 ± 4.6 %. These results complement the profile of the pharmacological actions of TMZ.

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PO-098

Sex differences in the antidepressant-like effect of ketamine, letrozole and their combination in adult male and female rats

Sandra Ledesma-Corvi, Jordi Jornet-Plaza and M. Julia García-Fuster

(1)IUNICS, University of the Balearic Islands, Palma, Spain
(2)Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain

E-mail: sandra.ledesma1@estudiant.uib.es

Introduction: Ketamine has been recently approved to treat resistant depression; however preclinical studies showed sex differences in its efficacy. Given that women suffer from major depression at twice the rate of men, it is important to better understand how ketamine induced this sex-specific antidepressant-like effect. Sex steroids, such as estrogens and testosterone, both in the periphery and locally in the brain, are regarded as important modulators of these sex differences(1). Therefore, the present study evaluated the role of inhibiting estrogens' biosynthesis with letrozole, an aromatase inhibitor that catalyzes the conversion of androgensinto estrogens, in the differential antidepressant-like-response induced by ketamine by sex.

Methods: We performed several consecutive studies in adult Sprague-Dawley rats to evaluate potential sex differences in the antidepressant-like effects of ketamine (5 mg/kg, 7 days, i.p.), letrozole (1 mg/kg, 8 days, i.p.) and their combination (letrozole pre-treatment 3 h before ketamine). Acute and repeated antidepressant-like responses were ascertained in a series of behavioral tests (forced-swim, novelty-suppressed feeding, two-bottle choice for sucrose preference).

Results: The main results proved clear sex differences in the antidepressant-like response induced by ketamine, which was observed following a repeated paradigm in adult male rats, but rendered inefficacious in female rats. Moreover, decreasing estrogens' production with letrozole induced on itself an antidepressant-like response in female rats, while also improved ketamine's response in male rats (i.e., quicker response, only after a single dose). Interestingly, both the antidepressant-like effects induced by ketamine in male rats or letrozole in female rats persisted over time up to 65 days post-treatment, suggesting long-term sexdirected benefits for these drugs.

Conclusion: The present results demonstrated a sex-specificrole for inhibiting estrogens' biosynthesis in the antidepressant-like response induced by ketamine in male rats. Moreover, letrozole presented itself as a potential antidepressant for females with persistent effects over time. Clearly, the production of estrogens is key in modulating, in a sex-specific manner, affective-like responses and thus deserve further studies.

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PO-099

Metformin, a principal actress in the global oxidative status in patients with calcific aortic stenosis and diabetes mellitus

Corbacho-Alonso N[1], Rodriguez-Sanchez E[2], Sastre-Oliva T[1], Perales I[1], Juarez-Alia C[1], Mourino-Alvarez L[1], Ruiz-Hurtado G[2], Barderas MG[1], Tejerina T[3]

[1] Hospital Nacional de Paraplejicos, SESCAM, 45071 Toledo, Spain; [2] Instituto de Investigación i+12, Hospital Universitario, Spain; [3] School of Medicine, Universidad Complutense de Madrid, Spain;

E-mail: teje@med.ucm.es

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 defense (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.

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