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BOOK OF ABSTRACTS

XLI National Meeting of the Spanish Society of Pharmacology

CAIXAFORUM PALMA | OCT 3-5 | 2024

Plaça de Weyler, 3, Centre,
07001 Palma, Illes Balears

UIB Universitat de les Illes Balears | Departament de Medicina | iUNiCS Institut Universitari d'Investigació en Ciències de la Salut | Govern Illes Balears | IdISBa

Sociedad Española de Farmacología | Fundación Española de Farmacología

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WELCOME

On behalf of the **Spanish Society of Pharmacology**, it is my pleasure to welcome you to the **XLI National Meeting of the Spanish Society of Pharmacology that will be held in Palma at CaixaForum from October 3-5 of 2024**.

As prior editions, the meeting is expected to gather a large number of pharmacologists and scientists from other academic disciplines, such as chemists, pharmacists, and physicians. We are organizing an amazing scientific program covering frontline areas of pharmacology and therapeutics. Since the future of our society relays on the younger generations, in this meeting we are directing our main focus to promote the participation of early career scientists through short presentations of their scientific results, as well as by covering their expenses to attend the meeting through grants.

Besides the scientific part, Palma is a resort city with over 400,000 inhabitants and capital of the Spanish island of Mallorca (Majorca), in the western Mediterranean. The massive Santa María cathedral, a Gothic landmark begun in the 13th century, overlooks the Bay of Palma, and has been the inspiration for the Conference Banner. The adjacent Almudaina is a Moorish-style Arab fortress converted to a royal residence. West of the city, hilltop Bellver Castle is a medieval fortress with a distinctive circular shape. Not to mention the pristine sands and turquoise waters that the island has to offer!

For all these reasons, we look forward to meeting you in **Palma at the XLI National Meeting of the Spanish Society of Pharmacology.**

M. Julia García Fuster

COMMITTEES

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CONGRESS TIME-TABLE

Day 1 03/10/2024	*Sala Lluis Dòmenec i Muntaner*	*Sala Miquel dels Sants Oliver*
8:00-9:00	Delivery of documentation	
9:00-10:30	**Session 1:** Advances in Neuropharmacology **Moderator:** M. Julia García Fuster (Universidad de las Islas Baleares) **Invited speaker:** Esther Berrocoso (Universidad de Cádiz) **Selected oral communications:** Marco Taddei-Tardón (Instituto de Parasitología y Biomedicina "López-Neyra" - CSIC, Granada; Celia Rodríguez-Casas (Universidad de Barcelona); Mercè Pallàs (Universidad de Barcelona); Borja García-Bueno (Universidad Complutense de Madrid)	**Session 2:** Professional Careers in Pharmacology **Moderator**: Rocío Muñoz-García (Universidad de Sevilla) **Invited speakers:** Mª Luisa Suárez Gea (AEMPS); Belén López Bouzo (Lab. Farmacéuticos ROVI); Eduardo Oliver Pérez (CSIC); Beatriz Artalejo Ortega (IQS y Universidad Ramón Llull)
10:30-11:00	Coffee-break	
11:00-11:30	Official Opening	
11:30-12:30	**Invited Lecture 1:** Gut Feelings: The Microbiome as a Key Regulator of Brain & Behaviour Across the Lifespan **Moderator:** Rubén García Cabrerizo (Universidad de las Islas Baleares) **Invited speaker: John F. Cryan** (University College Cork)	
12:30-13:30	**Round Table 1:** Translational Research in Addictive Disorders (RIAPAD) **Moderator:** Francisco Navarrete (Universidad Miguel Hernández) **Invited speakers:** Laura Orio (Universidad Complutense de Madrid); Fernando Rodríguez de Fonseca (IBIMA Málaga y Universidad Complutense de Madrid); Paola Zuluaga (Hospital Universitario Germans Trias i Pujol)	**Session 3:** Natural Products Pharmacology **Moderator**: Ricardo Borges (Universidad de La Laguna) **Invited speaker:** Beatriz de las Heras (Universidad Complutense de Madrid) **Selected oral communications:** Rocío Muñoz-García (Universidad de Sevilla); Ricardo Borges (Universidad de La Laguna)
13:30-15:00	Lunch	
15:00-16:00	**Session 4:** Sex Differences in Neuropsychopharmacology **Moderator:** Olga Valverde (Universidad Pompeu Fabra)	**Session 5:** Inflammation and Immunomodulation Pharmacology **Moderator:** Mª Jesús Sanz (Universidad de Valencia)

	Invited speaker: Olga Valverde (Universidad Pompeu Fabra) **Selected oral communications:** Guadalupe Rivero (Universidad del País Vasco); Evelyn P. Silva (Universidad de Cantabria)	**Invited speaker:** Mª Jesús Sanz (Universidad de Valencia) **Selected oral communications:** Javier Ávila Román (Universidad de Sevilla); Inés Valencia (Hospital Universitario Santa Cristina, Madrid)
16:00-17:00	**Session 6:** Vascular Pharmacology **Moderator:** Mercedes Salaices Sánchez (Universidad Autónoma de Madrid) **Invited speaker:** Concepción Peiró (Universidad Autónoma de Madrid) **Selected oral communications:** Cristina González-Correa (Universidad de Granada); Inés Roger (Universidad de Valencia)	**Session 7:** Novel Approaches to *In Vivo* Pharmacology **Moderator:** Francisco Ciruela (Universidad de Barcelona) **Invited speaker:** Francisco Ciruela (Universidad de Barcelona) **Selected oral communications:** Fernando Yáñez-Gómez (Universidad de las Islas Baleares); Mateo Ruiz-Conca (Universidad de Alicante)
17:00-18:30	**Poster Session** Real Academia de Medicina de las Islas Baleares (C/ Ca'n Campaner 4, Palma)	
20:00-21:30	**Welcome Cocktail** Centro Cultural Ca'n Balaguer (C/Unió, 3, Palma)	

Day 2 04/10/2024	***Sala Lluis Dòmenec i Muntaner***	***Sala Miquel dels Sants Oliver***
8:00-9:00	Delivery of documentation	
9:00-10:30	**Session 8:** Cardiovascular Pharmacology **Moderator:** Antonio Rodríguez Artalejo (Universidad Complutense de Madrid) **Invited speaker:** Ricardo Caballero (Universidad Complutense de Madrid) **Selected oral communications:** Lucía Núñez (Universidade da Coruña); Carlos Hermenegildo (Universidad de Valencia); Dolores Viña (Universidad de Santiago de Compostela); Ana Paula Dantas (Universidad de Barcelona)	**Session 9:** Respiratory, Gastrointestinal, and Pain Pharmacology **Moderator:** Julio Cortijo (Universidad de Valencia) **Invited speaker:** Paula Montero (Universidad de Valencia) **Selected oral communications:** Jesús Cosín-Roger (Universidad de Valencia); Carolina López-Marín (Universidad de Cádiz); Miguel A. Huerta (Universidad de Granada); Fermín Sánchez de Medina (Universidad de Granada)
10:30-11:00	Coffee-break	
11:00-12:00	**Invited Lecture 2:** Hemopatías malignas: Una nueva mirada diagnóstica y terapéutica **Moderator:** J. Javier Meana (Universidad del País Vasco) **Invited speaker: Xabier Martín Martitegui** (Servicio de Hematología y Hemoterapia, Hospital Universitario Cruces, Bizkaia)	

12:00-13:30	**Round Table 2:** Rational Drug Prescription and Dispensing. **Chairman:** Francisco Zaragozá (Universidad de Alcalá) **Invited speakers:** Arantxa Sancho (Farmaindustria); Francisco Abad (Hospital Universitario de la Princesa y Universidad Autónoma de Madrid); Quintiliano Pérez (Farmacéutico Oficina de Farmacia, Madrid)	
13:30-15:00	Lunch	
15:00-16:00	**Session 10:** Novel Approaches for the Design, Development, and Delivery of Drugs **Moderator:** Valentín Ceña (Universidad de Castilla-La Mancha) **Invited speaker:** María Isabel Loza (Universidad de Santiago de Compostela) **Selected oral communications:** Irene Rodríguez-Clemente (Universidad de Castilla - La Mancha); Verònica Casadó-Anguera (Universidad de Barcelona)	**Session 11:** Teaching Innovation in Pharmacology **Moderator:** Fernando Yáñez-Gómez (Universidad de las Islas Baleares) **Invited speaker:** Immaculada Bellido (Universidad de Málaga) **Selected oral communications:** Mª Luisa Ferrándiz (Universidad de Valencia): Víctor López (Universidad San Jorge, Zaragoza)
16:00-17:00	SEF Assembly	
17:00-18:30	**Poster Session** Real Academia de Medicina de las Islas Baleares (C/ Ca'n Campaner 4, Palma)	
21:30	**SEF Congress Dinner** Restaurante S'Àngel (Plaça de la Porta de Santa Catalina, 7A, 07012)	

Day 3 05/10/2024	***Sala Lluis Dòmenec i Muntaner***
9:00-9:30	Delivery of documentation
9:30-10:30	SEF Young Investigator Award and Lifetime Achievement Award in Pharmacology
10:30-11:00	Awards Ceremony
11:00-11:30	Coffee-break
11:30-12:30	**Invited Lecture 3:** Tratamiento farmacológico de la obesidad: El amanecer tras la larga noche. **Chairman:** Lucía Nuñez Fernández (Universidad Complutense de Madrid) **Invited speaker: Javier Salvador** (Universidad de Navarra)
12:30-13:30	Closing Ceremony and Remarks

ABSTRACTS
INVITED LECTURES

Gut Feelings: The Microbiome as a Key Regulator of Brain & Behaviour Across the Lifespan

John F. Cryan

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The microbiota-gut-brain axis is emerging as a research area of increasing interest for those investigating the biological and physiological basis of neurodevelopmental, age-related and neuropsychiatric disorders. The routes of communication between the gut and brain include the vagus nerve, the immune system, tryptophan metabolism, via the enteric nervous system or via microbial metabolites such as short chain fatty acids. Studies in animal models have been key in delineating that neurodevelopment and the programming of an appropriate stress response is dependent on the microbiota. Developmentally, a variety of factors can impact the microbiota in early life including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics. Stress can significantly impact the microbiota-gut-brain axis at all stages across the lifespan. Moreover, animal models have been key in linking the regulation of fundamental brain processes ranging from adult hippocampal neurogenesis to myelination to microglia activation by the microbiome. Finally, studies examining the translation of these effects from animals to humans are currently ongoing. Further studies will focus on understanding the mechanisms underlying such brain effects and developing nutritional and microbial-based psychobiotic intervention strategies and how these interact with various systems in the body across the lifespan.

Hemopatías malignas: una nueva mirada diagnóstica y terapéutica

Xabier Martín Martitegui

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El campo de la hematología ha experimentado un progreso extraordinario desde mediados del siglo XX. Los avances en el diagnóstico y tratamiento de las neoplasias hematológicas han transformado el pronóstico de muchas enfermedades que anteriormente eran letales.

Uno de los hitos más importantes en la historia de la hematología fue el descubrimiento en 1960 del cromosoma Filadelfia, una anomalía cromosómica específica en pacientes con leucemia mieloide crónica (LMC). Este cromosoma resulta de una translocación entre los cromosomas 9 y 22, produciendo el gen quimérico BCR-ABL, que codifica una proteína con actividad tirosina quinasa que impulsa la proliferación descontrolada de células hematopoyéticas malignas. El tratamiento de la LMC se basaba en quimioterapia convencional y trasplante hematopoyético hasta que en el año 2001 se aprueba imatinib, el primer inhibidor de tirosina quinasa (ITK), que revolucionó el tratamiento de esta enfermedad. Imatinib aumentó significativamente la supervivencia en pacientes con LMC, así como su calidad de vida. El descubrimiento del cromosoma Filadelfia y el desarrollo de los ITK marcaron el inicio de una nueva era en la medicina personalizada.

Con el tiempo, se han producido avances en las técnicas de diagnóstico: tanto la citometría de flujo como la secuenciación de nueva generación (NGS) han revolucionado el campo diagnóstico, permitiendo identificar marcadores y mutaciones con valor diagnóstico y pronóstico y facilitando la identificación de recaídas antes de que sean clínicamente evidentes. Del mismo modo, la identificación de determinadas alteraciones recurrentes ha conducido al desarrollo de terapias dirigidas.

Además de los ITK, en los últimos años hemos asistido al desarrollo de nuevas estrategias dirigidas a dianas específicas, que buscan en última instancia mejorar la eficacia de los fármacos minimizando la clásica toxicidad derivada de la quimioterapia. Entre los diferentes grupos de tratamientos disponibles destacan los anticuerpos monoclonales, los anticuerpos conjugados, los anticuerpos biespecíficos, los inhibidores del check point y las pequeñas moléculas inhibidoras de vías de señalización.
El ultimo hito al que hemos asistido es el desarrollo e integración en nuestro arsenal terapéutico de la terapia CART (quimeric antigen receptor T), que consiste en la reprogramación genética de los linfocitos T del propio paciente para que expresen un receptor quimérico capaz de reconocer antígenos en las células tumorales (como CD19 en las células B malignas). Tras la infusión de estas células modificadas, estas se expanden y atacan de manera efectiva al cáncer.

La integración de la medicina personalizada, las nuevas terapias dirigidas y enfoques inmunológicos ha transformado el panorama para los pacientes con neoplasias hematológicas, alterando la historia natural de estas enfermedades, permitiendo no solo mejorar la supervivencia, sino también ofrecer una mejor calidad de vida.

Tratamiento farmacológico de la obesidad: El amanecer tras la larga noche

Javier Salvador

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El tratamiento farmacológico representa un eslabón clave en el tratamiento de las personas con obesidad cuando el valor de IMC supera la cifra de 30 kg/m^2 o de 27 kg/m^2 con complicaciones asociadas en el marco de un programa terapéutico integral con nutrición clínica, estilo de vida, apoyo psicológico y cirugía bariátrica cuando se encuentre indicada.
La larga noche se extiende desde 1933 con fármacos basados en la modulación de neurotransmisores que fueron retirados, persistiendo únicamente orlistat (1999), de efecto periférico bloqueador de lipasas gastrointestinales. En 2012 y 2014 se aprueban por la FDA las combinaciones fentermina-topiramato y naltrexona-bupropion que pivotan sobre su efecto sobre distintos neurotransmisores. En 2005 se aprueba el primer agonista de receptor de GLP-1 (AR GLP-1), exenatida, para tratamiento de la diabetes tipo 2, lo que se sigue de la eclosión de diferentes ARs GLP-1 con el mismo fin demostrando su efecto central saciante y reductor del exceso de grasa con mejoría del control metabólico. El amanecer del tratamiento farmacológico de la obesidad se inicia en 2014 con la comercialización de liraglutida 3 mg (AR GLP-1) que en inyección diaria se asocia con reducciones ponderales inferiores al 10% en un año, asociado a una mejoría de factores de riesgo cardiovascular, apnea del sueño y reducción del riesgo de diabetes con un perfil de seguridad favorable. La ingeniería bioquímica dio paso a la síntesis de semaglutida (SEMA) y su formulación subcutánea semanal de 2,4 mg (2021), que constituye el primer AR GLP-1 que genera una reducción porcentual en peso corporal de dos dígitos (15-17,4%). El programa STEP de SEMA además documenta su capacidad de mejorar el control de la diabetes asociada a la obesidad y reafirma su eficacia sostenida a 2 años y la superioridad frente a liraglutida 3 mg. La mejoría en factores de riesgo cardiovascular por SEMA se ve corroborada en el estudio SELECT por la reducción en 48 meses del 20% de eventos cardiovasculares (MACE) y del 22% de objetivo compuesto renal en personas con obesidad sin diabetes. Recientemente, se ha demostrado una mejoría significativa en los síntomas de pacientes con insuficiencia cardiaca con fracción de eyección preservada en personas con obesidad, tanto sin como con diabetes tipo 2 asociada, por parte de SEMA 2,4 mg.
La ingeniería bioquímica hace posible el desarrollo de multiagonistas unimoleculares, cuya primera representante es tirzepatida (2023), un co-agonista GIP-GLP-1 de administración semanal. Tirzepatida es capaz de inducir una reducción ponderal de hasta 22,7% con la máxima dosis semanal de 15 mg, manteniendo un buen perfil de tolerancia y reduciendo los factores de riesgo cardiovascular, el índice de apnea-hipopnea, la esteatosis y levemente la fibrosis hepática. Estos nuevos fármacos representan un avance extraordinariamente significativo para el tratamiento de la obesidad. El futuro próximo se ve jalonado por nuevas formulaciones orales y otras de larga duración de AR GLP-1, co-agonistas GLP-1/glucagón, triagonistas GLP-1/GIP/glucagón y combinaciones AR GLP-1 con agonistas de amilina entre otras innovaciones, como los fármacos destinados a preservar la masa muscular, que van a revolucionar en conjunto el tratamiento de la obesidad y sus complicaciones.

Drucker DJ. Efficacy and safety of GLP-1 medicines for type 2 diabetes and obesity. Diabetes Care 2024; 47:1-16.
Gudzune KA, Kushner RF. Medications for obesity. A review. JAMA 2024; 332:571-84.

ROUND TABLES

Round Table 1: Translational Research in Addictive Disorders: RIAPAD

Chairman: Francisco Navarrete (Universidad Miguel Hernández)

Invited speakers:

Laura Orio (Universidad Complutense de Madrid)
Fernando Rodríguez de Fonseca (IBIMA Málaga y Universidad Complutense de Madrid)
Paola Zuluaga (Hospital Universitario Germans Trias i Pujol)

Round Table 2: Rational Drug Prescription and Dispensing

Chairman: Francisco Zaragozá (Universad de Alcalá)

Invited speakers:

Arantxa Sancho (Farmaindustria)
Francisco Abad (Hospital Universtario de la Princesa y Universidad Autónoma de Madrid)
Quintiliano Pérez (Farmacéutico de oficina de farmacia comunitaria; Madrid)


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SESSIONS

Session 1: Advances in Neuropharmacology

Moderator: M. Julia García Fuster (Universitat de les Illes Balears)

Invited speakers:

9:00-9:30 Esther Berrocoso (Universidad de Cádiz)
Designing a humanized murine model for bipolar disorder and lithium

9:30-9:45 Marco Taddei-Tardón *(Instituto de Parasitología y Biomedicina "López-Neyra" – CSIC, Granada)*
Role of the serotonin 5-HT2A receptor in neuroplasticity Induced by serotonergic psychedelics

9:45-10:00 Celia Rodríguez-Casas (Universitat de Barcelona)
Searching for opioids with low addictive side effects due to its weak potency on opioid-galanin receptor heteromers

10:00:10:15 Mercè Pallás (Universitat de Barcelona)
Searching for new inhibitors of soluble epoxides towards regulatory preclinical studies

10:15-10:30 Borja García-Bueno (Universidad Complutense de Madrid)
Alterations in the expression of apolipoproteins in a combined model of schizophrenia and periodontitis in rats

Designing a humanized murine model for bipolar disorder and lithium response

Esther Berrocoso[1,2,3], Irene Suarez-Pereira[1,2,3], María Hidalgo-Figueroa1[1,2,3], Alejandra Delgado-Sequera[1], Alejandro Torrillas de la Cal1[1,2,3], Clara García-Mompó[1], Rafael Luján[4], Cristina Romero López-Alberca[1,2,3], José I. Peréz-Revuelta[2,3,5], Anaid Pérez[1,3], Francisco González-Saiz[2,3,5], Víctor Pérez-Solá[3,6], Ana González-Pinto[3,7], Eduard Vieta[3,8]

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University of the Basque Country, Bilbao, Spain. [8] Bipolar and Depressive Disorders Unit, Hospital Clinic, Institute of Neuroscience, University of Barcelona, IDIBAPS, Barcelona, Catalonia, Spain.

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Bipolar disorder (BD) is a highly heritable psychiatric illness with a lifetime prevalence of 1-3%. It is a multifactorial condition that involves both genetic and environmental factors. The gold standard treatment for BD is lithium; however, patient response to lithium varies significantly, with 40-50% of patients experiencing inadequate clinical responses and/or serious side effects. The limited number of experimental models for BD makes it challenging to characterize novel biomarkers or develop treatments that could enhance our understanding of its neurobiology and improve drug responses. Our goal is to develop and characterize a novel murine humanized model of BD using human olfactory neuroepithelium (ONE) and evaluate its face and construct validity in relation to BD, as well as its predictive validity in response to lithium treatment. Neural progenitor cells from the olfactory neuroepithelium (ONE) were obtained from both control donors and patients with BD type-I, who either do or do not respond to lithium treatment based on the ALDA scale evaluation. These cells were grafted into a neurogenic niche in rodents. Four weeks after cell implantation, mania-like and depressive-like behaviors were assessed to determine if the mice developed a BD-like phenotype. Lithium was administered through chow food for three weeks prior to behavior testing. At the experiment's endpoint, brains were collected for immunofluorescence and electron microscopy assays. Our data demonstrate that animals grafted with ONE cells from BD patients exhibit a BD-like phenotype, characterized by depressive-like behavior and motor hyperactivity, four weeks after cell implantation. Remarkably, this phenotype was specifically reversed following chronic lithium treatment in animals grafted with ONE cells from lithium responders. Immunohistological studies further revealed that 75-80% of these human ONE cells expressed markers of mature GABAergic neurons and successfully integrated into the mouse neuronal circuitry, forming synaptic contacts. These findings indicate that the developed model is suitable for studying the neurobiology of BD, identifying predictive biomarkers for therapeutic response, and evaluating the efficacy of compounds against BD. Additionally, this research opens avenues for personalized medicine approaches in treating BD.

Acknowledgements: Supported by the "Programa Operativo de Andalucía FEDER, Iniciativa Territorial Integrada ITI 2014-2020" Conserjería de Salud - Junta de Andalucía" (PI-0009-2017); by the "Conserjería de Salud y Familias - Junta de Andalucía" (PEMP-0008-2020) and the "Ministerio de Sanidad, Consumo y Bienestar Social, Plan Nacional sobre Drogas," (2019I041).Cibersam (CB07/09/0033, SAM22TRF01); INiBICA (IN-CO9); FPU Fellowship (#FPU20-03072). Red Española de Investigación en Estrés/Spanish Network for Stress Research RED2022-134191-T financed by MCIN/AEI. /10.13039/501100011033;

Role of the serotonin 5-HT$_{2A}$ receptor in neuroplasticity induced by serotonergic psychedelics

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In recent years, serotonergic psychedelics have garnered considerable attention for their potential therapeutic effects on various mental disorders. These compounds primarily target serotonin (5-HT) receptors, with a particular emphasis on the 5-HT$_{2A}$ receptor, which is highly expressed in the brain. Administration of serotonergic psychedelics has been demonstrated to induce neuroplasticity. However, it remains unclear whether this neuroplasticity is exclusively mediated through interactions with the 5-HT$_{2A}$ receptor or if other receptors may also be involved.

We have generated a genetically engineered neural stem cell line wherein 5-HT$_{2A}$ receptor silencing can be induced via the expression of 5HT$_{2A}$ shRNA upon treatment with IPTG. Neuroplasticity in neurons derived from the differentiation of these stem cells, both in the presence and absence of IPTG, was assessed by measuring dendritogenesis through Sholl analysis. The assessment was conducted following treatment with the following serotonergic psychedelics: 2-Bromolysergic acid diethylamide (2Br-LSD), lysergic acid diethylamide (LSD), N,N-Dimethyltryptamine (DMT), psilocin, ketamine, lisuride, 1-(2,5-Dimethoxy-4-methylphenyl)butan-2-amine (ARIADNE), and 2,5-Dimethoxy-4-iodoamphetamine (DOI). Brain-derived neurotrophic factor (BDNF) was utilized as a positive control, while water (H_2O) and dimethyl sulfoxide (DMSO) served as vehicles.

5-HT$_{2A}$ receptor silencing was validated through qPCR and functional assays. 5HT$_{2A}$ receptor expression decreased by over 90% in differentiated cell cultures. When treating cultures with psychedelics in the presence of IPTG, no expression of the early gene c-Fos was observed, contrasting with an upregulation observed in cultures where IPTG was absent. Similarly, Sholl analysis indicated increased neuronal arborization when treated with serotonergic psychedelics, both hallucinogenic and non-hallucinogenic, exclusively in experiments conducted in the absence of IPTG. Additionally, there was a statistically significant difference in the effects of BDNF when comparing both experimental conditions, indicating a reduction in the effect of this neurotrophin when 5-HT$_{2A}$ receptor expression is silenced.

Our results indicate a crucial role of the 5HT$_{2A}$ receptor in mediating neuroplasticity induced by serotonergic psychedelics, irrespective of the hallucinogenic nature of these drugs. Additionally, the results regarding BDNF suggest a potential synergy between 5HT$_{2A}$ and tyrosine receptor kinase B (TrkB), both of which are endogenously expressed in this neural in vitro experimental model.

Acknowledgements:
Proyecto PID2021-125448OB-I00 financiado por MCIN/AEI/10.13039/501100011033/ y por FEDER. Una manera de hacer Europa

Searching for opioids with low addictive side effects due to its weak potency on opioid-galanin receptor heteromers

Cèlia Rodríguez-Casas[1], Natàlia Llopart[1,2], Ning-Sheng Cai[3], César Quiroz[3], Michael Michaelides[3], Michael H. Baumann[3], Sergi Ferré[3], Estefanía Moreno[1,2], Vicent Casadó[1,2], Verònica Casadó-Anguera[1,2]

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μ-opioid receptor (MOR) agonists are the most effective drugs to treat chronic pain[1]. MORs belong to the superfamily of G protein-coupled receptors (GPCRs) and mediate both the analgesic and addictive effects of opioids[1]. It has been shown that MOR and galanin 1 receptor (Gal1R) form heteromers in the ventral tegmental area (VTA) and nucleus accumbens (NAc) and that the dopaminergic cell function in these areas is modulated by MOR-Gal1R heteromer exerting an antagonistic effect on opioid reward[2-4]. Therefore, the main aim of this project was to search for non-addictive opioid drugs useful to treat chronic pain. To achieve this objective we used different pharmacological, biochemical, and functional techniques in transfected cells with MOR or MOR and Gal1R as well as *in vivo* assays in rodents. Using radioligand binding assay we have found that (S)-methadone in both MOR and MOR-Gal1R cells was the MOR agonist with worst affinity. By G-protein BRET activation and cAMP production assays we have found that all MOR agonists tended to exhibit better potency in MOR cells compared to MOR-Gal1R cells. Buprenorphine, PZM21 and (S)-methadone showed the most significant differences in potency between MOR and MOR-Gal1R cells. Additionally, morphine, buprenorphine and PZM21 are partial agonists in MOR-Gal1R cells. Using several behavioral assays such as drug self-administration as well as tests to assess the analgesic properties of (R)-methadone, (S)-methadone and (R,S)- methadone, we demonstrated that (S)-methadone induced analgesia with similar efficacy as (R)-methadone but was not self-administered, indicating low addictive profile. 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 addictive profile but the same analgesic properties and could be used clinically as analgesics. In a future study we will assess the analgesic and addictive properties of other opioids, such as PZM21 and buprenorphine.

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Acknowledgements:
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Searching for new inhibitors of soluble epoxides towards regulatory preclinical studies

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Soluble epoxide hydrolase (sEH) inhibition is considered a promising strategy for Alzheimer's disease treatment (Griñán-Ferré et al, 2021). We developed UB-SCG51 (4-[((1r,4r)-4-(3-(9-Chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)cyclohexyl)oxy]-benzoic acid) (Jarné-Ferré et al, 2023) as a hit, a process of lead nomination was performed pursuing higher efficacy and improving ADME profile. UB-SCG74 is the arginate of UB-SCG51, that envisage a best oral biodisponibility was selected to go through drug development steps including efficacy and safety issues as a non-regulatory approach that to go through Technology Readiness Level (TLR) 3 (experimental PoC) to TLR4 (Lab validated technology). UB-SCG74 showed a much better oral absorption than SCG51, showing brain concentrations higher than UB-SCG51 in mice.
Novel object recognition (NORT) and novel object location (NOLT) paradigms were used to evaluate improvement in cognitive impairment and Golgi staining to discern dendritic plasticity after UB-SCG74 administration to 5XFAD mice (males and females) (0,5, 1,5 and 3 mg/kg) as a model of early onset AD. Safety issues were addressed by studying hERG, off-targets panel, maximum tolerated dose, and aneuploidy cells to discard cardiovascular risk, toxicity, and genotoxicity, respectively.
Results showed that UB-SCG74 prevented cognitive impairment and preserve dendritic spines and in the synaptic branching. Safety issues results showed that UB-SCG74 has a very good safety profile, without hERG affectation, MTD up to 2000mg/kg and absence of mutagenesis and teratogenesis markers. In sum, those results, allowing us to nominate UB-SCG74 as a candidate for pursuing developing regulatory development, forward clinical trials.

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ACKNOWLEDGEMENTS. This study was supported by the Ministerio de Economía, Industria y Competitividad (Agencia Estatal de Investigación, AEI) and European Union NextGenerationEU/PRTR (PID2020-118127RB-I00/AEI/ 10.13039/501100011033; PDC2021-121096/AEI/10. 13039/5011000110 33) and Generalitat de Catalunya *Senior co-

Alterations in the expression of apolipoproteins in a combined model of schizophrenia and periodontitis in rats

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Background: Schizophrenia (SZ) is a severe mental illness with multiple symptomatology. Previous findings suggest that alterations in the inflammatory response could have a role in its physiopathology. The neurodevelopmental theory of SZ proposes a "2 hit model" where the first hit occurs pre/perinatally causing neurodevelopmental alterations, and the second hit during late adolescence triggers the first psychotic episode. Periodontitis is an irreversible inflammatory gum disease produced by bacterial oral dysbiosis whose progression is irreversible. Increasing studies suggest the existence of comorbidity between both pathologies, pointing to chronic low-grade inflammation as a common denominator.

Apolipoproteins (Apos) are a family of proteins that form lipoproteins (HDL, LDL,etc.) with a major role in the transport of cholesterol and in bacterial endotoxins (Gram - LPS and Gram + LTA) detoxification system. Recently, complementary functions for Apos in the regulation of LPS neuroinflammation, reelin-cognition connection, and neurophagy have been suggested.

Considering all this background we aimed to check whether there are alterations in the expression of several apolipoproteins/receptors in brain frontal cortex and liver samples from rats submitted to a combined model of SZ and periodontitis as a first step to disentangle new therapeutic strategies for the better management of SZ and comorbid periodontitis.

Methods: We designed an animal model based on maternal immune activation with the viral mimetic compound poly I:C administered iv on gestational day 14 as the first hit, and periodontitis in the offspring on late adolescence (postnatal day 45) as the second hit using oral gavages of a solution of periodontal pathogens. 48 offspring Wistar rats (male and female 5:3) divided in 4 experimental groups were used: control (n=9), perio (n=10), poly I:C (n=14), and poly I:C+Perio (n=15). To validate the animal model, behavioural tests (prepulse inhibition test (PPI), open field test (OF), and novel object recognition (NOR)) and biochemical plasma assays (corticosterone, LPS and LTA) were made. In addition, molecular analysis checking the expression in prefrontal cortex samples (Western Blot), liver (RT-qPCR) and plasma (ELISA) of Apos (A1, B, E, M and J) and their receptors (ApoER2, LDLR, SR-B1) were made. Two ways ANOVA was performed to assess the effect of the sex. When significant, data is reported divided in males and females. To analyse intergroup differences, ANOVA with Bonferroni corrections or Kruskal Wallis tests were used.

Results: poly I:C+Perio animals showed deficits in PPI test and increased levels of plasma corticosterone. ApoER2 and SRB1 receptors protein expression in frontal cortex increased in poly I:C+Perio group Vs control (no differences were found segregating by sex). In plasma, ApoM levels decreased in poly I:C+Perio group Vs control (no effects of sex were found). In addition, the levels of ox-HDL (a marker of oxidative stress over HDL related to changes in the composition of its Apos) increased only in males from the combined group compared to controls. No significant differences were found in liver samples between control and poly I:C+Perio groups.

Conclusions: Animals exposed to a combined model of SZ and periodontitis showed alterations at peripheral and central level in the expression of specific apolipoproteins and their receptors (some of them dependent of sex). Further studies are needed to correlate these alterations with the SZ-related behavioural changes found in this model and to disentangle the therapeutic potential of future pharmacological strategies focused on the modulation of Apos/lipoproteins expression and function at systemic and central level.

Acknowledgements:
This study has been supported by FEDER and the Spanish Ministry of Science, Innovation and Universities (ref: PID2021-127256OB-I00 and PID2019-109033RB-100) and Proyecto de Investigación Banco Santander/UCM (PR44/21-29921). JRM is a recipient of a FPI pre-doctoral scholarship (PRE2020-092642).

Session 2: Proffesional Careers in Pharmacology

Organizer: Association of SEF Young Researchers

Moderator: Rocío Muñoz (Universidad de Sevilla)

Invited speakers:

Mª Luisa Suárez Gea (Agencia Española de Medicamentos y Productos Sanitarios)
Belén López Bouzo (Laboratorios Farmacéuticos ROVI)
Eduardo Oliver Pérez (Consejo Superior de Investigaciones Científicas)
Beatriz Artalejo Ortega (IQS y Universitat Ramón Llull)

Session 3: Natural Products Pharmacology

Moderator: Ricardo Borges (Universidad de La Laguna)

Invited speakers:

12:30-13:00 Beatriz de la Heras (Universidad Complutense de Madrid)
Hispanolone derivatives: current achievements and future perspectives

13:00-13:15 Rocío Muñoz-García (Universidad de Sevilla)
Dietary supplementation with a novel *O*-methylated secoiridoid derivative (Met-OL) improved endothelial dysfunction and lupus nephritis in a murine model of pristine-induced systemic lupus erythematosus

13:15-13:30 Ricardo Borges (Universidad de La Laguna)
Novel laboratory tools for pharmacological characterization

Hispanolone derivatives: current achievements and future perspectives

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Bioactive natural products, in particular diterpenes, are very promising molecules for the development of new pharmacological agents, exhibiting a broad spectrum of biological activities. Hispanolone is a labdane diterpene firstly isolated from the aerial parts of *Ballota hispanica* (Labiatae). Based on extensive chemical structural modifications, a series of hispanolone derivatives with enhanced efficacy and selectivity has been developed. The pharmacological properties of these compounds have been actively investigated.
Inflammation has been identified as a critical factor in the development of numerous chronic inflamamatory conditions and autoimmune diseases. The great anti-inflammatory potential of these compounds has been largely attributed to their capacity to inhibit classical inflammatory signaling pathways, as the activation of NF-κB transcription factor and mitogen-activated protein kinases (MAPKs) in a range of experimental disease models.
In recent years, the NLRP3 inflammasome has emerged as a promising drug target for novel anti-inflammatory therapies. Hispanolone derivatives have been described as potent inhibitors of the NLRP3 inflammasome, thereby modulating the pathogenicity of inflammatory diseases. Of these, dehydroisohispanolone (DIH) has been extensively evaluated (1). Mechanistically, this compound blocks inflammasome assembly, leading to a reduction of IL-1β release and pyroptotic cell death. Molecular docking analysis revealed that DIH fits well into the ATP-binding site of the NLRP3 protein, forming a covalent bond with Cys415 and the β carbon of the α, β unsaturated carbonyl moiety. Recent evidence also suggests that the NLRP3 inflammasome plays a key role in myocardial injury associated with DOX-induced cardiotoxicity, with inflammation identified as a primary underlying mechanism. In addition to its anti-inflammatory effects, DIH also exhibits cardioprotective properties by reducing oxidative stress and apoptosis, with the activation of specific survival signals against ischemia/reperfusion injury, which improves cardiac function. Furthermore, DIH has been demonstrated to act as an effective inhibitor of NLRP3 in cardiomyocytes.

In conclusion, these findings underscore the promising potential of hispanolone diterpene derivatives, particularly DIH, as a pharmacological approach for the prevention and treatment of inflammation-based diseases.

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Acknowledgements. The study was funded by Instituto de Salud Carlos III (PI17CIII/00012, PI20CIII/00018) and Ministerio de Ciencia e Innovación (MCIN/AEI/10.13039/501100011033/FEDER, UE (PID2022-136549OB-100)

Dietary supplementation with a novel *O*-methylated secoiridoid derivative (Met-OL) improved endothelial dysfunction and lupus nephritis in a murine model of pristine-induced systemic lupus erythematosus.

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Systemic lupus erythematosus (SLE) is a women-prevalent chronic immunoinflammatory disease characterised by the production of autoantibodies and the deposition of immune complexes leading to multi-organ affectation [1]. Currently, nutritional therapy is presented as a useful tool in the management of SLE. In fact, dietary supplementation with EVOO polyphenols improved the progression of renal damage in pristane-induced mice [2,3]. However, the main handicap of these compounds is their chemical instability and their poor pharmacokinetic profile [4]. Furthermore, phenolic hydroxyl group methylation (*O*-methylation) could improve transport through cell membranes, increasing bioavailability [5]. The present study was designed to evaluate the preventive effects of met-OL on the development of SLE in BALB/c mice induced by pristane. 0.5 ml of pristane was intraperitoneally injected to BALB/c female mice (12 weeks-old) and animals received a met-OL enriched diet (0.01% (w/w)) for 6 months. Renal histopathological changes were evaluated by Haematoxylin-Eosin, Masson trichrome, and periodic acid-Schiff staining. In addition, renal deposition of immune complexes was explored by immunofluorescence and immunohistochemistry. Furthermore, epigenetic changes were evaluated by RT-qPCR in kidney tissue. Endothelial dysfunction has been examined in thoracic aortas and protein expression was measured by Western Blot. After 24 weeks of met-OL dietary treatment, renal damage and immune complexes deposition were reduced, additionally, an improvement in endothelial function was also found in met-OL treated mice. Moreover, met-OL supplemented diets modulated JAK/STAT and Nf-κB signaling pathways and regulated miRNA-126, miRNA-123, miRNA-146a and miRNA-24-3p expression. These promising results propose that a diet supplemented with met-OL may present a useful alternative to the management of SLE.

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Acknowledgements:
Authors gratefully acknowledge the support provided by the research grant AGL-2017-89342-P (Ministerio de Economía y Competitividad, Spain) and "PI19/01213" (Instituto de Salud Carlos III (ISCIII), co-funded by the European Union). We thank the assistance of Center for Technology and Innovation Research of the University of Seville and University of Jaen and Junta de Andalucía (CTS-259 and FQM-182) for their financial support. R. Muñoz-García and M. Alcarranza gratefully acknowledges the support provided by the from FPU fellowship and financial sponsorship from the Spanish Ministerio de Universidades.

Novel laboratory tools for pharmacological characterization

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Drug characterization using isolated organs was the gold standard for decades, as muscle contraction is one of the major effectors in physiology. However, these techniques have been progressively abandoned due to their expense, slow process, and high bench space consumption. Nowadays, isolated organs have been largely replaced by cultured cells or target-immobilized receptors. However, these experimental approaches cannot fully replicate the information provided by real tissues.

In this communication, we introduce two novel devices that analyze the contractile responses of smooth muscle preparations: the MuWOB and liquid chromatography with biological sensors.

i) The MuWOB is a platform that uses optical methods for the simultaneous online monitoring of the contraction of rat aorta or trachea rings. This platform utilizes 96-well plates, which work with 100 µL volumes. This means a reduction in the amount of drug and buffers required, the possibility of applying liquid handling systems used for multiwell plates, and it occupies just 60x60 cm of bench space. Measurements are taken by a bi-telecentric optic coupled to a USB camera and custom software. We are also developing a robotic arm for automatic drug delivery. This system is especially conceived for the drug screening of vasodilators or bronchodilators.

ii) Liquid chromatography with biological sensors. Extracts from natural products or combinatorial synthesis require separation into fractions and subsequent evaluation of the pharmacological properties of each one. Instead, we have used a physiological buffer (Krebs-HEPES) as the mobile phase for liquid chromatography, and the eluent from the column is conducted to a cascade of isolated organs (rat aorta, trachea, vas deferens, and ileum), with the contractile response monitored by force transducers. The system identifies, in a single run, the periods when the eluent contains active compounds. Only these fractions are collected for subsequent characterization.

These two devices have demonstrated enormous versatility for drug characterization in the laboratory of pharmacology.

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Acknowledgments.
Supported by Ministerio de Ciencia, Innovación y Universidades (PID2020-116589GB-I00).

Session 4: Sex Differences in Neuropsychopharmacology

Moderator: Olga Valverde (Universitat Pompeu Fabra)

Invited speakers:

15:00-15:30 Olga Valverde (Universitat Pompeu Fabra)
Cannabidiol modulates cocaine seeking behavior in a sex-dependent manner

15:30-15:45 Guadalupe Rivero (Universidad del País Vasco)
Histone-level epigenetic mechanisms are sex-dependently modulated in a two-hit murine model

15:45-16:00 Evelyn P. Silva (Universidad de Cantabria)
Role of TGF-β signalling and sex influence in mice models of Diabetic Peripheral Neuropathy

Cannabidiol modulates cocaine seeking behavior in a sex-dependent manner

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Cocaine is the second illicit drug most consumed in Europe after cannabis (WHO, 2020). However, despite the high prevalence and the devasting consequences of cocaine consumption, there are no effective pharmacological therapies to manage this disease to date. Drug addiction including cocaine addiction is influenced by sex/gender factors and epidemiological data and preclinical studies show that females have an accelerated course to addiction than males, acquiring faster the criteria for dependence after initial use (Castro-Zavala et al., 2021; Haas and Peters, 2000). In the last years, cannabidiol (CBD) has emerged as a new potential treatment for cocaine addiction (Luján et al., 2018), and different preclinical studies have evaluated the effects of CBD on acquisition of cocaine self-administration and several phases of the addictive process. However, many questions still arise regarding CBD effectiveness in treating cocaine-related negative symptomatology, notably whether there are sex differences in the potential beneficial effects of CBD regarding cocaine seeking.

The aim of our study was to investigate the effect of CBD on cocaine seeking in female mice using the intravenous self-administration model. We also assessed the molecular substrate of a CBD treatment, considering the expression of genes involved in motivation and aberrant plasticity processes in the dorsal striatum using Open arrays. To this end, we first analyzed the effective doses of CBD in models of anxiety and memory in females, as the elevated plus maze and the novel object recognition test. We show that acute CBD produces anxiolytic responses in the elevated plus maze at the dose of 10 mg/kg, ip, whereas results from the novel object recognition test indicates that CBD increases discriminative index of the novel object mainly at the doses of 10 and 20 mg/kg, ip. We then tested the effects of both doses of CBD (10 and 20 mg/kg, ip) in animals exposed to an operant procedure of intravenous self-administration to evaluate acquisition process, and we also evaluated the effects of CBD on cocaine consumption under the risk of punishment (Alegre-Zurano et al., 2023). Our findings reveal that CBD attenuated cocaine acquisition under fixed ratio 1 schedule of response, although the response pattern in females varies depending on the dose of CBD administered. Moreover, CBD was shown to be effective in reducing cocaine seeking under risk of punishment, a response that had not been evaluated to date in preclinical studies. The molecular results may help us to understand the neurobiological basis of the differential responses observed in males and females. Overall, our work emphasizes the beneficial potential of the use of CBD for the management of cocaine abuse and demonstrates the differential sex effects for CBD in these responses, a factor that should be always considered in preclinical studies related to neuropsychiatric disorders.

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Histone-level epigenetic mechanisms are sex-dependently modulated in a two-hit murine model

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The impact of environmental risk factors in the etiology of schizophrenia (SZ) may be mediated by epigenetics, which is sex-dependently modulated at histone posttranslational level[1]. Postmortem brain studies in SZ subjects found higher expression of permissive histone posttranslational modifications (HPTM)[2] and lower expression of metabotropic glutamate receptor-2 (mGluR2)[3]. Thus, we studied histone-level epigenetic mechanisms in both male and female mice of a two-hit SZ model combining the exposure to maternal immune activation (MIA) and the deletion of *Grm2* gene (encoding for mGluR2).

Grm2$^{+/-}$ 129Sv pregnant dams received intraperitoneally polyinosinic:polycytidylic acid (5 mg/kg, Invivogen®) or saline at gestational day 9.5. The offspring (n=48) were divided in 12 groups according to sex, MIA and *Grm2* genotype ($^{+/+}$, $^{+/-}$, $^{-/-}$). Cortical expression of permissive HPTMs: H3K4me3, H3K9ac and H3K27ac and of histone deacetylases HDAC1, 2 and 4 was quantified in adult mice by Western blotting. HDAC activity was determined by enzyme kinetic assays. Data were analyzed by three-way ANOVA and enzyme kinetic curves compared by F- test.

Female mice showed lower H3K9ac (-27%, p=0.049) and H3K27ac (-23%, p=0.027) and higher HDAC1 (+54%, p=0.0002) and HDAC2 (+29%, p=0.029) expression, as well as different HDAC enzyme activity ($F(2, 348) = 20.25$, $p<0.0001$) compared to male mice. Three- way ANOVA detected an interaction between MIA and sex in both H3K4me3 and H3K27ac expression. MIA-exposed male mice showed higher H3K4me3 (+67%, p=0.021) and H3K27ac (+70%, p=0.008) expression than saline-exposed male mice. In contrast, MIA-exposed female showed lower H3K27ac expression (-57%, p=0.002) than saline-treated counterparts. *Grm2* genotype did not have any influence on the expression of any of the assessed HPTM or HDAC proteins. However, HDAC enzyme kinetics fitted better to different curves for each genotype ($F(4, 346) = 6.736$, $p<0.0001$).

Epigenetic mechanisms at histone posttranslational level were differentially modulated in males and females as revealed by differences in HPTM expression and HDAC expression and activity. MIA caused a sex-dependent effect on histone-level epigenetic mechanisms. The impact of MIA in male mice reproduced the findings in postmortem brain of SZ subjects[2]. These observations highlight the need to consider sex differences in epigenetic studies of psychiatric disorders.

Acknowledgements: This work was supported by grants PID2022-137848OB-I00 (MCIU/AEI/FEDER), IT1512/22 (Basque Government), and predoctoral grants from UPV/EHU to OMP and JDCB and from the Basque Government to JAS. The authors report no conflict of interest.

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Role of TGF-β signalling and sex influence in mice models of Diabetic Peripheral Neuropathy

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Diabetes Mellitus (DM) is one of the most serious and common chronic diseases of our times, causing disabling, costly complications, and reducing life expectancy [1]. Around 50% of diabetic patients develop Diabetic Peripheral Neuropathy (DPN). This complication can cause pain, numbness, and loss of sensation in the extremities. It is characterised by a decline in peripheral innervations, increased neuronal inflammation, demyelination, axonal atrophy, and decreased neuronal regenerative capacity. Transforming growth factor-β (TGF-β) constitutes a large family of pleiotropic and multifunctional cytokines. Mice lacking the TGF-β pseudoreceptor BAMBI present an antiallodynic phenotype after sciatic nerve injury in male mice [2]. Our study aims to investigate whether BAMBI deficiency affects the establishment of DPN in two models of DM in male and female mice.

Type 1 DM was induced in C57BL6 and BAMBI-KO male and female mice by multiple low doses of streptozotocin (STZ) in citrate buffer (40mg/kg i.p, daily for 5 consecutive days). For type 2 DM induction the mice were fed "ad libitum" with a 5% fructose solution for 3 weeks, and then, treated with STZ (n=9-12 mice per experimental group). Blood glucose levels were measured at baseline and 4 weeks after STZ administration. Mechanical allodynia was assessed with the von Frey test.

Our results show that the increase in blood glucose levels was lower in female C57BL6 mice at 4 weeks post-STZ injections compared to male C57BL6 mice in both DPN models (Type 1 DM: 230±18 vs. 505±36, $p<0.001$; Type 2 DM: 326±29 vs. 500±41, $p<0.001$). Weight loss, polydipsia, and polyuria were less pronounced in females than in male C57BL6 mice. Female and male BAMBI-KO mice exhibited marked symptoms of diabetes with higher levels of blood glucose. All mice developed mechanical allodynia in both models of DM; however, in Type 2 DM model, the response intensity was significantly higher in female BAMBI-KO mice ($p<0.001$).

Our findings indicate that type 2 diabetic BAMBI-KO female mice are more susceptible to developing DPN. The present study suggests the role of BAMBI in the development of DPN and the existence of sex differences in glucose handling.

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Acknowledgements: Supported by PID2022-136418OBI00/AEI/10.13039/501100011033/ FEDER, UE and IDIVAL (INN-VAL 23/12).

Session 5: Inflammation and Immunomodulation Pharmacology

Moderator: Mª Jesús Sanz (Universidad de Valencia)

Invited speakers:

15:00-15:30 Mª Jesús Sanz (Universidad de Valencia)
Role of eotaxin-1 (CCL11)/CCR3 axis in atherosclerotic lesion development

15:30-15:45 J Ávila Román (Universidad de Sevilla)
Chrysin glucoside derivatives attenuate oxidative stress and inflammation through activation of Keap1/Nrf2/HO-1 signaling pathway in human macrophages

15:45-16:00 Inés Valencia (Hospital Universitario Santa Cristina, Madrid)
Pharmacological evaluation of novel non-nucleotide purine derivatives as P2X7 antagonists for the treatment of neuroinflammation in traumatic brain injury

Role of eotaxin-1 (CCL11)/CCR3 axis in atherosclerotic lesion development

Elena Domingo[1,3], Aida Collado[1,3], Patrice Marques[1,3], José Aparicio[3], Miguel Civera[2,3,4], José T. Real[2,3,4], Laura Piqueras[1,3,4], María Jesús Sanz[1,3,4]

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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 [1,2], however, its role in the atherosclerotic lesion development remains elusive. Therefore, we have evaluated the impact of an atherogenic diet in the lesion formation of eight-week-old male apolipoprotein E-deficient mice ($apoE^{-/-}CCR3^{+/+}$) *versus* those lacking eotaxin receptor (CCR3, $apoE^{-/-}CCR3^{-/-}$).

Material and Methods: $ApoE^{-/-}CCR3^{+/+}$ 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 in 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 ($apoE^{-/-}CCR3^{+/+}$). While eotaxin-1/CCL11 expression within the lesion of $apoE^{-/-}CCR3^{+/+}$ mice in a hypercholesterolemic scenario was clearly increased, it was barely detected in $apoE^{-/-}CCR3^{-/-}$mice.

Conclusion: Our findings suggest that in animals lacking CCR3 receptor, while increased lesion formation was detected, eotaxin-1 expression was nearly absent. Therefore, eotaxin-1 (CCL11)/CCR3 axis may exert a protective role in atherosclerosis development.

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Acknowledgements: This work was supported by the Spanish Ministry of Science and Innovation: [grant number PID2020-120336RB-I00]; the Carlos III Health Institute (ISCIII) and the European Regional Development Fund (FEDER) [grant number PI21-00220]; the Generalitat Valenciana [grant numbers APOTIP/2020/011, CIPROM/2022/45].

Chrysin glucoside derivatives attenuate oxidative stress and inflammation through activation of Keap1/Nrf2/HO-1 signaling pathway in human macrophages.

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Natural products such as phenolic compounds have been shown to have numerous beneficial effects for human health. Flavonoids are a huge family of phenolics with many biological activities, as for example antioxidant, anti-inflammatory, anti-cancer and anti-microbial (1, 2). However, these compounds present a low oral bioavailability, which is enhanced when administered in the form of flavonoid glycosides since they possess better absorption capability (3). The beneficial effects of the flavonoid chrysin can be reduced by its poor oral bioavailability. It has been shown that chrysin 8-*C*-glucoside (**1**) has better absorption capability. The aim of this study was to evaluate the antioxidant and anti-inflammatory activity of this glucoside, as well as the respective hexa-acetate derivative **1a** and the hexa-ethyl carbonate derivative **1b** since the inclusion of moieties in bioactive molecules can increase or modify their biological effects. Our data demonstrated, for the first time, that pre-treatment with the three compounds caused a marked reduction in lipopolysaccharide (LPS)-induced ROS, TNF-α and IL-1β levels, as well as in the expression of COX-2. The mechanisms involved in these effects were associated, at least in part, to the competitive molecular interactions of these phenolic compounds with Keap1-Nrf2, which allow the dissociation of Nrf2 and its translocation into the nucleus and subsequent up-regulation of HO-1 expression. Compared to the 8-*C*-glucoside parent chrysin, compound **1a** exhibited the strongest antioxidant and anti-inflammatory activity. We hypothesized that the incorporation of an acetate group may reduce its polarity and, thus, increase membrane permeability. Together, our results highlight that these phenolics may be used as Nrf2 activators against oxidative stress-related inflammatory diseases.

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Acknowledgements
We thanks to the Operative FEDER Program-Andalucía 2014-2020 (US-1380844) and the "VII Plan Propio de Investigación y Transferencia" of The University of Seville for the funding for this project

Pharmacological evaluation of novel non-nucleotide purine derivatives as P2X7 antagonists for the treatment of neuroinflammation in traumatic brain injury

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Traumatic brain injury (TBI) is an acute brain lesion acknowledged as one of the main causes of mortality and disability worldwide. Brain injury after TBI is spread in the long term, with neuroinflammation playing a key role in the development of secondary brain sequelae. Searching for therapeutic strategies that shrink the inflammatory response after TBI is key to prevent and/or ameliorate secondary brain injury and to improve the outcome of patients. After TBI there is a fast activation of innate immunity, where microglia is activated in response to damage-associated molecular patterns such as ATP, which is detected by P2X7 purinergic receptors. Indeed, the P2X7-NLRP3 inflammasome axis has been identified as one of the main actors in neuroinflammation. Thus, the aim of this study was to validate P2X7 as a potential therapeutic target in TBI, as well as to evaluate new non-nucleotide purine derivative compounds as P2X7 antagonists in models of TBI both *in vitro* and *in vivo*.
First, serum P2X7 levels were evaluated in patients after TBI, observing a significant reduction of P2X7 after 72h of brain lesion. P2X7 was then validated as pharmacological target by genetic inhibition, performing the closed head injury model of TBI in *p2x7*-deficient mice. *p2x7* -/- mice showed worse transcriptomic proinflammatory profile at brain level as well as poorer neuroconductual score 24h after TBI, in comparison to control mice. However, the animals that were treated with the potent and selective P2X7 antagonist JNJ-47965567 (30 mg/kg i.p.) 30 minutes after TBI, presented a tendency towards improvement of their neurological and inflammatory parameters. These apparently differing results depicted the relevance of P2X7 activation in a time-dependent control of traumatic lesion, which was further evaluated in a second pharmacological approach. A series of purine derivative compounds, divided into etanoil and sulfonil families according to their chemical structure, were evaluated as P2X7 antagonists in the context of TBI. Those compounds that prevented IL-1β release from primary mixed glial cultures in response to LPS+ATP, were further evaluated *in vivo*. In the etanoil family, ITH15004 (1 mg/kg i.p.) injected 30 minutes after TBI, improved inflammatory markers 24h after TBI. On the other hand, ITH15003 was the most effective compound from the sulfonil family and it will be shortly evaluated *in vivo*. Altogether, these results highlight the implication of P2X7 as potential therapeutic target to modulate neuroinflammation course in TBI.

Acknowledgements This work is supported by grant PI22/00362 funded by Instituto de Salud Carlos III (ISCIII, Spain) and co-funded by Fondo Europeo de Desarrollo Regional (FEDER), by Fundación Mutua Madrileña to J.E and Instituto Salud Carlos III Sara Borrell Grant (CD22/00101) to I.V. Proyect participant on COST Action CA21130 "P2X receptors as therapeutic opportunity (PRESTO)"

Session 6: Vascular Pharmacology

Moderator: Mercedes Salaices Sánchez (Universidad Autónoma de Madrid)

Invited speakers:

16:00-16:30 Concepción Peiró (Universidad Autónoma de Madrid)
The isolated SARS-CoV-2 S protein induces hyperinflammation, coagulant activity and premature senescence in human endothelial cell cultures

16:30-16:45 Cristina González-Correa (Universidad de Granada)
Critical role of T CD4+ cells in the development of endothelial dysfunction induced by gut microbiota from lupus patients with hypertension

16:45-17:00 Inés Roger (Universidad de Valencia)
Effects of antifibrotic agents pirfenidone and nintedanib on IL-11-induced pulmonary artery cells

The isolated SARS-CoV-2 S protein induces hyperinflammation, coagulant activity and premature senescence in human endothelial cell cultures

Alicia Villacampa[1, 2], Licia Shamoon[1, 2], Inés Valencia[1], Cristina Morales[1], Fernando de la Cuesta[1, 2], Dolores Sánchez-Niño[1, 3], Sofía Figueiras[1], Isabel Sánchez-Pérez[1,4], Salvador Moncada[1], José Luis Bartha[1, 2], Francisco López-Sánchez[2], Guillermo Díaz-Araya[5], Carlos F. Sánchez-Ferrer[1, 2], Óscar Lorenzo[1, 3], Concepción Peiró[1, 2]

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Background: COVID-19 is associated with hyperinflammation, hypercoagulation and endothelial injury, not only in acute forms of the disease but also in long COVID. Recent studies show that isolated SARS-CoV-2 S elements may be found in tissues or the circulation long after infection, which may open the field for deciphering new pathogenic mechanisms of this complex disease. We investigated whether the viral S protein induces by itself inflammation or pro-coagulant and pro-senescence responses in human endothelial cells cultures with focus on the role played by the NLRP3 inflammasome, a key component of the innate immune system and a fundamental source of interleukin (IL)-1b.

Material and methods: Primary cultures of human umbilical vein endothelial cells (HUVEC) were stimulated with increasing concentrations of recombinant native SARS-CoV-2 S protein. NF-□B, NLRP3 inflammasome components, senescence-related proteins (gH2AX histone, p53, p21, p16) and coagulation factors were quantified by Western Blot and indirect immunofluorescence. Von Willebrand factor (vWF) and IL-1 b were quantified by ELISA. A senescence associated-b-galactosidase (SA-b-gal) activity assay was used to confirm cell senescence.

Results: In HUVEC cultures, the S protein alone (7 to 70 nM) activated NF-□B as well as the priming and activation of the NLRP3 inflammasome system in a concentration-dependent manner from a threshold concentration of 35 nM. In parallel, coagulation factors such as vWF, factor VIII and tissue factor were equally induced. However, contrarily to the cytokine interleukin (IL)-1b that was used as a positive control in this study, the S protein did not increase the levels of ADAMTS-13, as a main physiological counteractor of the pro-coagulant activity of vWF. Moreover, the S protein provoked premature senescence, enhanced SA-b-gal activity and promoted the acquisition of a characteristic senescence-associated secretory phenotype (SASP) by HUVEC. This was associated to a marked reduction of key anti-oxidant and anti-aging proteins such as the Nrf2/heme-oxygenase-1 system or the klotho protein. The pharmacological inhibition of the NRLP3 inflammasome with the experimental drug MCC950 or the blockade of IL-1 receptors with anakinra partly prevented the stimulatory action of the S protein in terms of hypercoagulation and senescence, underlying a role for hyperinflammation in the direct deleterious action of the viral protein in human endothelial cells.

Conclusions: Isolated SARS-CoV-2 viral elements may directly damage endothelial cells and thus be causative agents for the complex vascular manifestations of both acute and long COVID-19. Hyperinflammation and cellular defense system exhaustion should be regarded as potential targets to attenuate the deleterious effects of the viral components on the human endothelium.

Acknowledgements: Supported by grants from REACT-EU-Comunidad de Madrid and the European Regional Development Fund (SPACE2-CV-COVID-CM), Plan Nacional I+D (PID2020-115590RB-100), Fondo de Investigación Sanitaria-FIS Carlos III (PI20/00923).

Critical role of T CD4+ cells in the development of endothelial dysfunction induced by gut microbiota from lupus patients with hypertension

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Lupus has been associated with several changes in gut microbiota, which play a crucial role in the development of hypertension in SLE murine models (de la Visitación et al., *Br. J. Pharmacol*. 2021, *178*, 3708-29). We previously demonstrated that gut microbiota from SLE patients with hypertension induces endothelial dysfunction linked to aortic Th17 infiltration. In the present study, we tested whether Th17 infiltration at the vascular wall is a key event to the development of endothelial dysfunction. Faecal samples were obtained from healthy controls subjects (SN), normotensive SLE patients (LN), and hypertensive SLE patients (LH), and were used as donors. Normotensive ten-week-old female C57Bl/6J mice and Rag $^{-/-}$ mice were used as recipient mice. For faecal microbiota transplantation (FMT) experiments, was performed as previously described (Toral et al., *Mol. Nutr. Food Res*. 2018, e1800033). The two set of animals (normal C57Bl/6J and Rag $^{-/-}$) were randomly divided to 3 different groups (n = 8): with control microbiota from SN, with microbiota from LN, and with microbiota from LH. In another experiment, C57Bl/6J germ-free (GF) mice were inoculated with faeces for two consecutive days and animals were maintained for 10 weeks. An increase of ≈ 10 mmHg in systolic BP were observed in mice after FMT from LN and LH patients at the end of the experiment, which was maintained in Rag$^{-/-}$ mice. Splenomegaly and plasma anti-ds-DNA were increased in normal LN and LH mice, as compared with SN, whereas no change was observed among the 3 groups of Rag$^{-/-}$ mice. As expected, no B and T cells was found in Rag$^{-/-}$ mice. The increased disease activity was linked with higher spleen and plasmatic plasma cells content. Moreover, in normal mice a significant increase in the proportion of Th17 was found in mesenteric lymph nodes, spleen and blood from both LN and LH mice as compared to SN. Tregs content in spleen and blood was lower in LH than in LN group. Aortic relaxation induced by acetylcholine were impaired in mice receiving microbiota from LH patients, whereas no significant changes were induced by FMT from LN patients. These results could be related with a higher production of reactive oxygen species from NADPH oxidase in the vascular wall in LH groups. Furthermore, higher aortic infiltration of Th17 and lower Tregs was found after FMT from LH patients. However, in Rag$^{-/-}$ mice FMT from LH patients did not impaired acetylcholine relaxation. Similarly, reduced endothelium dependent relaxation and Th17 infiltration was found in aorta from GF-mice inoculated with faeces from LH patients. In conclusion, increased Th17/Tregs cells in the vasculature is a key event in the development of endothelial dysfunction induced by microbiota from SLE patients with hypertension.

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

Effects of antifibrotic agents pirfenidone and nintedanib on IL-11-induced pulmonary artery cells

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Idiopathic pulmonary fibrosis (IPF) combined with pulmonary hypertension (PH) leads to a poor prognosis, characterized by fibrosis of lung parenchyma and remodeling of the pulmonary arteries. In patients with IPF-PH, both serum and lung parenchyma levels of interleukin 11 (IL-11) are elevated, contributing to pulmonary artery remodeling and the development of PH. The impact of currently approved IPF treatments on IL-11-induced pulmonary artery remodeling remains unclear. This study aims to investigate the effects of nintedanib (NTD) and pirfenidone (PFD) on IL-11-induced remodeling of pulmonary artery endothelial and smooth muscle cells *in vitro*. Our findings demonstrate that NTD and PFD mitigate endothelial to mesenchymal transition (EnMT), transformation of pulmonary artery smooth muscle cells into myofibroblast-like cells, and pulmonary remodeling in precision-cut lung slices. The study also reveals that PFD and NTD inhibit IL-11-induced proliferation and senescence of endothelial and muscle cells. Additionally, the effects of these drugs on monocyte arrest and angiogenesis were examined. Lastly, we observed that IL-11 activates the canonical STAT3 pathway and non-canonical ERK1/2 pathway, but PFD and NTD selectively inhibiting ERK1/2 phosphorylation. Consequently, this study provides evidence of the inhibitory effects of NTD and PFD on IL-11-induced markers of pulmonary artery remodeling.

Session 7: Novel Approaches to *In Vivo* Pharmacology

Moderator: Francisco Ciruela (Universitat de Barecelona)

Invited speakers:

16:00-16:30 Francisco Ciruela (Universitat de Barcelona)
Wireless remote control of pain using photopharmacology

16:30-16:45 Fernando Yáñez-Gómez (Universitat de les Illes Balears)
Creating an *in-situ* gelling hydrogel for targeted antidepressant release through subcutaneous application

16:45-17:00 Mateo Ruiz-Conca (Universidad de Alicante)
Combined therapy of repurposed drugs as a new strategy for the treatment of Retinitis Pigmentosa

Wireless remote control of pain using photopharmacology

Francisco Ciruela[1,2]

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During the last decade, the pharmacology of G protein-coupled receptors (GPCRs) has evolved significantly, providing a deep mechanistic and kinetic understanding of ligand-receptor interactions. This improved understanding has facilitated the design of drugs with tunable spatiotemporal intrinsic activity, allowing for more precise modulation of receptor functions. One of the most innovative approaches to achieving such precision in drug activity is based on the use of light to modify the physicochemical properties of drugs in vivo, a field known as *in vivo* photopharmacology. This technology uses light-sensitive drugs, or photodrugs, that can be activated or deactivated by specific wavelengths of light, thus allowing for a highly controlled and localized drug action.

We have developed several photodrugs that have shown success in animal models of disease, including pain. However, while these photodrugs offer a powerful tool for understanding disease mechanisms and developing potential new light-based treatments, there is a critical challenge in translating these discoveries into practical clinical applications, particularly due to the invasiveness of delivering light to target tissues. To address this challenge, we embarked on an ambitious research program grounded in the concept of "Wireless Remote Pharmacology." This pioneering approach involves the development of compact, implantable and battery-free µLEDs for light delivery. These implantable devices can deliver precise doses of light to activate or deactivate photodrugs in specific tissues or organs in response to external commands.

In one notable example, we remotely photoactivate a morphine photoderivative (photocaged morphine or pc-Mor) using a battery-free µLED implanted in the spinal cord of mice. This remote photoactivation of the pc-Mor generated antinociceptive effects while avoiding common undesired effects related to systemic opioids. In general, light-dependent opioid-based drugs in conjunction with battery-free µLEDs as a source of light may allow effective analgesia with minimal invasiveness and side effects.

Creating an *in-situ* gelling hydrogel for targeted antidepressant release through subcutaneous application

Fernando Yáñez-Gómez[1,2], María Antonia Vallés-Sansó[1], M. Julia García-Fuster[1,2]

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Background: Using controlled drug-release systems could improve *in vivo* pharmacological experimental procedures by providing a better therapeutic administration without distorting aspects (handling and/or animal stress), while improving the interpretation of the experimental results. In this study, and in the context of our line of research aiming at characterizing novel antidepressants for adolescent depression [1], we aimed at developing an in-situ gelling hydrogel for the controlled release of sub-anesthetic doses of ketamine in adolescent rats.
Materials and methods: Main components of the polymeric matrix were Pluronic, F127, and sodium hyaluronic acid (HA; Guinama, Spain). Ketamine (Richter Pharma, Austria) was chosen as the active compound. Separate solutions of Pluronic F127 at concentrations of 26% and 29% (w/v), respectively, were prepared at 4 ºC with magnetic stirring overnight, along with HA solutions at 0.5 or 1% (w/v). Ketamine was incorporated into these hydrogels to investigate its integration and subsequent controlled release of 5 mg/kg per day to mimic the daily dose that proved antidepressant-like efficacy in adolescent rats [1].
Results: Systems composed predominantly of 29% pluronic F-127 in different percentages, 0.5 or 1% HA, demonstrated optimal *in-situ* gelation properties *in vitro* at both 20 and 37 ºC. These systems effectively encapsulated the initial ketamine dosage, within a final hydrogel volume of 1 ml, suitable for subsequent *in vivo* analysis. Notably, these polymeric matrices exhibited rapid gelation at ambient temperature and sustained structural integrity over a 7-day period at 37 ºC, showcasing their potential for prolonged drug release applications. Differential scanning calorimetry (DSC), viscosimetry, and scanning electron microscope (SEM) characterization was carried out on these hydrogels. All the exhibited gelation temperatures, close to physiological levels, along with favorable mechanical properties indicated high injectability potential. Moreover, all these features coupled with an adecuate pore size suggest our hydrogels as strong candidates for controlled drug delivery systems.
Conclusions: We have found an *in-situ* hydrogel that could be used as a drug release platform at the body temperature of adolescent rats. Moreover, sub-anesthesic doses of ketamine incorporated perfectly in this structure, since experimental studies have demonstrated ketamine's control release from the polymer *in vitro. In vivo* studies are currently ongoing to ensure the control release capabilities of our hydrogel.

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Acknowledgements: Fundación Jané Mateu; PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033).

Combined therapy of repurposed drugs as a new strategy for the treatment of Retinitis Pigmentosa

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Retinal neurodegenerative diseases such as retinitis pigmentosa (RP) cause vision loss and blindness, leading to a high degree of disability. The treatment of these diseases represents a great challenge to improve the health and quality of life of visually-impaired people in our society. In the basic mechanisms of cell death, RP do not differ from other retinal degenerative diseases such as glaucoma, age-related macular degeneration, or diabetic retinopathy, nor other CNS degenerative diseases, including Parkinson's and Alzheimer's. In this sense, the alteration of calcium homeostasis appears as a common factor in the different pathways that lead to cell death in neurodegenerative diseases. Hence, we hypothesize that mitigating the main calcium entry pathways in degenerating retinal cells may be a mechanism to slow the progression of neurodegeneration. As therapies directed to a single target have not been very effective in previous preclinical studies, we also hypothesize that a combined therapy can lead to a greater neuroprotective efficacy. Our main goal is to find a combination of drugs acting in different targets of the calcium entry pathways that retard the course of RP or delay its onset. We propose to use repurposed drugs, what allows reducing the tests to be carried out and the average time spent in the drug search and development process. These drugs, which are currently in clinic use, have well defined pharmacokinetic, safety and efficacy profiles. The drugs will be selected within the Fundación Teófilo Hernando drug repurposing program. A mouse model of RP (rd10) will be used. The three drugs will be intraperitoneally administered at two different doses to the animals both alone or in combination, from postnatal day (P)18 to P25. We will analyze their effect on the retinal function by means of electroretinogram, and on the retinal morphology by immunohistochemical analysis. We will also analyze the grade of inflammation by studying the phenotype of activated microglia by flow cytometry. To date, we have selected the first combination of three drugs directed to three different calcium entry targets. Our preliminary results show that the molecules are compatible, well tolerated, and that we have a suitable animal model for drug repositioning approaches. Due to the common mechanisms of cell death present in neurodegenerative diseases, the findings of this project could be extrapolated, to a greater or lesser extent, to other neurodegenerative diseases as far as they are directed to common targets.

Acknowledgements: Fundación Teófilo Hernando de I+D del Medicamento

Session 8: Cardiovascular Pharmacology

Moderator: Antonio R. Artalejo (Universidad Complutense de Madrid)

Invited speakers:

9:00-9:30 Ricardo Caballero (Universidad Complutense de Madrid)
Recent advances in the pharmacological treatment of ventricular arrhythmias

9:30-9:45 Lucía Núñez (Universidade da Coruña)
Novel variant in Kv1.5 channel possible risk factor for the development of ventricular fibrillation during acute myocardial infarction

9:45-10:00 Carlos Hermenegildo (Universidad de Valencia)
Sex and aging regulation of angiogenesis related genes through miR-149-5p in an experimental myocardial infarction

10:00-10:15 Dolores Viña (Universidad de Santiago de Compostela)
Sodium nitroprusside pretreatment protects cerebral microvascular endothelial cells under OGD conditions through a NO-independent mechanism

10:15-10:30 Ana Paula Dantas (Universitat de Barcelona)
Pleiotropic effects of lipid-lowering therapies on progenitor endothelial cells function in myocardial infarction

Recent advances in the pharmacological treatment of ventricular arrhythmias

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Ventricular arrhythmias (VA) such as ventricular tachycardia and fibrillation are the main cause of sudden cardiac death (SCD), especially in patients with structural heart disease (e.g. coronary artery disease, cardiac hypertrophy, and heart failure-HF) or inherited arrhythmogenic syndromes. Indeed, mortality due to VA is a problem of epidemic proportions. Antiarrhythmic drugs (AADs) still play a pivotal role for the treatment of VA, although their efficacy and safety (they can produce severe proarrhythmic effects) are quite limited. According to current guidelines, they are no longer the treatment of choice for several VA as they have been replaced by implantable cardioverter defibrillators (ICDs) or catheter ablation even though the latter tools cannot be used in all the patients due to eligibility and safety reasons. For instance, ICD shocks may cause impairment in the quality of life, are associated with an increased risk of death, HF, and hospitalizations. As a result, some life threatening VAs do not have an optimal treatment and are considered as unmet medical needs and it is evident that novel antiarrhythmic strategies are warranted. Our aim is to give an overview on the most recent advances in this field including small molecules (e.g. sodium-glucose cotransporter 2-SGLT2 inhibitors), biologics (peptides, antibodies), RNA interference, and gene therapy or editing. We are especially interested in HF-associated VAs since they carry an unacceptable high risk of arrhythmia-induced SCD, despite the use of guideline-recommended therapies. HF produces anatomical, histological, and electrical remodelling that increases the susceptibility to arrhythmias and AADs-induced proarrhythmia, and makes arrhythmias resistant to AADs. HF-associated electrical remodelling is mainly characterized by a reduced excitability and action potential duration prolongation due to the decrease in the Nav1.5 and Kir2.1 channel expression at the cardiomyocyte membrane. In the last years, our group has been interested in obtaining novel therapies to target the electrical remodelling searching for safer and more efficient alternatives than classical AADs. The success of these novel therapies would produce a paradigm shift in the field allowing the discovery of novel and long-pursued antiarrhythmic mechanism of actions.

Acknowledgements (This work was funded by Grants from the Spanish Ministry of Science and Innovation (PID2020-118694RB-I00), Comunidad de Madrid (ARCADIA S2022/BMD-7229), and Instituto de Salud Carlos III [CB16/11/00303])

Novel variant in Kv1.5 channel possible risk factor for the development of ventricular fibrillation during acute myocardial infarction.

Ricardo Pan-Lizcano[1], Maren Schrölkamp[2], Bo Bentzen[2], Nicole Schmitt[2], Manuel Hermida-Prieto[1], José Manuel Vázquez-Rodríguez[1,3], Lucía Núñez[1,4]

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Introduction: *KCNA5* gene encodes a voltage-gated potassium channel (hKv1.5) responsible for the ultrarapid delayed rectifier potassium current (I_{Kur}), which plays a fundamental role in the normal cardiac repolarization. Pathogenic variants in this gene have been associated with the development of different cardiac arrhythmias and sudden cardiac death. Having this into account, the genetic screening of rare genetic variants in this gene should be considered in patients with cardiac arrhythmias.

Methods: A total of 55 patients diagnosed with an acute myocardial infarction with elevation of the ST-segment along with a ventricular fibrillation were sequenced using NGS technologies, to obtain the genetic variants in *KCNA5*. The possible effect of the rare variants identified was assessed using five bioinformatics tools: Mutation Taster, SNAP2, SIFT, Polyphen2 and PhD-SNP. Variants considered as pathogenic or significant by, at least, 3 of the 5 programs, were tested in *in vitro* electrophysiology models: whole cell patch clamp with HEK293 cells and two-electrodes voltage clamp (TEVC) with *X. laevis* oocytes.

Results: A total of six rare variants were identified in this set of patients, two synonymous: p.P532= and p.S599=, and four missense: p.P37S, p.G183R, p.E211D, and p.P310L. Only the p.G183R novel mutation was classified as pathogenic for at least three of the prediction tools. Both electrophysiology models showed an almost complete loss-of-function of the $KCNA5_{G183R}$ channel in comparison to the wild type. In the whole cell patch clamp model, current density at +20 mV in wild type was 399.6±163.6 pA/pF and 44.5±14.7 pA/pF for p.G183R (*P* value: 0.000005). In the TEVC model, the mean steady state currents measured at +20 mV for cells injected with the wild type gene was 13.8±0.7µA and 0.28±0.1µA for cells injected with the p.G183R gene (*P* values <0.00001).

Conclusions: Both *in silico* and *in vitro* models had showed that the novel missense variant G183R in *KCNA5* is a loss-of-function mutation, affecting the correct function of the channel encoded by the gene. This variant therefore would be classified by ACMG criteria as a possibly pathogenic variant and could be related to the development of the cardiac arrhythmia presented in this patient.

Acknowledgements: This work was supported by a grant from Instituto de Salud Carlos III (PI18/01737)-FEDER funds, the scholarship for doctoral stays INDITEX-UDC 2023, and a non-conditional grant from Abbott Vascular. Moreover, we want to acknowledge the help of the GRINCAR-UDC group.

Sex and aging regulation of angiogenesis related genes through miR-149-5p in an experimental myocardial infarction

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***Background*:** miRNAs are proposed as systemic regulators in aging, and change after an acute myocardial infarction (AMI). Sex influences cardiovascular disease incidence and clinical manifestations. However, the potential contribution of miRNAs to sex differences in AMI remains poorly understood. This study analyses sex and aging-related variations in miR-149-5p expression in a Senescence-Accelerated Mouse (SAM) undergoing experimental AMI and analyse the functional contribution of miR-149-5p target genes.

***Material and methods*:** AMI was surgically induced by coronary artery ligation in six-months-old male and female SAM-Resistance (SAMR1; n = 16) and SAM-Prone (SAMP8; n = 16) mice (University of Valencia, 2020/VSC/PEA/0128). Sham-operated group was used as control. Four hours after surgery, mice were euthanized, and serum and cardiac tissue were collected. RNA was isolated and miR-149-5p and gene expression were determined by qRT-PCR, using snRNAU6 and GAPDH as endogen controls. Bioinformatic analysis of miR-149-5p target genes was performed: those predicted by at least 2 of 3 databases which were conserved in human and mouse, were used in DAVID database to perform an enrichment analysis in GO terms (biological process).

***Results*:** AMI raised circulating levels of miR-149-5p in SAMP8 females ($p < 0.05$) without changes in SAMR1. In cardiac tissue, AMI decreased miR-149-5p levels ($p < 0.05$) in SAMR1 and SAMP8 males with no changes in females. miR-149-5p predicted target genes by bioinformatics were related with angiogenesis pathway ($p < 0.05$), and angiogenesis-related targets FZD5 and PPAP2B showed an increased cardiac expression in all AMI groups except in aged AMI females. PPAP2B cardiac levels were decreased in aged sham males and females, consistent with a miR-149-5p inhibition.

***Conclusions*:** Expression of miR-149-5p increases with aging and may present sex-bias in response to AMI. Angiogenesis-related miR-149-5p targets, PPAP2B and FZD5, increase after AMI and could be associated to the repair response after an ischemic injury in the heart.

Acknowledgements:
This work was supported by the Spanish Ministry of Science and Innovation (ISCIII) PI22/1083 co-financed by the European Regional Development Fund (ERDF), and by the Generalitat Valenciana (CIAICO 2021/211; CIGE/2021/158). Bianca Descals-Beltrán is a predoctoral researcher (CIACIF/2022/331) from the Generalitat Valenciana.

Sodium nitroprusside pretreatment protects cerebral microvascular endothelial cells under OGD conditions through a NO-independent mechanism

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Background: The reduction in nitric oxide (NO) signalling pathways has been observed following ischemic stroke, particularly within the blood vessel wall. NO is a vasoactive molecule essential for maintaining vascular homeostasis, regulating endothelial function and vasomotor tone. Furthermore, NO also presents anti-inflammatory effects and reduces reactive oxygen species (ROS) production.

Objective: To evaluate the potential protective role of a classic NO donor, sodium nitroprusside (SNP), in cerebral microvascular bEnd.3 cells subjected to oxygen and glucose deprivation (OGD) and to identify the underlying mechanisms of this effect.

Methods: Monolayer cultures of bEnd.3 cells were grown to confluence and then subjected to OGD conditions or treated with SNP prior to, during, or both before and during OGD. Subsequently, cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.
To determine the mechanism of action of SNP on cell viability, the cell monolayers were treated under the previously described conditions with nitroglycerin, SNP metabolites (Fe^{+2}, CN^{-}), AICAR, and/or compound C (AMPK inhibitor).

Results: Pre-treatment of bEnd.3 cells with SNP showed a dose-dependent protective effect against OGD-induced apoptosis. However, in the absence of pre-treatment, SNP did not have a protective effect. Nitroglycerin did not show effects on viability under any of the studied conditions. However, ferrous ion and AICAR showed a similar effect to SNP. Pretreatment with compound C partially reversed the protective effects demonstrated by SNP, ferrous ion, and AICAR. If compound C is maintained during OGD conditions, this protective effect disappears.

Conclusion: SNP protects bEnd.3 cells from OGD conditions through a mechanism mediated by the release of ferrous ion rather than by nitric oxide production as expected. The adaptations induced by SNP in these cells are partially dependent on the AMPK pathway. Further studies are needed to determine if ferritin induction mediates the observed response.

Acknowledgements: This project was funded by the Ministry of Science and Innovation (PID2020-119178GB-I00) and the 'Xunta de Galicia: Grants for the consolidation and structuring of competitive research units of the SUG, 2023-2026' (EDT431C 2023/22. Research group GPC GI-1862).

Pleiotropic effects of lipid-lowering therapies on progenitor endothelial cells function in myocardial infarction

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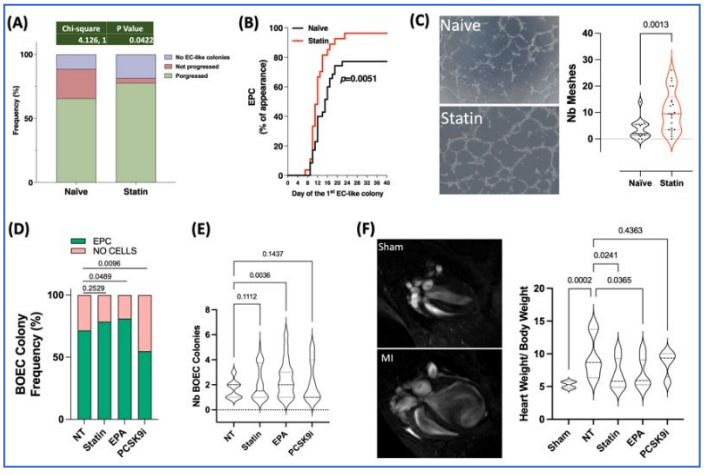
Objective: Early revascularization in acute coronary syndrome (ACS) is crucial to improve patient's outcomes. Despite their primary actions, major lipid-lowering drugs may also reduce cardiovascular events by pleiotropic mechanisms. We aimed to determine if conventional (statin) and novel lipid-lowering therapies [(PSCK9-inhibitor (PCSK9i) and eicosatetraenoic acid (EPA)] can improve endothelial progenitor cells (EPC) phenotype and favor neovascularization in infarcted hearts.
Methods: The study was separated into 3 work packages (WP). In WP,1 EPC was isolated from the buffy coat of peripheral blood from ACS patients (n=62) who were statin-naïve (n=34) or chronic statin treatment (n=28) at the time of inclusion. In WP2, EPC was isolated from statin-naïve ACS patients (n=51), and the buffy coat was separated into groups treated in vitro with vehicle (NT), simvastatin (20nM), PCSKi (1µM) and EPA (3µM). In both WPs, EPC function was determined based on colony progression, number of colonies, proliferative rate, and time of appearance. In WP3, myocardial infarction (MI) was induced in C57BL/6 mice (n=32) by left coronary artery ligation and confirmed by EKG. Four mice underwent sham surgical procedures without coronary artery ligation. Two weeks before MI, mice were randomly treated with vehicle (NT), simvastatin (10mg/Kg, p.o.), PCSKi (140µM/Kg, s.c.), and EPA (180mg/Kg, p.o.), and treatment was maintained until the end of follow-up (12 weeks)
Results: The frequency of EPC appearance was higher in samples of the simvastatin group (78%) compared to the naïve group (66%) (Fig A). They also appeared much earlier (Fig B), in greater quantity (4.7 vs. 2.1 colonies, p=0.0009),and had a higher proliferative rate than EPCs of the naïve group. Besides, they had increased potential for tube formation and shaped more branched vessel-like structures (Fig C). In vitro, treatment of buffy coat with simvastatin does not modify the frequency of EPC colonies (Fig E) and the number of colonies per sample (Fig E), while EPA improved EPC function and PCSK9i impaired EPC function (Fig D, Fig E). In MI mice, statin and EPA treatments significantly attenuated heart hypertrophy compared to NT MI (Fig F).



Conclusions: Statin and EPA have pleiotropic effects on EPC, boosting their potential to form endothelial cell colonies and induce angiogenesis. In mice, both treatments improved hypertrophic remodeling post-MI. However, histological studies are still in progress to define the effects of these drugs on vessel formation in the infarcted heart of mice.

Acknowledgments: Research has been supported by the Spanish Health Institute (Instituto de Salud Carlos III -FEDER-ERDF; PI19/00264). PhD student Francisco Jimenez-Trinidad is a fellow of the University Professor Training (Formación de Profesorado Universitario) fellowship (Spanish Ministerio de Ciencia Innovación y Universidades, FPU19/04925)

Session 9: Respiratory, Gastrointestinal, and Pain Pharmacology

Moderator: Julio Cortijo (Universidad de Valencia)

Invited speakers:

9:00-9:30 Paula Montero (Universidad de Valencia)
Therapeutic innovations in airway smooth muscle diseases: Use of human ex vivo models

9:30-9:45 Jesús Cosín-Roger (Universidad de Valencia)
Differences in metabolite-sensing GPCRs, metabolomic profile and microbial dysbiosis characterize intestinal surgical resections from IBD patients

9:45-10:00 Carolina López-Marín (Universidad de Cádiz))
Exploring the potential of pupillometry in translational pain research as a pain biomarker

10:00-10:15 Miguel A. Huerta (Universidad de Granada)
Synergistic analgesic effect of sigma-1 antagonism and soluble epoxide hydrolase inhibition in pain associated with rheumatoid arthritis

10:15-10:30 Fermín Sánchez de Medina (Universidad de Granada)
Mice lacking tissue non-specific alkaline phosphatase in intestinal epithelium have an altered immunological response and lipid metabolism

Therapeutic innovations in airway smooth muscle diseases: Use of human *ex vivo* models

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Since 1840, isolated airway smooth muscle has been extensively studied to understand the pharmacology of airway diseases such as Chronic obstructive pulmonary disease (COPD) and asthma. The use of isolated human airways is a logical approach to optimizing the development of innovative molecules for treating respiratory diseases. In this regard, experiments using isolated human bronchial tissues in vitro and ex vivo, such as isolated organ bath systems, replicate many key anatomical, pathophysiological, mechanical, and immunological characteristics of the human tissue. Furthermore, precision-cut lung slices (PCLS) have emerged as a sophisticated and physiologically relevant ex vivo model for studying human airways, offering researchers a more accurate representation of the lung's intricate microenvironment. Considerable research has been conducted to identify novel classes of bronchodilator drugs and to further optimize the use of existing bronchodilator classes. Detailed analysis of intracellular signaling pathways regulating smooth muscle contraction has highlighted the critical roles played by small GTPases of the Rho superfamily. ROCK inhibitors are a potential next-generation class of drugs in the treatment of asthma and COPD because of their bronchodilatory and anti-inflammatory properties. In conclusion, the advanced understanding of airway smooth muscle biology and the identification of novel therapeutic targets such as ROCK inhibitors hold significant promise for the development of more effective treatments for asthma and COPD. Continued research in this field is essential for translating these findings into clinical practice and improving patient outcomes.

Differences in metabolite-sensing GPCRs, metabolomic profile and microbial dysbiosis characterize intestinal surgical resections from IBD patients

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Background: Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease which affects the gastrointestinal tract. Among other alterations, microbiota dysbiosis and altered metabolomic profiles have been reported in IBD patients. Recently, G-protein coupled receptors (GPCRs) have been identified as promising pharmacological targets since they could be involved in inflammatory and fibrotic processes associated to IBD. We aim to characterize microbiota composition, tissue metabolomic profile and metabolite-sensing GPCRs expression, in intestinal surgical resections from IBD patients.

Material and methods: Intestinal surgical resections from Ulcerative colitis (UC) (n=18), Crohn's Disease (CD) (n=21), non-IBD colon (n=20) and non-IBD ileum (n=12) patients were obtained. Metabolomic analysis was performed by NMR. Results are expressed as µg metabolite/g tissue. Microbiota characterization was performed by 16S rRNA gene Illumina Miseq sequencing. Gene expression was analysed by qPCR and data expressed as fold induction (mean±SEM) and compared by a t-test.

Results: First, microbiota characterization revealed a reduction in bacterial diversity and load in UC, whereas no differences were found in CD. Next, significant differences at genus and species levels between UC, CD and its respective non-IBD controls were also detected. For instance, *Cellulosimicrobium NA* was increased in UC patients. Regarding CD, *Ruminococcus bromii* and *Faecalibacterium prausnitzii* were decreased in CD patients.
Next, altered levels of GPCRs-agonists were found in surgical resections from IBD patients as shown in the following table (Table 1):

	β-hydroxybutyrate	Succinic acid	Propionic acid	Acetic acid	Phenylalanine
Non-IBD (colon)	6.18±0.47	7.29±0.94	4.71±0.74	7.72±0.88	5.16±0.54
UC	8.27±0.85	7.97±0.88	7.71±1.13	7.47±0.8	8.77±1.55
Non-IBD (ileum)	9.05±0.88	7.17±0.83	4.09±0.45	10.81±1.33	12.09±1.59
CD	13.13±1.07	14.06±1.70	7.82±0.97	16.01±1.82	25.09±3.21

Table 1: Levels of metabolites expressed in µg metabolite/g tissue of fibrotic CD patients vs non-IBD.

Finally, IBD patients exhibited differential expression of metabolite-sensing GPCRs vs non- IBD as summarized in the following table (Table 2):

	GPR35	TAAR1	GPR91	GPR109A	GPR109B	GPR43	GPR41
Non-IBD (colon)	1.83±0.44	2.05±0.55	1.51±0.27	1.39±0.24	1.60±0.44	1.39±0.25	1.18±0.14
UC	0.52±0.11	45.46±26.68	13.24±5.65	19.05±5.30	42.30±29.01	21.52±9.06	4.73±1.73
Non-IBD (ileum)	6.59±2.07	48.13±17.58	5.59±1.25	28.50±13.89	20.07±8.06	6.33±1.78	44.27±15.18
CD	1.74±0.28	814.2±283.5	63.29±22.71	400.6±130.6	295.7±53.41	118.7±35.88	269.5±90.24

Table 2: Human gene expression of metabolite-sensing GPCRs expressed as fold induction.

Conclusions: Altered metabolite-sensing GPCRs expression, levels of GPCRs agonist and microbial dysbiosis is detected in intestinal resections from IBD patients pointing new promising pharmacological targets which need to be further studied.

Exploring the potential of pupillometry in translational pain research as a pain biomarker

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Pain is a major health challenge with high incidence of treatment-resistant and difficulty in finding new effective analgesics [1]. Thus, to advance the development and discovery of new drugs, it is essential to establish novel pain-like readouts and physiological markers. In this context, studies have found strong correlations between pupil dilation response to pain stimuli and pain intensity [2,3]. Pupillometry offers a non-invasive, cost-effective method applicable in both humans and rodents.

This study aims to evaluate the pupil size as a correlate for pain assessment and analgesic response. For this purpose, formalin pain model was induced by the injection of 20µl of a 4% formalin solution subcutaneously into the plantar surface [4]. The biphasic pain response of this model (a short early phase followed by a second late phase) was evaluated in male and female mice after morphine (10mg/kg, intraperitoneally), duloxetine (20mg/kg, intraperitoneally) or ibuprofen (150mg/kg, orally) administration. Pupil response to formalin and analgesic drugs was monitored binocularly under light isoflurane anesthesia. The pupil dilation response induced by mechanical hindpaw compressions was also evaluated.

Findings revealed that formalin administration produced an early and late pupillary dilation response coinciding with the nociceptive behavior without changes between sexes. As previously described, treatment with morphine and duloxetine reduced nociceptive response in both phases, while ibuprofen only decreased it in the second phase in both sexes. Interestingly, sex differences were evidenced in the second phase of formalin after morphine treatment, likely due to sex variations described in the opioid system. Regarding pupillometry, morphine reduced the pupillary response in the early phase, while in the late phase, the reduction was observed only in males, similar to the nociceptive response. Duloxetine also reduced pupillary response similarly to its effect on nociception, while ibuprofen did not. Additionally, all drugs attenuated pupil dilation caused by mechanical hindpaw compressions in the formalin-induced pain model. Therefore, these results showed a correlation between pain intensity and pupillary response to formalin and analgesic drugs in a sex-dependent manner in certain cases. Thus, pupillometry could represent an innovative tool with high translational value as pain biomarker and predictive potential of analgesic response.

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Acknowledgements: "Fondo Europeo de Desarrollo Regional-UE, A way to build Europe" from "Ministerio de Economía y Competitividad" (PID2022-142785OB-I00; PDC2022-133987-I00); "Consejería de Transformación Económica, Industria, Conocimiento y Universidad, Junta de Andalucía" (CTS-510); "Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz-INiBICA" (LI19/06IN-CO22; IN-C09); "CIBERSAM: CIBER-Consorcio Centro de Investigación Biomédica en Red (CB07/09/0033), "Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación"; EU's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955684 and Red Española de Investigación en Estrés/Spanish Network for Stress Research RED2022-134191-T financed by MCIN/AEI/10.13039/501100011033.

Synergistic analgesic effect of sigma-1 antagonism and soluble epoxide hydrolase inhibition in pain associated with rheumatoid arthritis

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Introduction: Pain associated with rheumatoid arthritis (RA) is highly prevalent and the main concern of patients with this disease [1]. However, there are no specific drugs for its treatment, and classic analgesics, mainly NSAIDs, are used. These are often ineffective and have adverse effects that limit their use [2]. Both sigma-1 receptor (S1R) antagonism and soluble epoxide hydrolase (sEH) inhibition have shown robust analgesic efficacy in different models, however, the effects induced by their association had not been explored [3].
Methods: We used the collagen-induced arthritis (CIA) model in female Wistar rats. Mechanical allodynia was evaluated with the von Frey test and cold allodynia with the acetone test. The S1R antagonist S1RA, the sEH inhibitor EC-5026 and the dual compound EPB-117 were administered subcutaneously at different doses on day 8 or 13 after immunization. Behavioral assessments were performed 1, 2 and 3 h after administration. We also associated those treatments with PRE-084, a sigma-1 agonist, and MS-PPOH, an inhibitor of microsomal CYP450s which avoids the beneficial effect of sEH inhibition.
Results: Both S1RA and EC-5026 reduced mechanical and cold allodynia in a dose- and time-dependent manner. The maximum analgesic efficacy was moderate (50-60%) in all the cases. When two ineffective doses of S1RA (20 mg/kg) and EC-5026 (2.5 mg/kg) were combined a robust synergistic effect was observed in mechanical and cold allodynia. This synergism was confirmed by using the dual compound EPB-117, which also showed a dose- and time-dependent effect. Moreover, the synergistic effects were reversed by either PRE-084 or MS-PPOH, which confirmed that both targets are necessary for the potentiation.
Conclusion: We observed a clear synergistic analgesic effect of sigma-1 antagonism and soluble epoxide hydrolase inhibition in a rat model of pain associated with rheumatoid arthritis. The dual compound also exhibited robust analgesic efficacy suggesting that could be useful for treating pain associated with rheumatoid arthritis.

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Acknowledgements: FPU and project PID2021-123058NA-I00 funded by MCIN/AEI/10.13039/501100011033

Mice lacking tissue non-specific alkaline phosphatase in intestinal epithelium have an altered immunological response and lipid metabolism

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Tissue non-specific alkaline phosphatase (TNAP) is an enzyme encoded by *Alpl* gene which catalyzes hydrolysis of phosphomonoesters. TNAP plays a key role in bone mineralization and its deficiency leads to hypophosphatasia. However, the fact that it is expressed in many other tissues suggests the enzyme has multisystemics effects that need to be investigated (1). Our main objective is to study the effects of TNAP deficiency on the response to a high-fat diet in murine models.

In this study, we used wild type C57BL/6J (WT) mice and mice carrying a conditional intestinal epithelial deletion of TNAP ($Alpl^{IEC-/-}$), a *knock out* line generated in the group. They were split in four groups and fed a control diet (CD) or a high fat diet (HFD) for 94 days. During this time mice weights, glucose levels and food and water consumption were recorded. After sacrifice, liver slices were stained with hematoxylin and eosin. Hepatic glycogen levels were measured spectrophotometrically. In addition, liver and jejunum samples were preserved in RNAlater solution. Finally, RNAseq and RT-qPCR analysis were performed to measure the expression of different genes related to metabolism and inflammatory markers in both tissues. $Alpl^{IEC-/-}$ HFD mice were compared to WT HFD.

$Alpl^{IEC-/-}$ mice fed with HFD did not gain weight compared to WT fed with the same diet. Histological analysis revealed fat microdeposits in $Alpl^{IEC-/-}$ mice. Also, levels of hepatic glycogen were higher in $Alpl^{IEC-/-}$ mice. RNAseq analysis in jejunum of $Alpl^{IEC-/-}$ mice fed a HFD showed an upregulation of defensin codifying genes and the downregulation of immune response genes related to interferon and B cell responses in jejunum when compared to WT mice. In addition, *Nt5e* and *P2ry4* were upregulated in jejunum of *knock out* mice. As for the liver, genes that encode proteins related to lipid (*Mid1ip1*) and line glycogen (*Ppp1ccb*) metabolism pathways were upregulated. The results also revealed inhibition of the expression of genes related to inflammatory processes like *Ptrn3, Ltf* and, *Cxcl1*.

In conclusion, the elimination of TNAP from the intestinal epithelium modifies the immunological defense in the jejunum and has effects on gene expression in the liver, modifying the immunological response, lipid and glucid metabolism. These data support our hypothesis that this enzyme has effects on immunity and metabolism and could be a pharmacological target.

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Session 10: Novel Approaches for the Design, Development, and Delivery of Drugs

Moderator: Valentín Ceña (Universidad de Castilla-La Mancha)

Invited speakers:

15:00-15:30 María Isabel Loza (Universidad de Santiago de Compostela)
Novel findings in antipsychotic modulation of signalling pathways

15:30-15:45 Irene Rodríguez-Clemente (Universidad de Castilla-La Mancha)
Dihydropyridine-based nanoparticles efficiently transfect siRNA in glioblastoma cells and are transported into the Central Nervous System

15:45-16:00 Verónica Casadó-Anguera (Universitat de Barcelona)
Targeting GPCR heteromers in Parkinson's disease: Design and characterization of a tetravalent A2AR-D2R ligand

Novel findings in antipsychotic modulation of signalling pathways

María Isabel Loza, José Brea, Laura Gómez-García, Sonia Gómez, Iria Brocos-Mosquera, Raquel Lama, Antón Leandro Martínez

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Schizophrenia is a complex disorder characterised by diverse symptoms that often stem from multiple neural pathways and neurotransmitter dysregulation. Most of the current drugs were developed from target-based approaches acting on concrete signalling pathways. Thus, current antipsychotic drugs target G protein-coupled receptors (GPCRs), particularly the dopamine D_2 and serotonin 5-HT_{2A} receptors. Given that the expression of the D_2/5-HT_{2A} heterodimer has been reported, we demonstrate here that the heterodimerization of dopamine D_2 and serotonin 5-HT_{2A} receptors induce differential intracellular pharmacological signalling compared with monomeric D_2 and 5-HT_{2A} receptors, which may influence the effect of certain antipsychotic drugs. To investigate the intracellular crosstalk of the D_2/5HT_{2A} heterodimer, we employed a HEK 293 Flp-In^{TM} T-Rex^{TM} cell line constitutively expressing the D_2 receptor and an on/off inducible expression of 5-HT_{2A} receptor by doxycycline treatment.

Activation of 5-HT_{2A} protomer of the D_2/5-HT_{2A} heterodimer by the hallucinogenic agonist (±)DOI modulated the D_2 protomer response to dopamine via crosstalk between D_2 and 5-HT_{2A} receptors, which, in turn, was mediated by the protein kinase A/CREB mediated pathway. This crosstalk is dependent on G_q protein and phospholipase C. This modulation conditioned the antipsychotic effect, indicating the different antagonistic profiles of typical and atypical antipsychotics.

To better characterize the translationality of this modulation in vitro, we generated both 2D and 3D neuronal models, included neurospheres, that will eventually be employed for in vitro screening related with such signalling target looking for compounds able to revert the insult-mediated alterations on either the neuron excitability or the neuron morphology.

Acknowledgements: We acknowledge grant support from Agencia Estatal de Investigación (PID2020-119428RB-I00) and Xunta de Galicia (ED431C 2022/20) and European Regional Development Fund (ERDF). RL is recipient of a Juan de la Cierva fellowship (JDC2022-049537-I) from the Ministry of Science and Innovation and European Union (*NextGenerationEU*/PRTR)

Dihydropyridine-based nanoparticles efficiently transfect siRNA in glioblastoma cells and are transported into the Central Nervous System

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Although different approaches have been used to increase Blood-Brain Barrier (BBB) crossing by nanoparticles (NPs) [1], there are still significant restrictions for NPs to access the central nervous system (CNS) in significant amounts, limiting their usefulness for being part of the therapeutic approaches to neurological diseases and brain tumors such as glioblastoma (GBM) [2]. Small interference RNAs (siRNAs) are gaining increasing interest as therapeutic options in several diseases [3]. siRNAs require a vehicle (i.e., NPs) to be protected from degradation and transported into the target cells. On the other hand, dihydropyridines (DHP) are widely used drugs in cardiovascular pharmacology.

We have synthesized several DHP-based NPs and studied the cytotoxicity induced by the NPs on commercial GBM cell lines, astrocytes and neurons as well as on patient-derived GBM cells using lactate dehydrogenase release assays. The effect of specific siRNAs, transported into the cells by DHP-derived NPs, on the levels of the proteins (p42-MAPK, Rheb, MGMT) encoded by the mRNAs targeted by the siRNAs were determined by the Western Blot technique. Finally, we labeled the NPs with a fluorescent probe and studied the biodistribution in mice using an IVIS equipment.

We found that the DHP-based NPs bound siRNA and protected it from degradation by RNAses. The NPs were not toxic by themselves to GBM cells, astrocytes and neurons. Moreover, they were able to transport specific siRNAs into the GBM cell interior and decrease the intracellular levels of the target proteins to about 10% of control values in 72 hours. In addition, biodistribution studies showed a marked accumulation of the labeled NPs in both brain and spinal medulla.

The results strongly suggest that the DHP-derived NPs can represent a very efficient scaffold to transport siRNA and drugs into the CNS.

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Acknowledgements:
The authors acknowledge the excellent technical support from Vanesa Guijarro. This research was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and MICIN (project PID2020-120134RB-I00 Financiado por MCIN/AEI/10.13039/501100011033).

Targeting GPCR heteromers in Parkinson's disease: Design and characterization of a tetravalent $A_{2A}R$-D_2R ligand

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G protein-coupled receptors (GPCRs) represent the most significant superfamily of membrane proteins targeted pharmacologically, with approximately 35% of FDA-approved drugs acting on these receptors [1,2]. GPCRs can form oligomeric complexes, resulting in diverse allosteric interactions within their orthosteric and allosteric centres. A GPCR heteromer that fulfills the established criteria for its existence *in vivo* is the complex between adenosine A_{2A} ($A_{2A}R$) and dopamine D_2 (D_2R) receptors, which has an important role in the treatment of Parkinson's disease (PD). The quaternary structure of the $A_{2A}R$-D_2R oligomer, comprising homodimers of each receptor, not only underpins the therapeutic use of the $A_{2A}R$ antagonist istradefylline with L-dopa for the treatment of PD, but also explains the locomotor depression caused by high doses of potent $A_{2A}R$ antagonists such as caffeine[3–5]. Our study aimed to design, synthesize, and characterize a tetravalent ligand targeting the $A_{2A}R$-D_2R heteromer.

Here, we have designed and synthesized tetravalent ligands constituted by four antagonist pharmacophores selective for each protomer linked by a polyethylene glycol-based linker with various lengths. These ligands have been designed to find the optimized structure able to simultaneously bind to the four orthosteric centres of the complex to further create a compound with the appropriate pharmacophores for effective PD treatment. We have assessed the simultaneous binding of these ligands to the different orthosteric sites within the heteromer 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 has allowed us to identify a compound able to simultaneously bind with subnanomolar affinity to the four different orthosteric sites of the $A_{2A}R$-D_2R heterotetrameric complex. While the ability of multivalent ligands to traverse the blood-brain barrier (BBB) is typically limited, this challenge can be addressed through emerging drug-delivery systems, such as biodegradable polymeric nanoparticles and virus-derived peptides capable of transporting drugs across the BBB[6,7]. These advancements hold promise for enhancing the therapeutic potential of our designed tetravalent ligand in PD treatment.

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Acknowledgements

This work was supported by the grant PID2020-113938RB-I00 (VCA, NL, EM, VC) and RTI2018-093831-B-I00 (DP, MR), the grant 2021-SGR-00230 from the "Generalitat de Catalunya" and UBPredocs fellowship from the University of Barcelona (NL)

Session 11: Teaching Innovation in Pharmacology

Moderator: Fernando Yáñez Gómez (Universitat de les Illes Balears)

Invited speakers:

15:00-15:30: Inmaculada Bellido (Universidad de Málaga)
The Objective Structured Clinical Examination (OSCE), another tool to assessment and training pharmacology

15:30-15:45 Mª Luisa Ferrándiz (Universidad de Valencia)
Chair for the Rational Use of Medicines MICOF-UV, students and professionals working together towards safe drug therapy

15:45-16:00 Víctor López (Universidad San Jorge, Zaragoza)
Blended Intensive Programmes in Pharmacy at Universidad San Jorge: a new innovative international teaching and learning experience within higher education

The Objective Structured Clinical Examination (OSCE), another tool to assessment and training pharmacology

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The Objective Structured Clinical Examination (OSCE) is a multidisciplinary, multipurpose, mandatory assessment at the end of Medicine Degree. It is very helpful in nursing, pharmacy, podiatry, and psychology, between others Degrees. OSCE stations may include: 1) Clinical interactions (face to face, or virtual) with standardized patients; 2) Examination of mannequins and interpretation of findings; 3) Test and Probes interpretation; 4) Clinical reasoning; 5) Diagnosis; 6) Computerized cases; 7) Telemedicine cases, 8) Reports' writing; 9) Patient education/counselling…
It is always required: 1) Good organization, which guarantees that all students access the ECOEs with the same means and possibilities; 2) Some minimal material and human resources; 3) Evaluators perfectly trained and capable of evaluating exactly the competencies and skills that students must develop in each OSCE. And well-trained students showing competencies and integration of medical knowledge and skills.
We currently develop 5 different types of OSCE at the School of Medicine and Health Sciences of Malaga in which Pharmacology subject participates.
1. Classical OSCE designed for the assessment of Final Rotatory from Medicine Degree. A circuit of 12 stations to be solved face to face in 3 hours, one of which is the Pharmacology Station. In it, students must review and renew the treatment of a polymedicated patient with several comorbidities, indicating the drugs they would suspend, change (drug/dose/posology), add and keep as is, and the reason why they perform each of these actions.
2. Virtual National OSCE designed for the assessment of Final Rotatory from Medicine Degree.
In this case, a draw in which 12- 14 stations are chosen to be virtually solve in 2,5 hours. This OSCE circuit takes place on the same day for all faculties, and allows students from all participating faculties to be virtually evaluated with the same criteria.
3. OSCE performed by students. In this case, 4-6 students group from the Pharmacology subject from 3rd and 5th year of Medicine Degree and 2nd year of Podiatry Degree have been encouraged to design their own OSCE with the supervision and help of teachers following flipped classroom methodology. Each student team designs a 10 min OSCE station showing a clinical situation involving drugs, has to look for correct and incorrect items related to drugs uses, and people behaviours related to prescriptions and explanations about medicines, and teach the different OSCE station to the rest of the course.
4. Iterative OSCE station. It is a variant of the previous OSCE. In this case, the student teams work with a patient with the same initial basic characteristics for all groups, but based on the patient's answers to each team's questions, the OSCE station solution will be different from one group to another, allowing so each group could compare how different interactions with the same patient could condition different treatments and responses, and thus understanding the need and seeing cases of personalized medicine.
5. OSCE in second life/metaverse. We are preparing an ECOE circuit in second life to make this way of working a little more attractive for students.

Chair for the Rational Use of Medicines MICOF-UV, students and professionals working together towards safe drug therapy

Ferrándiz ML[1,2,4], Sendra-Lillo J[3,4], García-Arnandis I[1,4], Vicedo-Salort MT[3,4], Pereda J[1,4], Martí-Rodrigo A[3,4], Montesinos MC[1,2,4]

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Inappropriate use of medicines constitutes a costly health problem. Given the prominent role of pharmacists in promoting that patients receive and understand the proper use of medications depending on their clinical needs, the new Chair for the Rational Use of Medicines (RUM) was introduced to the students of the Degree in Pharmacy of the University of Valencia (UV) as a collaborative venue with the Very Illustrious Official College of Pharmacists of Valencia (MICOF).

The UVCàtedres program is an exceptional resource for collaboration between the University of Valencia and society. In December 2023, the two institutions, UV and MICOF, signed the agreement to strengthen their collaboration in three fundamental lines related to RUM: training, research and dissemination. The functions of the mixed monitoring committee include: the approval of the annual program of activities to be carried out, at the proposal of the director; the approval of the budget; monitoring and evaluation of the academic activities; and the approval of the annual report of the chair.

The RUM MICOF-UV Chair is a great tool from a teaching point of view in two very important aspects for our students: on the one hand, it greatly facilitates their contact with pharmaceutical professionals and, therefore, in real life scenarios; and on the other hand, it helps us to reinforce the training of our students at the RUM, offering them an exceptional opportunity to work with real data regarding the use of medications in different care levels. The Chair was presented at the Pharmacy Student Congress in February 2024, sponsoring 2 awards for the best RUM- related communications by undergraduate and graduate students. Some of the results of the first Doctoral Thesis that is being carried out under the umbrella of the Chair were also presented. Degree Final Projects will be offered for the 2024-25 academic year, and several fourth-year students have already shown interest in in this line of work. It has also been presented to the teaching staff of the Degree in Pharmacy and to the members of the MICOF, promoting collaborative work between pharmaceutical professionals, teaching staff and students.

In conclusion, the RUM MICOF-UV Chair is a space for collaboration that will allow us to reinforce our students' knowledge and skills to promote the appropriate use of medications in any situation to optimize patients' pharmacotherapy and improve results in health.

Acknowledgments:
Funded by Chair for the Rational Use of Medicines MICOF-UV

Blended Intensive Programmes in Pharmacy at Universidad San Jorge: a new innovative international teaching and learning experience within higher education

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Blended Intensive Programmes (BIPs) in higher education are short academic programmes that use innovative ways of learning and teaching where transnational teams work together to reach learning outcomes through short-term physical mobility abroad combined with a compulsory virtual component. In this context, Universidad San Jorge organised its first BIP during the academic year 2023-2024.

The BIP entitled "DEVELOPMENT OF PHYTOPHARMACEUTICAL PRODUCTS FOR PHARMACOLOGICAL, COSMETIC OR FOOD APPLICATIONS" was organised for international students of different academic levels (Master and PhD) of Pharmacy and Pharmaceutical Sciences. During the course, the participants follow a virtual component with lectures given by experts in the field of Pharmaceutical Sciences whereas the physical mobility was held at the Faculty of Health Sciences with lecturers from Universidad San Jorge.

20 participants from 6 different institutions belonging different countries (University of Camerino-Italy, University of Helsinki-Finland, University of Split-Croatia, University of Kaunas-Lithuania, University of Porto-Portugal, University of Coimbra-Portugal) enjoyed the programme in which Universidad San Jorge was the host and organizing institution. The language of teaching was English. The virtual component was developed in September 2023 with 7 lectures given through the Microsoft Teams platform by international lecturers from different universities whereas the physical mobility was developed in the Campus of Villanueva de Gállego of Universidad San Jorge during 5 working days. The working days consisted of laboratory work, lectures and a cultural agenda for facilitating the integration of the students in the Spanish context. The laboratory work consisted in 20 hours in which the students learned different extraction techniques for natural products, basic analytical techniques, pharmacological and biological assays using enzymes, cell cultures and living organisms, innovative pharmaceutical forms through pharmaceutical technology formulation. Many of the laboratory sessions were performed using bioactive compounds and extracts used in pharmaceutical products like medicines, food supplements and cosmetics. The satisfaction of the participants in the BIP was evaluated through surveys, in which it was remarked that the Blended Intensive Programme was a very positively international experience with an interesting content for their academic background.

Acknowledgements:
Universidad San Jorge thanks SEPIE (Servicio Español para la Internacionalización de la Educación), Erasmus + and the European Comission for finantial Support for the development and implementation of Blended Intensive Programmes.

POSTER SESSIONS

NEUROPHARMACOLOGY

P1

Investigating the role of the orexinergic system in the pathogenesis of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive and heterogeneous neurodegenerative disease that appears in adult life and inevitably leads to death within 3 to 5 years after diagnosis. Currently, only two treatments are available in the market for ALS patients, riluzole and edaravone, but none of them can prolong patients' lives for more than three months[1]. Therefore, it is of great medical interest to find new treatments for this pathology.

The orexinergic system, consisting of the orexin peptides (OX-A and OX-B) and the orexin receptors (OX_1 and OX_2), plays a crucial role in sleep-wake cycle regulation, energy balance, and addiction[2]. During the last 20 years, this system has been associated with several neurodegenerative diseases, but its role in the pathogenesis of ALS remains unclear[3–5].

The present project aims at identifying the role of the orexinergic system in the pathogenesis of ALS. In the first part, the orexinergic system was studied in the NSC-34 cell line, an established *in vitro* model of ALS[6]. For the first time, the presence of orexin receptors was confirmed in this cell line using western blot and immunofluorescence techniques, which represents a valuable finding and allows us to understand how the modulation of this system may affect motor neurons. In the second part of the project, animal studies will be conducted using the $SOD1^{G93A}$ mouse model of ALS. Animals will be treated with orexin receptor modulators and compared with traditional ALS treatments and placebo at pre- and post-symptomatic stages (postnatal day 60 and 90, respectively). Motor coordination, general neurological status, and weight loss will be assessed to monitor treatment effects on disease progression. Moreover, samples of the brain (cortex, cerebellum, and lateral hypothalamus) and the spinal cord will be analyzed using different techniques to evaluate several hallmarks of ALS pathology. Understanding the involvement of the orexinergic system in ALS could pave the way for new therapeutic strategies, potentially improving outcomes for these patients.

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Acknowledements:
This work is funded by Ministerio de Ciencia, Innovación y Universidades (Juan de la Cierva postdoctoral contract Ref: JDC2022-049555-I, and Proyectos de Generación de Conocimiento Ref: PID2020-117127RB-I00

P2

Target validation of antibodies against serotonin 5-HT$_{2a}$ receptors in mouse brain

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Generating selective antibodies against G-protein coupled receptors (GPCR) is particularly challenging due to their complex structure, membrane embedding, and imbricate sequences. Unfortunately, most commercially available antibodies for research purposes undergo minimal selectivity testing. Therefore, target validation of anti-GPCR antibodies is critical to ensure reliability and reproducibility of the results. The present study aimed to (1) characterize commercially available antibodies against serotonin 5-HT$_{2A}$ receptors (HTR2A) using brain tissues from wild-type (WT) and HTR2A knockout (KO) mice, and (2) quantify HTR2A cortical immunodensity in mice subjected to maternal immune activation (MIA), a procedure known to alter the hallucinogenic response to HTR2A agonists.

Eight different affinity-purified "selective" antibodies raised against HTR2A were purchased from six different manufacturers. HTR2A-KO mice were obtained from Shanghai Model Organisms. MIA was induced in WT pregnant dams at gestational day 9.5 by intraperitoneal administration of polyinosinic:polycytidylic acid (PIC; 5 mg/kg) or saline. The offspring (n=8 per group) were sacrificed on postnatal day 84, and brains were processed for Western blotting (WB) or immunohistochemistry (IHC) under standard conditions.

Of all tested commercial antisera, only one antibody (ImmunoStar, Catalogue# 24288) reacted against potentially specific antigens in WT brain samples, which were absent in HTR2A-KO samples. Although the predicted molecular mass of HTR2A is 53 kDa, the antibody detected two selective bands of ~65 and ~80 kDa, respectively, in WB assays. *In vitro* pre-incubation of brain homogenates with peptide:N-glycosidase F (PNGase) did not alter HTR2A migration across the gel, indicating that the observed gain in molecular size cannot be attributed to receptor N-glycosylation. IHC assays with the WB-validated anti-HTR2A antibody showed intense staining of frontal and parietal cortices, with minimal staining in other cortical or subcortical areas. Within the frontal cortex, the highest HTR2A density was seemingly in layers III and V, while layers I and IV displayed minimal immunoreactivity. No significant staining was observed in brain slices from HTR2A-KO animals. Finally, cortical HTR2A immunondensity in PIC-exposed mice during gestation did not differ from that of controls.

In conclusion, we successfully validated an anti-HTR2A antibody for the detection and quantification of HTR2A in murine brain tissues using WB and IHC. The larger molecular mass of native HTR2A in brain tissue may be attributed to posttranslational modifications other than N-glycosylation. The observed staining pattern in IHC experiments aligns with previous autoradiography studies showing preferential localization of HTR2A at cortical layer V. Our findings do not support the hypothesis that increased responsiveness to HTR2A agonists in the MIA model is due to greater HTR2A protein expression in mouse cortex.

Acknowledgements:
This work was supported by grants PID2022-137848OB-I00 (MCIU/AEI/FEDER) and IT1512/22 (Basque Government). EH-H holds grant FJC2022-048338-I, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR. The authors report no conflict of interest.

P3

Combined impact of adolescent ethanol and cocaine exposure on voluntary ethanol consumption and hippocampal neurotoxicity in adult male and female rats

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The effects exerted individually by cocaine and alcohol have been extensively studied, nevertheless there is lack of knowledge about the impact of its combination. If key risk factors such as sex and early drug use are added to this combination, the susceptibility to develop addiction-liability increases. In this context, we utilized rodents to model an early combined initiation of both drugs to study the long-term development of an addictive-like phenotype (i.e., voluntary alcohol consumption) during abstinence in adulthood and its possible effects on hippocampal neurotoxicity while including sex as a biological variable. For this purpose, 4 experimental groups of male and female adolescent Sprague-Dawley rats were treated i.p. with either vehicle (0.9% NaCl), ethanol (2 g/kg, 2 consecutive days at 48-h intervals, 3 cycles), cocaine (15 mg/kg, 6 consecutive days), or a combination of both. Rats were left in forced abstinence until adulthood, when they were granted voluntary access to 20% ethanol (two-bottle choice test) for 3 consecutive days/week during six weeks, as previously characterized [1]. Subsequently, after the last day of the sixth week, brains were collected to study potential hippocampal neurotoxicity (i.e., FADD, cyt-c) by western blot. Statistical analyses were done through two-way ANOVAs (independent variables: Sex, Adolescent treatment). The main results showed that adolescent ethanol exposure was a risk-factor for later developing an increased voluntary ethanol consumption in adulthood, both for male and female rats. This risk was similar when ethanol was combined with adolescent cocaine exposure, since cocaine alone showed no effects on later ethanol intake. Moreover, when correcting ethanol intake by weight to calculate the dose consumed per day (g/kg/24 h), female rats with a prior history of adolescent ethanol exposure consumed higher doses than their male counterparts. Interestingly, this higher consumption in females paralleled an increased level of the apoptotic marker, cyt-c, in hippocampus. No changes were observed in FADD protein content. Taken together, our findings revealed that adolescent ethanol exposure increased later voluntary ethanol consumption in in adult male and female rats. However, females end up being exposed to higher ethanol doses than males, and therefore suffering more adverse consequences at the neurochemical level. To better understand these effects, current studies are evaluating potential decreases in hippocampal neurogenesis (NeuroD: early neuronal survival) induced by ethanol consumption and prior adolescent exposure.

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Acknowledgements: Funded by Delegación del Gobierno para el Plan Nacional sobre Drogas (2020/001, Ministerio de Sanidad) to MJG-F. CC-R and MJG-F are members of RIAPAd (RD21/0009/0008; ISCIII, MICIN). Pre-doctoral scholarship (FPU2022-012-A; Conselleria de Fons Europeus, Universitat i Cultura del Govern de les Illes Balears) to CC-R.

P4

Pharmacological characterization of 1,3,5-trisubstituted and 1,3,4,5-tetrasubstituted pyrazoles as cannabinoid ligands

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The endocannabinoid system participates in the regulation of numerous physiological processes. It has been also involved in the pathophysiology of different diseases, highlighting the therapeutic potential of modulating this system. Thus, cannabinoid compounds may be potentially useful as analgesics, antiemetics, antispasmodics, appetite stimulants, and in the treatment of epilepsy and glaucoma. Therefore, developing new cannabinoids as drugs for the treatment of several diseases is a hot topic for research nowadays.

In this context, the main goal of the present study was the pharmacological characterization of 1,3,5-trisubstituted and 1,3,4,5-tetrasubstituted pyrazoles as cannabinoid compounds.

The affinity of 34 new compounds towards the CB1 receptors was measured *in vitro* by competition binding experiments. Specific [^{3}H]SR141716 binding (4 nM) was measured in postmortem human brain prefrontal cortex membranes homogenates in the absence or presence of increasing concentrations of competing compounds (10^{-11} M to 10^{-4} M, 11 concentrations). Of all the tested compounds, only **1d**, **2a**, **2c**, **3a**, **3c**, **3f**, **5a**, **6d**, **6g**, **6i**, **7d** and **7f** displayed moderate affinity (pKi > 5) over CB1 receptors. They were therefore evaluated by *in vitro* functional [^{35}S]GTPγS binding assays with human prefrontal cortex membranes homogenates to determine their activity as agonists, antagonists or inverse agonists over CB1 receptors. Compounds **3a** and **6g** displayed an antagonistic effect, while **6d** (-log EC_{50} = 5.8) and **6i** (-log EC_{50} = 6.7) behaved as agonists. All the other tested compounds demonstrated an inverse agonistic activity.

These preliminary results highlight the possibility for compounds **6d** and **6i** to act as CB1 agonists, which may open a new door to the therapeutical approach for several disorders. Furthermore, it seems that a styryl group at position 4 of the pyrazole ring might be of high importance when designing new agonistic cannabinoid drugs.

Acknowledgements:
Catarina M. Correia thanks FCT/MCTES for her PhD grant 2022.11584.BD. Thanks are also due to the National NMR Network (PT NMR) partially supported by Infrastructure Project No. 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC). Vera L.M. Silva thanks FCT for funding through the Scientific Employment Stimulus – Institutional Call (Ref. CEECINST/ 00026/ 2018 https://doi.org/10.54499/CEECINST/00026/2018/ CP1521/CT0013). Supported by the Basque Government (IT1512/22).

P5

Gene expression profile in the cerebral cortex of the Niemann-Pick type C mutant mouse

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Niemann-Pick type C (NPC) is a disease with a low incidence that belongs to the lysosomal storage disorders (LSD). The hallmark of NPC is the accumulation of cholesterol and sphingomyelin, sphingosine, and gangliosides (GM2 and GM3). The accumulation is multisystemic and is evident in the cerebral cortex: increased cholesterol and various sphingolipids in neuronal membranes, aggregates of hyperphosphorylated tau protein, α-synuclein, β-amyloid peptide. NPC is an autosomal recessive disease caused by mutations in the NPC1 and NPC2 genes. Both genes encode proteins involved in the release of cholesterol from the endosome-lysosome system. In the present work, we sought to determine the expression profile in the cerebral cortex of the mutant mouse with NPC. We performed a microarray analysis of 22,000 genes from the cerebral cortex compared to the WT mouse to achieve this goal. Based on the data obtained from the microarray, bioinformatic analysis and significant genes were selected and confirmed by qPCR with Taqman probes. Western blot (WB) was used to analyze the expression of proteins involved in cortical circadian rhythm and ubiquitination in neurodegenerative diseases. Microarray analysis revealed an under-expression of 611 genes and an over-expression of 341 genes. Bioinformatic analysis revealed that genes involved in ubiquitination, apoptosis, fatty acid metabolism, differentiation and development, and mitochondrial genes are deregulated in the cerebral cortex of the NPC mouse. Using qPCR, we confirmed the underexpression of the genes *Bmp4, Ppm1f, Ptpmt1, Bbs9,* and *Rarg* and the overexpression of the gene *Bmal1*, which was detected in the microarray. In Western blot, we observed altered protein levels in the cortical circadian rhythm pathway and overexpression of proteins involved in ubiquitination and TDP-43. These results reveal groups of altered genes in NPC, which helps us to understand the altered molecular mechanisms and to identify new therapeutic targets.

P6

Impact of Spinocerebellar Ataxia type 3 in postnatal cerebellar developement

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Spinocerebellar Ataxia type 3 or Machado Joseph disease (SCA3/MJD) is a neurodegenerative disease caused by an abnormal expansion of the CAG triplet in the coding region of the ATXN3 gene, which leads to a polyglutamine stretch that and the agreggation of the protein ataxin-3[1]. SCA3/MJD is the most prevalent ataxia worldwide, especially in Europe and the Iberian Peninsula (20%-50%)[2,3]. This condition causes severe disability, and normally ends with premature death of the patients due to progressive appearance of alterations in movement, cognition, equilibrium and organ motility. 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]. Furthermore, there is no cure or effective treatment, so research on unveiling when neural alterations exactly appear are of utmost importance so as to establish pharmacological strategies as early as possible. This is why, we hypothesize that mutant ataxin-3 protein could modify cerebellar morphogenesis during early postnatal development.
For this research project, we use a mouse model of SCA3/MJD which faithfully mirrors human hallmarks and progression of the disease, at early postnatal stage. 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], and we have observed differences in individual cell behaviour parameters. We have also characterized these progenitors electrophisiologically by patch clamp these and have concluded that SCA3/MJD cells show a profile of slightly more mature neurons than its WT counterparts. In addition, we have combined these methods with immunohistochemical assays which suggest differences in postnatal cerebelar architecture, as well as gene and protein expresión assays or viability assays.
In addition, our research group has a long-lasting experience in the stydy of purinergic system, which is known to have a key role in developement, homeostasis and survival of neuronal populations of the cerebellum[6].Consequently, we have analysed the expresssion and modulation of some components of this purinergic system, as a potential and promising strategy to treat SCA3/MJD.

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Acknowledgements: This work was supported by funding received from the Spanish Ministerio de Ciencia e Innovación (PID2019-109155RB 100).

P7

Effects of cannabinoids on ameliorating affective-like behavior in mice exposed to peripartum depression model

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Major depressive disorder (MDD) is one of the most diagnosed psychiatric disorders, with a rising tendency among women especially after the COVID19 pandemic [1]. The World Health Organization reported that 1 out of 5 women will experience a mental health condition during pregnancy or in the year after giving birth [2]. In this context, peripartum depression (PPD) represents a subtype of MDD with a prevalence of around 20% in women in childbearing years. Along this line, the role of the endocannabinoid system (ECS) in the neurobiology of neuropsychiatric disorders is remarkable since it modulates the stress and anxiety response. Additionally, the neuroendocrine and neuroimmune systems are known to be dysfunctional in individuals with a depressive symptomatology [3], particularly in PPD due to their crucial impact in neurotransmission. The present study aims to target the ECS in a mouse model of PDD, to reduce the negative effects induced by stress and alleviate depressive-like symptoms. For this, we implemented a model of PPD that emulates human conditions which has been well-characterized by our group. The model is characterized by the combination of maternal separation with early weaning (MSEW) which combines both psychological and social stress. Briefly, litters selected to MSEW were daily separated from their mothers for 4 h between postpartum day (PD) 2-5 and for 8 h between PD 6-16. Then, the offspring were weaned prematurely on PD 17. Meanwhile, the offspring of the standard nest group remained undisturbed with their dams until the standard weaning day at PD 21. Two phytocannabinoids were selected as therapeutic options to ameliorate/revert the MSEW-induced depressive-like phenotype on dams. Given their therapeutic potential on treating diverse psychiatric disorders, Δ9-tetrahidrocannabinol (THC) and cannabidiol (CBD) were chosen to treat dams at low and higher dose, respectively. The main results show that (1) untreated dams exposed to MSEW displayed increase anxiety-like behavior. (2) The combination of MSEW and THC did not modulate behavior, but THC in control mice increased immobility in tail suspension test. (3) Based on prior studies indicating the role of CBD in animal models of psychiatric conditions, we hypothesize that CBD will modulate the behavior of the dams exposed to MSEW, possibly reverting its negative affect. This supports the growing literature regarding the potential of the administration of cannabinoids as treatment for improving affective-like behavior in an animal model of PDD.

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Acknowledgements: This work was supported by a grant from the Ministerio de Ciencia, Innovación y Universidades (PID2022-136962OB-100 - MICIU/AEI/10.13039/501100011033 and ERDF/EU), by Ministerio de Sanidad, Delegación del Gobierno para el Plan Nacional sobre and Fondos de Recuperación, Transformación y Resiliencia (PRTR) Unión Europea (#Exp2022/008695). OV is recipient of an ICREA Academia Award (Institució Catalana de Recerca i Estudis Avançats, Generalitat de Catalunya).

P8

Expression of the miR-143/145 cluster changes in RRMS patients, is sexually dimorphic and changes with the use of DMTs.

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Relapsing-remitting multiple sclerosis (RRMS) is a neurodegenerative and autoimmune disease, that is characterized by a clear clinical sexual dimorphism. Although the response to some therapies (DMT) has been studied, much remains to be known. In this context, epigenetics in MS plays one of the most important roles in the pathophysiology and response to DMT. miRNAs are epigenetic regulators that have become very important in the disease and have been proposed as biomarkers in various pathologies. To this end, we extracted total serum miRNA from 80 RRMS patients and 60 healthy volunteers (HC) and performed qPCR analysis using the probes hsa-miR-143-5p and hsa-miR-145-5p as target miRNAs and hsa-miR-320a-3p as endogenous control. Non-parametric analyses were performed. We found that miR-143-5p is upregulated by 1.56-fold in RRMS patients compared to HCs, whereas miR-145-5p is downregulated by 63%. Moreover, we show that miR-143-5p expression can change under different treatments and exhibits sexual dimorphism, while miR-145 expression depends mainly on pathology but can increase over the years. Furthermore, we performed an *in-silico* analysis and found that the major target genes of this cluster belong to the immune system. The present study provides new information on the expression of the cluster. To our knowledge, we are the first to determine the expression level of miR-143-5p in the context of MS. In contrast, miR-145-5p is a miRNA that has been very little studied in the context of DMTs, and sex-specific changes in expression have never been considered. Further studies are needed to determine the exact role in the utilization of DMTs and the possible pathways involved in the sexual dimorphism in the expression of this cluster.

Acknowledgements: This project was supported by CONACyT-Mexico Grant Ciencia de Frontera-2019-552265 to D.O.-S. and by Universidad de Guadalajara grant 269639-PROSNI to D.O.-S.

P9

Psychedelic potential of 25C-NBF: Hallucinogenic effects, antidepressant action, and neural plasticity in mice

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Background: The recent surge in new psychoactive substances (NPS) has brought renewed interest to the therapeutic potential of psychedelics for neuropsychiatric disorders. This study investigated the effects of 25C-NBF, a novel phenethylamine psychedelic, in mice. We aimed to characterize its mechanism of action, hallucinogenic profile, assess its antidepressant properties, and explore potential mechanisms through neural plasticity.
Methods and results: Membrane preparations expressing human 5-HT2AR were incubated with radiolabeled [^{3}H]ketanserin, and ligand displacement by 25C-NBF at different concentrations was measured. Moreover, 5-HT2AR functional assays were performed with CHO/K1 cells expressing h5-HT2AR using the Invitrogen™ Fluo-4 NW Calcium Assay Kit (Thermo Fisher, Waltham, MA, USA). Our results showed nanomolar affinity of 25C-NBF for 5-HT2AR and full agonism at this receptor (Emax ± SD = 93.710 ± 4.92 %5-HT). Male Swiss CD-1 mice (6-11 weeks old) were used for behavioural experiments. The head-twitch response (HTR), a marker of 5-HT2A receptor activation and hallucinogenic effects in rodents, was measured following 25C-NBF administration. We observed a dose-dependent increase in HTR ($n=12$, $p < 0.001$), confirming the hallucinogenic potential of 25C-NBF. To evaluate antidepressant effects, mice were subjected to chronic stress using corticosterone (40 mg/kg) for 21 days. On day 22, they received a single dose of 25C-NBF (10 mg/kg). Antidepressant activity was assessed using the sucrose preference test (SPT). The SPT revealed a significant antidepressant effect of 25C-NBF 24 hours after a single injection ($n=12$; Stressor $p < 0.001$, Treatment $p > 0.05$, Stressor x Treatment $p < 0.05$). Given the potential link between neural plasticity and antidepressant action, we investigated the effects of 25C-NBF on dendritic spine density in the prefrontal cortex (PFC), a brain region implicated in mood regulation. Golgi-Cox staining revealed a significant increase in dendritic spines in both the anterior cingulate cortex (ACC) and prelimbic cortex (PL) 24 hours after 25C-NBF administration ($n=6$; $p < 0.05$).
Conclusion: Our findings demonstrate that 25C-NBF acts as a full agonist at h5HT2AR and exhibits hallucinogenic properties and rapid antidepressant effects in mice. These effects may be mediated, at least in part, by its ability to promote neural plasticity in the PFC. This study contributes to the exploration of novel therapeutic strategies for treatment-resistant depression.

Acknowledgements:
Grant/Other Support: European Union (EU) Home Affairs Funds, NextGenPS project (number: 101045825); MICIU/AEI/10.13039/501100011033 y ERDF/EU (PID2022-137541OB-I00)

P10

Regulation of cyclin-dependent kinase 5 activators p35 and p25 in the posterior putamen of Memory and Aging Project participants.

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The role of the CDK5 activator p25 via p35 cleavage in the pathogenesis of aging and Alzheimer's disease (AD) remains controversial. While most preclinical studies associated p25 overexpression with tau hyperphosphorylation, others reported that p25 formation may be necessary for memory formation and synaptogenesis. Human postmortem brain studies addressing p25/p35 expression levels showed contrasting results. Using cortical samples from 150 participants of the Rush Memory and Aging Project (MAP), we found that lower p25 density was associated with greater risk for AD, poor cognitive outcomes, and low indices of synaptic functionality, including lower fusogenecity of SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) complexes. Preservation of intact SNARE complex functionality was also reported to significantly contribute to cognitive reserve [1]. The present study aimed to extend our knowledge on CDK5/p25 pathophysiology to the posterior putamen (pPut), a brain region particularly degenerated in other age-related neuropathologies such as Parkinson's disease (PD). To this end, CDK5, p35 and p25 protein levels were quantified by Western blotting in pPut samples from 250 MAP participants. Multivariate correlations and analyses of covariance (ANCOVA) surveyed putative associations between CDK5/p35/p25 immunodensities in pPut and cognitive status, burden of neurodegenerative pathologies (i.e., AD and PD), and local levels of several presynaptic proteins (CPLX1/2, STXBP1, SNAP25, STX1, and VAMP). Results showed no correlation between CDK5/p35/p25 densities and cognitive status or AD-related neuropathologies. However, subjects with dementia had higher levels of p35 (+15% $p < 0.01$) compared to subjects with mild cognitive impairment. In addition, MAP participants with PD had greater densities of p35 (+26%, $p < 0.01$) in pPut samples. Additionally, p25 pPut immunodensities were positively correlated with all synaptic proteins examined (from $r = 0.129$, $p < 0.05$; to $r = 0.394$, $p < 0.0001$). In conclusion, while loss of p35/p25 in cortical areas may be disadvantageous for synaptic preservation and cognitive performance in old age, excessive production of p35 in pPut (and perhaps other striatal areas as well) may increase the risk for PD and cognitive impairment. The present findings also support a potentially important role for p25 during aging in safeguarding both synaptic density and functionality, as its loss appears to be a common event in different brain regions and correlates with synaptic protein loss.

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Acknowledgments: This work was supported by grants PID2022-137848OB-I00 (MCIU/AEI/FEDER) and IT1512/22 (Basque Government). EH-H holds grant FJC2022-048338-I, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

P11

Preclinical evaluation of the psychedelic mechanism of action of psilocybin in mice

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Psilocybin has recently been proposed to be efficacious in the treatment of different neuropsychiatric illnesses, such as depressive and anxiety disorders. Extensive evidence supports that acute psychedelic effects of psilocybin are primarily mediated by serotonin 2A receptor (5HT2AR) activation. However, controversy exists on the involvement of the endogenous neurotransmitter serotonin (5-HT) in these actions, and also on the effect mediated by serotonergic receptors different to 5HT2AR. Head-twitch response (HTR) is the best characterized behavioural assay to evaluate psychedelic effects in mice, and shows robust correlation with subjective potency of hallucinogens in humans.

The aim of this study was to evaluate modulatory mechanisms involved in psilocybin-induced HTR in mice, focusing on the role of endogenous 5-HT and serotonin 1A receptor (5HT1AR).

Adult male wild-type (WT) C57BL/6J mice (8 weeks old) were used. Pharmacological treatments consisted of: citalopram (20 or 40 mg/kg i.p.), or 8-OH-DPAT (0.1 or 1 mg/kg i.p.) or vehicle, administered 30 minutes prior to psilocybin (1 mg/kg i.p.) or saline. For 5-HT depletion, p-chlorophenylalanine (PCPA, 400 mg/kg i.p.) was administered 24 h before psilocybin (1 mg/kg) or saline. Subsequent to psilocybin administration, HTR was evaluated for 20 minutes. Immediately after HTR assessment, mice were euthanized and brain cortices were harvested and processed for noradrenaline (NA), dopamine (DA) and 5-HT content determination by HPLC. HTR data were analysed by two-way ANOVA followed by Bonferroni *post hoc* test. Monoamine content determination data were analysed using unpaired *t* test and simple linear regression was used to analyse correlation between monoamine content and HTR.

Citalopram pre-treatment dose-dependently attenuated psilocybin-induced HTR (20 mg/kg: $t=2.88$, $p=0.055$; 40 mg/kg: $t=10.72$, $p<0.0001$). On the contrary, 5-HT depletion by PCPA augmented psilocybin-induced HTR ($t=8.67$, $p<0.0001$). PCPA pre-treatment selectively reduced cortical 5-HT concentration (right ctx: $t=4.72$, $p<0.01$; left ctx $t=4.03$, $p<0.01$) but not NA or DA. Moreover, a significant inverse correlation was found between cortical 5-HT and HTR elicited ($F(1,13)=11.95$, $p<0.01$). 8-OH-DPAT pre-treatment attenuated psilocybin- induced HTR only at the highest dose administered (1 mg/kg) ($t=11.15$, $p<0.0001$).

In conclusion, endogenous 5-HT exerts an inhibitory effect on psilocybin-induced HTR. This effect may be related to competitive mechanisms between 5-HT and psilocybin at 5HT2AR, or alternatively be mediated by activation of 5HT1AR. Care should be taken regarding pharmacological interactions between psilocybin and antidepressant drugs whose mechanism of action targets synaptic 5-HT concentration or 5HT1AR.

Acknowledgements:
Funded by Grant PID2021-123508OB-I00 funded by MICIU/AEI/ 10.13039/501100011033 and Basque Government (IT- 1512/22). I.E-S. and N.M-A. received a predoctoral fellowship from the UPV/EHU (PIF19/308) and the Basque Government (PRE_2022_1_0256), respectively.

P12

Cofilin mediates methamphetamine-induced neurotoxicity

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Metamphetamine (Meth) is a drug of abuse that causes neurological deficits and nigrostriatal damage similar to Parkinson's disease, characterized by mitochondrial dysfunction and oxidative stress (1). Cofilin is an actin depolymerising factor that plays a central role in severing actin filaments and promoting actin dynamics. In the central nervous system, cofilin is involved in growth axonal transport (1) and cell cycle control in the cerebral cortex (2), but also in mediating neuronal apoptosis by promoting the release of Bax proapoptotic following its translocation to mitochondria in response to excitotoxic stimulus (3).

In the present work we have studied the role of cofilin in Meth-induced neuroblastoma cells as a model of Parkinson's disease-like cells.

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 reactive oxygen species (ROS) production by fluorescence microscopy; the levels of glutathione spectrophotometrically, the activation of the intrinsic apoptotic pathway and the activity of caspases -3,-9 by fluorometry, the ratio of phosphorylated/dephosphorylated cofilin and its intracellular location by western blot, and its interaction by different proapoptotic proteins by co-immunoprecipitation assays. Finally, we determined the involvement of different phosphatases in the cofilin activation by using non-specific and specific-pharmacological inhibitors.

We found that Meth induced neuroblastoma cell-death in a time- and concentration-dependent manner, increased mitochondrial ROS production and depleted glutathione intracellular levels activating the intrinsic apoptotic pathway. Moreover, cofilin was activated by dephosphorylation and translocated from mitochondrial membrane to cytosol accompanying cytochrome c releasing. The slingshot inhibitor D3 prevented cofilin activation and Meth-induced neuroblastoma cells suggesting that this is the phosphatase mainly involved in cofilin activation in response to Meth toxicity in neuroblastoma cells.

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P13

Thrombospondin-1 as a potential new biomarker in traumatic brain injury

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Traumatic brain injury (TBI) is the second most common cause of acquired brain injury in adults. It is estimated that in Spain, there are a total of 420,000 individuals with this type of injury, with an annual incidence of 100,000 new cases. TBI is caused by an external force, and its consequences have a wide-ranging impact on the quality of life of affected individuals. Predicting the medium to long-term evolution of a TBI patient is a challenging task, prompting various research groups to focus on the search for biomarkers to obtain key information in this regard. Considering the above, this study focuses on investigating the role of thrombospondin-1 (TSP-1) as a potential biomarker in the serum of TBI patients. The choice of TSP-1 is based on previous observations indicating that TSP-1 KO animals exhibit increased barrier breakdown following trauma, suggesting an important role in TBI pathophysiology.
Our data show that TSP-1 decreases in all TBI patients compared to control patients, regardless of the severity of the trauma. However, when analyzing these data in relation to the prognosis at 6 months post-injury, a significant difference was observed at 1-day post-TBI.
Furthermore, the role of TSP-1 was examined in a murine model of TBI. The results revealed that TSP-1-deficient animals exhibited increased BBB integrity loss and exacerbated inflammation. This exacerbation could be attributed to basal astrocytic activation in these animals, potentially triggering an exaggerated response to brain damage. TSP-1-deficient animals were observed to have apparent dysregulation of the vasculature at baseline, which may explain the increased BBB disruption following TBI. These findings suggest a protective role for TSP-1 in preserving BBB integrity and modulating the inflammatory response following trauma.
Finally, an N-terminal fragment of TSP-1 was employed, which has shown in vitro to increase vessel formation and eNOS phosphorylation. Additionally, in our in vivo TBI model, this fragment has shown to protect against BBB disruption in WT animals. These results suggest a therapeutic potential for the N-terminal fragment of TSP-1 to preserve BBB integrity after TBI.

Acknowledgements: Este estudio está financiado por la Fundación Mutua Madrileña, por el Fondo de Investigaciones Sanitarias (FIS) (ISCIII/FEDER) (PI22/00362).

P14

Regulation of groups i and iii metabotropic glutamate receptors in the prefrontal cortex of schizophrenia subjects: a postmortem brain study

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Protein densities of group II metabotropic glutamate receptors (mGluR2 and mGluR3) were reduced in postmortem samples of the dorsolateral prefrontal cortex (DLPFC) of subjects with schizophrenia (SZ). These alterations were apparently exacerbated (mGluR2) or reversed (mGluR3) by antipsychotic (AP) medication[1]. Other mGluRs have also been proposed as promising targets for novel AP drug development[2]. This work further evaluated gene and protein expression levels of group I (mGluR1 and mGluR5) and group III (mGluR4 and mGluR7) mGluRs in SZ postmortem brain. Postmortem samples from the DLPFC (Brodmann's area 9) of age-, sex-, and postmortem delay-matched pairs of SZ cases and control subjects (n=21 pairs) were obtained at autopsies in the Basque Institute of Legal Medicine. SZ subjects were classified as AP-positive (n=10) or AP-free (n=11) according to the presence or absence of APs in blood by the time of death. Protein immunodensities of group I mGluR1 and mGluR5, and group III mGluR4 and mGluR7 were quantified by Western blot (WB) using selective antibodies. Gene expression of *GRM4*, *GRM5* and *GRM7* was measured by reverse transcription quantitative PCR (RT-qPCR) in 19 SZ-control pairs. Data were analyzed by paired (WB) and unpaired (RT-qPCR) *t*-test.

WB experiments detected specific immunoreactive bands for mGluR1a (~150 kDa), mGluR5 (~150 kDa), mGluR4 (~100 kDa), and mGluR7 (~90 and ~100 kDa) in human postmortem brain samples. Density of 100 kDa mGluR4 was significantly lower in SZ subjects (-28%, $p<0.05$) than in matched controls. Similarly, immunodensities of both 90 and 100 kDa species of mGluR7 were dramatically downregulated in SZ subjects (-64%, $p<0.001$ and -31%, $p<0.05$; respectively). Subgroup analyses in AP-positive and AP-free SZ subjects revealed that mGluR7 downregulations of the 90 and 100 kDa protein species were mainly attributed to AP exposure (-71%, $p<0.001$ and -43%, $p<0.05$; respectively), whereas similar effects on mGluR4 were observed across both SZ subgroups. Gene expression levels of *GRM4* and *GRM7* were not significantly altered in SZ subjects, regardless of AP medication. No significant differences were observed in the immunodensities of mGluR1a or mGluR5, or in *GRM5* gene expression levels, between SZ subjects and their matched control subjects, regardless of AP medication. Similar to group II (mGluR2/3), protein densities of group III (mGluR4/7), but not group I (mGluR1a/5) mGluRs were reduced in SZ subjects. Lower densities of group III mGluRs may not be a result of transcriptional downregulation, as no changes in gene expression were observed across groups of subjects. In turn, SZ-related cortical downregulation of mGluR7 protein density may be associated with AP treatment.

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Acknowledegments: This work was supported by grants PID2022-137848OB-I00 (MCIU/AEI/FEDER) and IT1512/22 (Basque Government), PRE_2022_1_0075 (Basque Government to JAS) and PIF19/306 (UPV/EHU to OMP). The authors report no conflict of interest.

P15

Evaluating the antidepressant-like potential and neurochemical effects of cannabidiol in adolescent male and female rats

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Although cannabidiol has the potential to be a novel antidepressant for the treatment of adolescent depression, recent preclinical studies have demonstrated that its efficacy depends on some variables, such as biological sex, age and dose [1,2]. In fact, most of the prior studies on this topic have focused mainly on adult male rodents, plus, the mechanism of action by which this drug exerts its antidepressant effect is not yet elucidated. In this context, our group has recently shown that a dose of 10 mg/kg of cannabidiol induced an acute [1] and sustained antidepressant-like response in adolescent male rats [1,2]. This follow-up study aimed at evaluating the potential antidepressant-like response of higher doses of cannabidiol (30, 60 mg/kg) in adolescent rats of both sexes, to better understand female unresponsiveness, as well as the changes taking place in hippocampus (i.e., cell proliferation and a brain marker associated with antidepressant-like responses: BDNF). To do so, adolescent Sprague-Dawley rats of both sexes were treated with cannabidiol (30 or 60 mg/kg) or vehicle (DMSO, 1 ml/kg) for 7 consecutive days (i.p., n=7-8 per group/sex). Acute (30 min post-first injection) and repeated (24 h post-treatment) antidepressant-like responses were measured under the stress of the forced-swim test. In parallel, groups of rats were also treated with the same administration paradigm (cannabidiol 30 or 60 mg/kg vs. DMSO, 1 ml/kg, n=5-7 per group/sex/time of study; 1 vs. 7 days, i.p.). Brains were collected 30 min post-first injection or 24 h post-repeated treatment. While BDNF regulation by western blot was assessed at both time points, the study of cell proliferation by immunohistochemistry using Ki-67 as a marker was only evaluated following the repeated treatment. Data was analysed with two-way ANOVAs in which Sex and Treatment were the independent variables at each time point of study. Together with our prior study proving efficacious effects with the dose of 10 mg/kg in male adolescent rats [1], the main results showed a lack of acute and/or repeated antidepressant-like responses of the higher doses tested (30 and 60 mg/kg) for both sexes (no significant Treatment x Sex interactions). This lack of antidepressant-like response paralleled the absence of regulation of the markers evaluated (no significant Treatment x Sex interactions). In conclusion, together with our prior data [1,2], the present results extend the lack of efficacy of cannabidiol for adolescent female rats. This lack of response was observed both at the behavioural and neurochemical level. Moreover, our data proved that the effects of cannabidiol on male adolescent rats are dose-dependent since only the dose of 10 mg/kg proved to be efficacious [1,2]. Further experiments are needed to find efficacious doses for adolescent female rats and to better characterize the molecular mechanisms behind its actions.

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P16

Combination of repurposed drugs as a new approach for the treatment of amyotrophic lateral sclerosis

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Neurodegenerative diseases (NDD) are among the most studied pathologies in the present century and constitute one of the biggest challenges in medicine. Latest studies have estimated around 57.4 million cases worldwide by 2019 and expect an increase of up to 152.8 million cases by 2050[1]. Amyotrophic lateral sclerosis (ALS) is a progressive and heterogeneous neurodegenerative disease that appears in adult life and inevitably leads to death within 3 to 5 years after diagnosis. Currently, only two treatments are available in the market for ALS patients, riluzole and edaravone, but none of them can prolong patients' lives for more than three months[2]. Therefore, it is of great medical interest to find new treatments for this pathology. Moreover, single target-directed therapies have failed to provide clinical benefits for ALS patients. Consequently, the combination therapy, which is already established as the primary approach for treating complex diseases like cancer or human immunodeficiency viruses (HIV), has become a promising strategy for finding new treatments for NDD[3].

Our main objective in this project is to find a combination of clinically approved medicines to target different pathways in the pathophysiology of ALS (i.e., calcium-mediated excitotoxicity, mitochondrial dysfunction due to oxidative stress, and P2X7 receptor-mediated neuroinflammation) and boost neuroprotection. To do so, we conducted an animal study on the $SOD1^{G93A}$ mouse model of ALS. Animals were treated with a combination of three drugs, named "The triad", at pre-symptomatic stages (postnatal day 60). During the course of the study, animals were subjected to different tests, including motor coordination, general neurological status, and weight loss, to follow the influence of the treatment on disease progression. Samples of the brain (cortex, cerebellum, and hippocampus) and the spinal cord were analyzed using different techniques to conduct a neuropathology evaluation of several markers like immunoreactive choline-acetyltransferase (ChAT), Iba-1, GFAP, and overexpression of P2X7 receptor.

Our preliminary results by using a combination of memantine (blocker of NMDA receptors; to target excitotoxicity), procyclidine (blocker of muscarinic receptors, indicated in the treatment of extrapyramidal symptoms in Parkinson's disease; to target: mitochondrial oxidative stress) and prochlorperazine (dopaminergic D2 receptor antagonist widely used in the prevention and symptomatic control of nausea, vomiting, vestibular and psychiatric disorders; selected for its ability to block the P2X7R) showed that these compounds are well tolerated, however, the administration of the triad was unable to improve phenotypic outcomes (loss of body weight, motor and neurological scores) when administered ip from P60 to P180.

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Acknowledgements Funded by Ministerio de Ciencia, Innovación y Universidades (PID2020-117127RB-I00), and Juan de la Cierva postdoctoral contract, (Ref: JDC2022-049555-I).

P17

The E193K LRRK2 variant regulates autophagy in Parkinson´s disease

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Parkinson's disease is a neurodegenerative condition clinically characterized by movement-associated symptoms such as tremor, muscle stiffness and impaired balance. PD is mainly related to dopamine deficiency in the striatum due to selective loss of dopaminergic neurons in the substantia nigra pars compacta. In this scenario, autophagy is an essential neuronal mechanism to restore homeostasis. Aetiology of PD is complex due to the interaction between ageing, genetic and environmental factors. Different gene variants are linked to PD, being the mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene one of the most frequent causes. LRRK2 is a complex protein that consists of 7 domains, including an Armadillo domain (ARM) at the N-terminal part of the protein. E193K variant, which is located at the ARM domain, modifies LRRK2 protein folding and LRRK2 interactome by affecting both mitochondrial dynamics and proper vesicle trafficking[1]. In this study we analyzed the impact of E193K variant on autophagy regulation. We found that basal autophagy is increased in primary fibroblast obtained from a E193K carrier compared to a healthy subject. We also found that E193K mutation modifies cellular toxicity upon 1-methyl-4-phenylpyridinium exposure and LRRK2 binding to Dynein-1 complex, an essential protein adaptor of retrograde transport of autophagosomes. In conclusion, our data demonstrate a crucial role of LRRK2 as a scaffolding protein controlling autophagy pathway.

Acknowledgements:
The authors acknowledge the excellent technical support from Vanesa Guijarro. This research was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and project PID2020-120134RB-I00 (MCIN/AEI/10.13039/501100011033)to V.C. and project SBPLY/19/180501/000060 funded by JCCM to M.D.P-C.

P18

Study of the interaction between human pericytes and endothelial cells in the neurovascular unit for maintaining blood-brain barrier integrity in cerebral ischemia

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Acute brain injuries, including stroke and traumatic brain injury, are leading causes of disability worldwide. Ischemic stroke in particular accounts for 80-85% of all stroke cases and is characterized by a reduction in blood flow to specific regions of the brain, triggering an inflammatory response and subsequent cell death. A key aspect of this process is the disruption of the blood-brain barrier (BBB), leading to the loss of function of the neurovascular unit. This impairment can cause irreversible damage and worsen the prognosis of stroke patients. One key player in this inflammatory response is the activation of the NLRP3 inflammasome, a multiprotein complex responsible for the production of pro-inflammatory cytokines (IL-1β and IL-18).

Our research group hypothesizes that protecting the BBB and reducing the post-ischemic inflammatory response is crucial for maintaining communication and function within the neurovascular unit, thereby minimizing inflammation-induced brain damage.

We aim to investigate the interaction between pericytes and endothelial cells, two important components for maintaining the permeability of BBB after cerebral ischemia. For this purpose, we used two models:
1. An *in vitro* model, where we developed a protocol to form microvasculature in a three-dimensional culture and subject it to hypoxia to simulate cerebral ischemia.

2. An *in vivo* model, using a mouse model of transient middle cerebral artery occlusion (tMCAO) with intraluminal filament. The occlusion is performed for one hour and BBB disruption is observed after 4 and 24 hours of reperfusion in wild-type (WT) and NLRP3 knockout (KO) animals.

Using the *in vivo* model, we aim to visualize the physical and functional interactions between pericytes and endothelial cells after the ischemic process in both WT and NLRP3 knockout mice by immunofluorescence with specific markers for both cell types. Using the *in vitro* model, we aim to reproduce cerebral ischemia and evaluate whether different treatments can achieve similar results as in the *in vivo* model.

Acknowledgements:
This study has been funded by Ministerio de Ciencia, Innovación y Universidades (Consolidación Investigadora CNS2023-145023), Fondo de Investigaciones Sanitarias (FIS) (ISCIII/FEDER) (PI22/00362) and Fundación Mutua Madrileña

P19

MicroRNA-30c-5p as an etiopathogenic agent, therapeutic target and biomarker of oxaliplatin-induced peripheral neuropathy

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Chemotherapy-induced peripheral neuropathy (CIPN) is the most frequent complication of cancer treatment. CIPN is a complex pain syndrome that includes sensory symptoms, autonomic, and motor dysfunction. CIPN manifestations are often highly refractory to current analgesics, impacting the function and quality of life of patients [1]. MicroRNAs (miRNAs) are small noncoding RNAs that modulate post-transcriptionally gene expression. Previous results of our group support a major role for miR-30c-5p in neuropathic pain development after traumatic nerve injury [2]. Our translational study aims to investigate the potential value of microRNA-30c-5p as an etiopathogenic agent, therapeutic target and biomarker of peripheral neuropathy induced by oxaliplatin, an antineoplastic widely used for the treatment of cancer. The study was performed in female and male Sprague Dawley rats and a cohort of colorectal cancer patients. CIPN was induced in rats by five oxaliplatin intraperitoneal injections (4mg/kg) on alternate days. Mechanical and thermal allodynia were assessed with von Frey and acetone tests. Plasma samples were obtained under basal conditions and on day 14 after oxaliplatin administration when maximal neuropathic pain-related behaviours were evident. To determine the functional role of miR-30c-5p on pain development, oxaliplatin-treated rats received five injections of miR-30c-5p inhibitor (200 ng/10 ul) or mismatch inhibitor into the cisterna magna on days 0 (start of the treatment), 2, 5, 7 and 9 of oxaliplatin treatment, and mechanical allodynia was evaluated. Plasma samples from patients with colorectal cancer were collected before starting the oxaliplatin treatment, and each time a patient was administered a new cycle. All plasma samples were processed for miR-30c-5p quantification by qPCR.
Our results showed that oxaliplatin-treated rats developed a sex-independent mechanical allodynia after two weeks of treatment ($p<0.001$), and a sex-dependent thermal allodynia, that lasted 4 weeks ($p<0.05$). Furthermore, the response intensity was significantly higher in treated female rats compared to male rats ($p<0.05$). Pharmacological treatment with miR-30c-5p inhibitor significantly delayed the onset of mechanical allodynia. Both female and male oxaliplatin-treated rats exhibited a significant up-regulation of miR-30c-5p expression in plasma. Patients who developed peripheral neuropathy after treatment with oxaliplatin presented significantly higher plasma expression of miR-30c-5p compared to baseline values. Our findings suggest a potential value of miR-30c-5p as a biomarker and therapeutic target for treating chemotherapy-induced neuropathic pain, both in experimental models and in patients.

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Acknowledgements: Supported by PID2022-136418OBI00/AEI/10.13039/501100011033/ FEDER, UE and IDIVAL (INN- VAL 23/12).

P20

Receptors with implications in opioid tolerance: characterization of the μ- opioid-dopamine 1 receptor heteromer

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Chronic pain in Europe is a major health issue, compounded by the opioid crisis due to the widespread use of opioids for managing severe pain[1]. While opioids alleviate pain by binding to the mu opioid receptor (MOR), they also have adverse effects, such as tolerance and addic- tion. MOR can form higher-order complexes known as heteromers, which modify its func- tions. Of particular interest is the MOR-dopamine D1 receptor (D_1R) heteromer[2], potentially contributing to adverse effects like opioid tolerance and abuse liability. Therefore, our objec- tives involve elucidating the localization, pharmacology and function of the MOR-D_1R heter- omer. We first investigated the possibility of intermolecular interactions between MOR with D_1R using BRET assays. Saturable BRET curves were observed in transfected cells, demon- strating heteromer formation *in vitro*. Furthermore, *ex vivo* evidence of the complex in mouse brain striatum was obtained using proximity ligation assays. Following this, we investigated its fingerprint, starting with competition radioligand binding assays using [^{3}H]-naloxone *ver- sus* fentanyl. Sheep striatum samples were treated with SKF38393 or SCH23390, D_1R agonist and antagonist, respectively. The results suggested negative allosteric modulations in native tissue, as evidenced by changes in K_{DB1}, K_{DB2}, and K_{DAB}. Additionally, cAMP accumulation assays were conducted to examine secondary signaling effects upon receptor activation in transfected cells. Anticipating an increase in cAMP levels upon D_1R activation and a decrease upon MOR activation due to their coupling to Gαs and Gαi subunits, respectively, we ob- served that endomorphin-1 reduced cAMP levels induced by SKF38393, indicating negative crosstalk at the adenylyl cyclase (AC) level. Additionally, we observed bidirectional cross- antagonism between MOR and D_1R, suggesting their proximity and potential oligomer for- mation influencing the same AC activity. Literature supports that chronic D_1R blockade post- spinal nerve ligation preserves MOR responsiveness to opiate drugs, preventing tolerance[3]. The knowledge acquired in this study, particularly when leveraging cross-antagonism, will provide valuable insights for designing improved chronic pain treatments.

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Acknowledgements:
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P21

Evaluation of serotonin 2A receptor expression and function following psilocybin administration in mice

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The psychedelic 5-HT2A receptor (5HT2AR) agonist psilocybin has recently been posited as effective for the treatment of various neuropsychiatric illnesses, including mood disorders. Several clinical trials have proven that administration of single or multiple hallucinogenic doses of psilocybin induce robust reductions in depressive symptoms and anxiety. Acute psychedelic effect is mediated by 5HT2AR activation in brain cortex and can be measured in rodents by head-twitch response (HTR), a behavioural proxy of acute psychedelic activity. However, long- term molecular fingerprints of psilocybin in rodent brain cortex are undeciphered and could be associated to persisting behavioural effects that outlast the drug's presence in the organism.

The present study aimed to (1) characterise the acute psychedelic effect of psilocybin in animals with partial or total 5HT2AR deletion and (2) evaluate long-term effect of single or double administration of the drug on the expression of 5HT2AR.

Adult (8 weeks old) C57BL/6J wild-type (+/+) (WT), 5HT2AR heterozygous (−/+) (HET) and knockout (−/−) (KO) mice were used. Single dose or double dose (7 days apart) of systemic psilocybin (1 mg/kg, i.p.) or saline was administered, and HTR was evaluated for 20 minutes. Fourteen days after last administration, brain cortices were extracted for assessment of 5HT2AR mRNA and protein expression trough quantitative PCR (qPCR) and Western blot. Data were analysed using one-way ANOVA or unpaired or paired *t* test.

Psilocybin (1 mg/kg, i.p.)-induced HTR was significantly influenced by genotype, with decreased number of HTR elicited in WT (17.88±2.61) vs HET (9.71±1.41) vs KO (0.75±0.31) mice ($F=24.99$, $p<0.0001$). Single administration of psilocybin in WT mice induced 20.14±1.51 HTR, and subsequent to second administration, 25.21±1.47 HTR were observed. Significant difference was revealed in HTR induced by first vs second administration ($t=3.11$, $p<0.01$). No significant differences were found in cortical 5HT2AR expression after single psilocybin vs saline administration ($t=0.40$ $p=0.70$). However, increased levels of *ht2ar* mRNA ($t=2.46$, $p<0.05$) and 5HT2AR protein expression ($t=2.27$, $p<0.05$) were found following two systemic psilocybin administrations compared to saline.

The present data confirms correlation between 5HT2AR expression and psychedelic-like acute effects of the drug. Moreover, repeated administrations of psilocybin are able to induce an increase of 5HT2AR expression in brain cortex accompanied by functional hypersensitivity, suggesting long-term modulatory effects in the receptor's dynamics. Such modifications could potentially be linked to the long-lasting therapeutic effects of psilocybin.

Acknowledgements: Supported by Grant PID2021-123508OB-I00 funded by MICIU/AEI/ 10.13039/501100011033 and by Basque Government (IT-1512/22). I.E-S. and N.M-A. received a predoctoral fellowship from the UPV/EHU (PIF19/308) and the Basque Government (PRE_2022_1_0256), respectively.

P22

Neuropathic pain induces time-dependent changes in the Locus Coeruleus and stress-related behaviors in a sex-specific manner

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Chronic pain often coexists with stress-related disorders such as anxiety and depression, affecting the quality of life for 20-30% of patients (Dueñas et al., 2016; De la Rosa et al., 2024), with a higher prevalence in women (Bartley & Fillingim 2013). While the neurobiological mechanisms remain unclear, emerging evidence suggests that the sexually dimorphic noradrenergic Locus Coeruleus (LC) is a crucial hub in this comorbidity (Bangasser et al., 2016; Llorca-Torralba et al., 2019). The modulation of noradrenergic activity is a crucial mechanism of action for drugs with analgesic and/or antidepressant effects. Hence, we aim to investigate the changes induced by neuropathic pain in the LC, and how its chemogenetic modulation contributes to sensory, anxiety, and depressive-like behaviors, focusing on sex differences.

Pain sensitivity, cognition, fear conditioning and anxiodepressive-like consequences of neuropathic pain (Chronic Constriction Injury, CCI) were characterized in adult male and female C57BL/6J mice at different time points after nerve injury: short-term CCI (2/3w) and long-term CCI (7/11w). We also explored the number of noradrenergic LC cells, their somato- dendritic volume, as well as the electrophysiological properties of LC neurons. In addition, we evaluated the effect of chemogenetic DREADD-mediated inhibition of LC neurons on the sensory and emotional components of neuropathic pain by using TH-Cre transgenic mice.

We found that nerve injury led to sensory hypersensitivity from the onset of the injury, while depressive-like behaviors and cognitive impairment emerged at long-term CCI, similarly in both sexes. Interestingly, anxiety-like behavior and increased fear conditioning were observed exclusively in long-term CCI males. The onset of the emotional impairments temporally coincided with structural and functional alterations in the male LC, including an increased number of noradrenergic cells, larger somato-dendritic volume, and increased electrophysiological excitability in patch clamp recordings, changes not observed in females. Additionally, the chemogenetic blockade of the LC induced analgesia in both sexes, but alleviated anxiodepressive-like behaviors and fear conditioning specifically in male CCI mice. Our results suggest that neuropathic pain induces time-dependent changes in the LC, leading to alterations in stress-related behaviors in a sex-specific manner. These findings highlight the significance of elucidating sex-dependent alterations in the LC in pain and stress related disorders, with the goal of developing sex-specific personalized treatment.

Acknowledgements: "Fondo Europeo de Desarrollo Regional-UE, A way to build Europe" from "Ministerio de Economía y Competitividad" (PID2022-142785OB-I00; PDC2022-133987-I00); "Consejería de Salud y Familias, Junta de Andalucía" (PI- 0134-2018); "Consejería de Transformación Económica, Industria, Conocimiento y Universidad, Junta de Andalucía" (P20_00958 and CTS-510); "Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz-INiBICA" (LI19/06IN- CO22; IN-CO9); "CIBERSAM" CIBER-Consorcio Centro de Investigación Biomédica en Red (CB07/09/0033), Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación; European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement Nº 955684; Red Española de Investigación en Estrés/Spanish Network for Stress Research RED2022-134191-T financed by MCIN/AEI /10.13039/501100011033; Grant PRE2019-091106 and grant PTA2021-019890-I funded by MICIU/AEI/10.13039/501100011033 and FSE+".

P23

Alterations in brain Akt/mTOR/S6 signaling pathway and transcriptomic fingerprint after maternal immune activation and adolescent cannabis exposure in rodents

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Schizophrenia usually develops in late adolescence or early adulthood. Its causes are linked to a mix of genetic and environmental factors that exist before the disease occurs. Maternal immune activation during pregnancy has been identified as a trigger that increases the risk of developing schizophrenia. Similarly, cannabis use during early adolescence, a time of increased susceptibility, increases also the risk for schizophrenia in genetically predisposed individuals. In this way, the Akt/mTOR/S6 signaling pathway has been implicated in the impact of cannabis on this disease. In this context, we conducted a study involving a transcriptomic assessment and the evaluation of the Akt/mTOR/S6 signaling pathway in a "double-hit" rodent model of schizophrenia-like symptoms.

The "double-hit" model consisted of inducing a prenatal neurodevelopmental deficit through maternal immune activation (MIA) during pregnancy, followed by administration of THC during puberty. Specifically, female mice were administered poly(I:C) (PIC) (5 mg/kg i.p) or its vehicle on gestation day
9.5. After weaning on postnatal day 21, the mice received either THC or its vehicle (10 mg/kg/day i.p.) for 30 days. Following a 5-day withdrawal period, mice were euthanized, and RNA from the brain cortex was extracted for sequencing library preparation using the NEBNext Ultra II Directional RNA Library Prep kit for Illumina. Subsequently, samples were sequenced on Novaseq6000 (Illumina) using single- read sequencing with a read length of 101 nucleotides. Differential expression analysis was performed using the CUFFDIFF tool, while Metascape tool was employed to analyze the enrichment of specific gene ontologies for each experimental group. Additionally, to examine the signalling pathway, we measured the levels of Akt, ERK, mTOR, S6, and their phosphorylated forms in the mice's brain cortex using the AlphaLISA® technique.

Transcriptomic results revealed several groups of genes differentially expressed in every experimental group, most of them involved in the regulation of behaviour, learning and synaptic transmission. Furthermore, when assessing Akt/mTOR/S6 signaling pathway, in AlphaLISA® assays, no significant differences were found in the phosporilation ratio of Akt, ERK and mTOR proteins. However, in the case of phospho(Ser235/236)-rpS6 / rpS6 ratio protein, after conducting Bonferroni's post-hoc test, a significant decrease (-35.54%) was observed in the Poly(I:C)/vehicle group when compared to the saline/vehicle group (saline/vehicle: 1.64 ± 0.18; Poly(I:C)/vehicle: 1.05 ± 0.07, p=0.0009). Additionally, a decrease (-31.75%) was noted in the saline/THC group in comparison to the saline/vehicle group (saline/vehicle: 1.64 ± 0.18; saline/THC: 1.12 ± 0.08, p=0.0031). Furthermore, a decrease (-40.85%) was observed in the Poly(I:C)/THC group when compared to the saline/vehicle group (saline/vehicle: 1.64 ± 0.18; Poly(I:C)/THC: 0.97 ± 0.07, p=0.0001). All these differences were irrespective of sex. In summary, this model has shed light on the multifaceted nature of the disease, revealing changes not only in transcriptomic testing, but also in the Akt/mTOR/S6 signaling pathway. These findings suggest that both genetic predisposition and chronic THC exposure contribute to an alteration in the normal development of these animals.

Acknowledgments: The authors declare they have no conflicts of interest. This study was funded by the Spanish Ministry of Science and Innovation (PID2019- 106404RB-I00), Spanish Ministry of Health (PNSD 2019I021), and Basque Government (2019111082, and IT1512/22)

P24

Electroconvulsive seizures minimize voluntary ethanol consumption in adult rats caused by a prior adolescent exposure

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Binge drinking has become a prevalent form of alcohol misuse, particularly among adolescents. Adolescence is considered a critical period for brain development, and early alcohol initiation has been identified as a factor increasing vulnerability to long-term alcohol-related issues [1]. Previous studies performed in our research group demonstrated that early ethanol exposure was a risk-factor for later developing an increased voluntary ethanol consumption in adult male and female rats [2]. Therefore, it is crucial to explore therapeutic options to mitigate the long-term negative effects of early ethanol exposure. In this line, neuromodulation therapies such as electroconvulsive seizures (ECS) have shown significant promise for treating certain neuropsychiatric disorders [3], but the effectiveness on treating substance use disorders remains unclear. The present study aimed to evaluate ECS as a potential treatment intervention to mitigate long-term increased ethanol consumption following an adolescent drug exposure, while considering a sex perspective. A total of 33 Sprague-Dawley rats (15 males, 18 females) were used (male saline n=8, male ethanol n=7; female saline n=10, female ethanol n=8). Initially, we examined how adolescent ethanol (2 g/kg twice at 48-hour intervals over 3 cycles, from postnatal days 22-38) influenced voluntary ethanol consumption (20% ethanol vs. water, using a two-bottle choice model, for 3 consecutive days of ethanol access; [2]) in adulthood after 1 month of forced abstinence. Subsequently, all rats received ECS treatment once daily for 5 consecutive days (95 mA for 0.6 seconds at 100 Hz with a pulse width of 0.6 ms) [4]. One week following the intervention, their voluntary ethanol consumption was reassessed to determine the potential effects of ECS on voluntary intake. The findings validated that adolescent ethanol exposure led to increased voluntary ethanol preference in adulthood (two- way ANOVA detected a main effect of ethanol history; $F_{1,29}=17.33$; ***$p<0.0001$) similarly for both sexes. Additionally, ECS treatment significantly reduced voluntary ethanol preference in both male and female rats with a previous adolescent ethanol history (two-way ANOVA no longer detected a main effect of ethanol history; $F_{1, 29}=4.113$; $p>0.05$, n.s.). Overall, this work validated that early ethanol exposure during adolescence has lasting effects on voluntary ethanol consumption in adulthood. Moreover, ECS treatment in adulthood prior to early ethanol exposure was able to mitigate these adverse effects. The results underscored the susceptibility of adolescents to drug exposure and suggested that ECS might be a promising intervention to reduce long-term alcohol-related issues both in males and females.

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Acknowledgements: Delegación del Gobierno para el Plan Nacional sobre Drogas (grant 2020/001, Ministerio de Sanidad, Spain). Spanish Ministry of Universities and the University of the Balearic Islands through the Beatriz Galindo program (BG22/00037).

P25

Optimizing the dose-intensity needed for electroconvulsive seizures to induce an antidepressant-like effect in adolescent male and female rats

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The induction of electroconvulsive seizures (ECS) is a safe antidepressant option for treatment- resistant depression in adolescence, however further research is needed in terms of better characterizing the observed sex-differences in efficacy. In our preliminary work, we compared the antidepressant-like response of ECS (95 mA for 0.6 s at a frequency of 100 Hz square wave pulses, pulse width 0.6 ms, 1 shock/day, 5 days) in adolescent rats of both sexes, proving females to be unresponsive to the evaluated parameters [1]. Later on, and advised by the clinical literature suggesting that lower intensity-doses were needed in females to observe efficacy, we demonstrated that lowering the pulse used from 95 mA to 75 or 55 mA was enough to induce an antidepressant-like effect in adult female rats, while still no beneficial response was detected for adolescent female rats [2]. The current follow-up study aimed at lowering even more the dose-intensity used of ECS to try to observe certain efficacy in adolescent female rats. To do so, adolescent male and female Sprague-Dawley rats were exposed to even lower dose- intensities of ECS (35, 45 mA) as compared to the intensity that proved efficacy in adolescent male rats (95 mA) [1]. The other parameters were kept as in previous experiments (0.6 s, 100 Hz, 1 session/day, 5 days) [1,2]. Control rats were exposed to SHAM-treated conditions. Antidepressant-like responses were evaluated under the stress of the forced-swim test 1-, 3-, and 7-days post-treatment. Reduced immobility during the 5-min test paired with increased active behaviours (climbing and/or swimming) were indicative of an antidepressant-like response. Given the expected sex-differences in efficacy, two-way ANOVAs (independent variables: Treatment and Time of Analysis) were used to analyse the response for each particular sex across time. Besides the expected efficacy induced by the dose of 95 mA 1-day post-treatment (-45±17 s, **p*=0.026 vs. SHAM), no other effects were observed in male adolescent rats, in terms of the lower doses tested and/or time of analysis. Interestingly, the lower doses tested (35 and 45 mA) induced an antidepressant-like effect in female rats 1-day post-treatment (-42±15 s, **p*=0.020 and -37±14 s, **p*=0.042, respectively vs. SHAM); these effects that dissipated over time. Also, and as expected, the 95-mA dose was inefficacious in adolescent female rats [1,2]. Moreover, the particular features of the induced convulsions (tonic, clonic, recovery times) were monitored during treatment and the mean across days compared through one-way ANOVAs for each sex. Overall, lower dose-intensities of ECS were associated with a faster recovery time for both sexes. Taken together, these results emphasized the importance of fine-tuning the parameters for ECS to achieve efficacy, while considering sex as a crucial variable for treatment response. Ongoing experiments are evaluating the brains of these rats to deepen into the molecular mechanisms behind the observed sex-differences.

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Acknowledgements: Funded by PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033) and PDR2020/14 to MJG- F, "Programa JUNIOR del proyecto INTRES: Invetir, Investigar e Innovar" to SL-C.

P26

Sex differences in the pharmacological modulation of the EP3 LC receptor in a murine model of neuropathic pain

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It is clinically known that the comorbidity of chronic pain and stress-related disorders is more prevalent in women than in men, with symptoms being more severe and prolonged in women, but the underlying neurobiological mechanisms remain unclear (Baron-Cohen et al., 2005; Bekker and Van Mens-Verhulst, 2007). In this context, preclinical research has identifieda significant role for the noradrenergic locus coeruleus (LC) and its projection areas, such as the dorsal reticular nucleus (DRt) and the basolateral amygdala (BLA) (Camarena-Delgado et al., 2022; Llorca-Torralba et al., 2019). Notably, the LC is a sexually dimorphic nucleus, and the PTGER3 gene, which encodes the prostaglandin receptor EP3, is differentially expressed between sexes, making it a potential neurobiological target in neuropathic chronic pain. Thus, we hypothesize that changes in the EP3 pathway of the LC could be implicated in the sex differences observed in the development of stress-related disorders triggered by chronic pain. We employed pharmacological and neuronal cell type-specific targeting with viral vectors approaches to assess the role of LC on nociceptive and emotional-related behaviours inmale and female mice with nerve injury (chronic constriction injury, CCI). The EP3 receptor agonist (sulprostone) and its antagonist (L798,106) were administered intra-LC of wild-type mice by bilateral cannula implantation. Additionally, the EP3 receptor was overexpressed in the LC of TH:Cre mice. In addition, EP3 expression in the LC was determined by western blot,while pCREB level in the DRt and BLA was evaluated by immunohistochemistry. The results showed that CCI increased the expression of EP3 receptor level in the LC of male mice compared to sham counterparts, while females had no differences. Sulprostone administration intra-LC induced analgesic, anxiolytic effects and an enhanced welfare in CCI males, while no differences were observed in CCI females. In contrast, while overexpressing the EP3 receptor in the LC induced analgesia in both sexes, anxiolytic effect was just observedin CCI males. Interestingly, sulprostone administration intra-LC reduced neural activity of theDRt and BLA in CCI males, while both areas were overactivated in CCI females.

This pharmacological study could help to elucidate the role of EP3 LC receptor in the comorbidity of chronic pain and stress-related disorders, as well as to explain the increased vulnerability in women, ultimately leading to better personalized medical approaches.

Acknowledgements: “Fondo Europeo de Desarrollo Regional-UE, A way to build Europe” from “Ministerio de Economía y Competitividad” (PID2022-142785OB-I00, PDC2022-133987-I00); "Consejería de Salud y Familias, Junta de Andalucía" (PI-0134-2018); "Consejería de Transformación Económica, Industria, Conocimiento y Universidad, Junta de Andalucía" (P20_00958 and CTS-510); “Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz-INiBICA” (LI19/06IN- CO22; IN-CO9); “CIBERSAM” CIBER-Consorcio Centro de Investigación Biomédica en Red (CB07/09/0033), Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación; European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie Grant agreement Nº 955684; Red Española de Investigación en Estrés/Spanish Network for Stress Research RED2022-134191-T financed by MCIN/AEI /10.13039/501100011033; Grant PTA2021-019890-I and predoctoral fellowship PRE2019-091106 funded by the MICIU/AEI/10.13039/501100011033 and FSE+.

NATURAL PRODUCTS PHARMACOLOGY

P27

Effect of extra virgin olive oil (EVOO) polyphenols on vascular inflammation caused by hyperglycemia.

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The heart-healthy effect of extra virgin olive oil (EVOO), an indispensable food in the Mediterranean diet, is related to its polyphenol content, based mainly on its antioxidant effect (1, 2, 3).
The main purpose of this study is to evaluate the effect of the main polyphenols in EVOO in a experimental model of vascular inflammation.
Aortas from adult Wistar rats were incubated in normoglycemia (100 mg/dL) and hyperglycemia (300 mg/dL) conditions, with different concentrations of polyphenols (0 µmol/L, 1 µmol/L, 10 µmol/L, 100 µmol/L, and 500 µmol/L). The incubated polyphenols were the followings: tyrosol, hydroxytyrosol, 3,4-dihydroxyphenylglycol, oleocanthal and olacein. Oxidative stress levels (lipid peroxidation (quantification of TBARs), determination of antioxidant capacity: total glutathione (oxidized and reduced)), nitrosative stress (3- nitrotyrosine) and prostacyclin were determined in the supernatant of the artery homogenate. Thromboxane was determined in serum, after incubation of the blood with the different polyphenols, with the following induction of their production with collagen.
The concentration-effect curves for the different polyphenols studied show a range of effect within the amounts usually consumed with EVOO, mainly related to the inhibition of the production of TBARs and 3-nitrotyrosine. Polyphenols show a marked effect on the reduction of thromboxane production in a concentration range between 1-150 µM (with oleacein and oleocanthal presenting the lowest IC50).
The administration of polyphenols present in EVOO, in addition to lowering tissue oxidative stress, reduces the thrombogenic capacity in the experimental model of arterial inflammation.

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Acknowledgements: Funding project: PROYECTO OPERATIVO FEDER ANDALUCÍA 2014-2020 UMA-20-FEDERJA-054. Consejería de Transformación Económica, Industria, Conocimiento y Universidades. Dirección General de Investigación y Transferencia del Conocimiento.

P28

Black mulberries (*Morus nigra*) act as a nutraceutical agent: effect *in C. elegans* neurodegenerative and obese model

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The fruits of *Morus nigra* L. (*Moraacea*), commonly known as black mulberry, are renowned for their nutritional value and are also appreciated in traditional medicine. This is explained by their hight content in bioactive polyphenolic compounds, particularly anthocyanins.[1] The objective of this work is to study the potential neuroprotective and anti-obesity effect of black mulberry extract. For this purpose, *Caenorhabditis elegans* was used as a model organism. Due to its simplicity and the conservation of many genes and metabolic pathways with humans, it is ideal for preliminary studies of pharmacologic effect.[2]

The extract was obtained by microwave hydrodiffusion and gravity extraction. The neuroprotective effect was evaluated using *C. elegans* CL4176, an Alzheimer disease (AD) model, through paralysis assay[3] which measure the extract's protection against β-amyloid toxicity. Moreover, the capability to inhibit CNS enzymes (monoamine oxidase, acetylcholinesterase) was assessed. For the anti-obesity potential, *C. elegans* were exposed to a high concentration of glucose, either in the absence or presence of the extract, measuring fat accumulation[4] and the ability to inhibit *in vitro* lipase and α-glucosidase

M. nigra extract decreased the toxicity induced by β-amyloid accumulation in *C. elegans* by ameliorating AD-like symptoms ($p \leq 0.001$). The extract had no effect on AchE but exhibited an inhibitory effect on MAO A, which supports the neuroprotective potential of the extract shown *in vivo*. Furthermore, in obese model, fat deposits were significantly reduced (approx. 50%) when the worms were treated with the extract. The observed effect might be partly explained by the ability to inhibit digestive enzymes of the extract.

Our results demonstrate the neuroprotection and anti-obesity activity of black mulberry extract in *C. elegans* model and its potential use as functional foods and nutraceuticals.

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Acknowledgements: The authors thank Gobierno de Aragón for financial support (Phyto-Pharm Group B44_23R).

P29

Effect of a triterpene-rich olive oil on chronic kidney disease in an experimental model of diabetes mellitus

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It has recently been postulated that triterpenic derivatives of EVOO may have a beneficial effect on various cardiovascular conditions (1), which is why we proposed this study to try to find out the possible effect of these derivatives on diabetic nephropathy. The aim of the present study is to evaluate the possible effect of an olive oil with a high content of triterpenic derivatives, on various biomarkers of oxidative and nitrosative stress and renal function and morphology in an experimental model of diabetes mellitus. For this purpose, two types of oil were used, all of them from the picual variety olive: pitted olive oil (POO) and pitted and dehydrated olive oil (PDOO). This is an ex vivo study performed on 40 Wistar rats, the animals were randomly distributed into four experimental groups, consisting of 10 rats in each group:
Group of normoglycemic control animals (NCR), group of diabetic control animals (DCR), group of diabetic animals treated with pitted olive oil (POO) at a dose of 0.5 mL/kg/day orally and group of diabetic animals treated with pitted and dehydrated olive oil (PDOO) at the same doses. Diabetes mellitus was induced by a single dose of 40 mg/kg streptozotocin intraperitoneally. As results we obtained that both types of oils reduced both parameters: POO by 31% the glomerular volume and 61% the glomerulosclerosis index, PDOO by 40% the glomerular volume and 68% the glomerulosclerosis index. The administration of PDOO also reduced the concentrations of oxidative and nitrosative stress variables, its effect was significantly greater than the pitted olive oil in all variables except total antioxidant capacity in renal tissue. It is concluded that the administration of an olive oil rich in triterpenic derivatives, shows a greater nephroprotective effect in an experimental model of diabetes type 1, relating this effect to the antioxidant one.

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P30

Anti-atopic dermatitis effect of two semisynthetic flavonoids and their zinc coordination complexes via modulation of Nrf2/HO-1 antioxidant pathway in HaCaT keratinocytes

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Atopic dermatitis (AD) is a chronic inflammatory skin disease with a high worldwide prevalence, especially in children, and is associated with numerous comorbidities, affecting the quality of life of patients. Given there is currently no cure for AD, the search for new therapeutic alternatives is an area of interest[1]. In this regard, the study of natural ingredients is a significant part of the research with new bioactive compounds, and, among all, phenolic compounds, especially flavonoids, represent one of the most studied groups. On the other hand, micronutritional deficiencies of zinc (Zn) have been described in patients suffering from atopic dermatitis; thus, supplementation with Zn may be an interesting strategy to improve the clinical evolution and quality of life of this population[2]. The present study aimed to evaluate the anti-atopic effect of two semisynthetic flavonoids and their respective Zn-coordination complexes in an in vitro model of AD in HaCaT human keratinocytes. To evaluate the possible cytotoxic effects of the compounds, HaCaT cells were treated with different concentrations (6.25, 12.5, 25, 50, and 100 µM) and cell viability was measured by MTT assay after 24 h. In addition, HaCaT keratinocytes were pre-treated with the compounds at 5, 25 and 50 µM for 1h and then stimulated with tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (10 ng/mL) for 24 h. Antioxidant activity was evaluated by H2DCFDA technique. Interleukin-6 (IL-6) and different chemokines levels were determined by using ELISA. Moreover, the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling pathway and COX-2 levels were measured by Western-blot. Results demonstrated that none of the compounds exhibited toxicity towards the keratinocytes at the tested concentrations, demonstrating a favorable safety profile. In addition, all compounds markedly reduced the production of ROS, as well as the pro-inflammatory cytokine IL-6 and different chemokines in TNF-α/IFN-γ-stimulated HaCaT cells. Interestingly, Zn-coordination complexes showed the highest anti-inflammatory and antioxidant activity. Furthermore, the pretreatment with tested compounds down-regulated COX-2 expression and modulated Nrf-2/HO-1 signaling pathway. In conclusion, these flavonoids effectively attenuated the inflammatory response and oxidative stress triggered in TNF-α/IFN-γ-stimulated HaCaT keratinocytes, the Zn-coordination complexes being the most active. Therefore, our findings suggest the potential of these compounds as therapeutic candidates for chronic inflammatory diseases like atopic dermatitis.

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Acknowledgements:
We thanks to the "VII Plan Propio de Investigación y Transferencia" of The University of Seville for the funding for this project.

P31

Polyphenolic fraction from *Cannabis sativa* regulates antioxidant system and neuroinflammation under oxidative stress conditions

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In the last decade, the study of the cannabis plant has increased exponentially. The understanding of cannabinoids and their pharmacological activity has led to the growth of the market for cannabis-based products. This growth is mainly due to the commercialization of products based on cannabidiol (CBD) [1]. Although *Cannabis sativa* L. is known by its phytocannabinoid composition, the phytochemical characterization reveals that are many other bioactive compounds such as polyphenols, alkaloids or lignanamides [2,3]. Particularly, polyphenols have been widely studied due to its antioxidant properties but have not been studied as much in hemp. These metabolites help to prevent chronic disorders such as cardiovascular and neurodegenerative diseases. These come from the imbalance between oxidants and antioxidants, a condition known as oxidative stress. Uncontrolled oxidation can disrupt redox signalling and cause injury to cellular mechanisms.

First, the cytotoxicity of the aqueous extract was tested in the human neuroblastoma cell line SH-SY5Y using the MTT assay. Subsequently, we proceeded to evaluate its neuroprotective effect after exposing the cells to a neurotoxic agent (hydrogen peroxide). After this screening, the activity of the antioxidant enzymes catalase and superoxide dismutase (SOD) was measured. In addition, the expression of nuclear factor erythroid 2 (Nrf2) under oxidative stress conditions was quantified by Western Blotting. On the other hand, parameters related to neuroinflammation such as nitric oxide (NO) and IL-6 and SIGMA-1 receptor (Sig1R) production were also evaluated.

MTT cell viability assay revealed that the polyphenolic cannabis extract was not cytotoxic. When neurons were exposed to hydrogen peroxide, the cannabis extract enhanced cell viability levels relative to the positive control, indicating its ability to protect cells from mitochondrial damage. The activity of superoxide dismutase and catalase enzymes, was significantly enhanced in cannabis extract-treated cells, suggesting an effective antioxidant result of these compounds. The upregulation of Nrf-2 was observed to be upregulated in the cannabis sativa-treated oxidative stress model, which could contribute to cellular protection against oxidative damage. At the inflammatory level, cannabis extract was also shown to reduce the levels of nitric oxide, and to modulate the activity of IL-6 as well as the expression of the Sigma-1 receptor, which plays a role in modulating inflammation and oxidative stress.

In summary, *Cannabis sativa* polyphenols appear to have significant therapeutic potential in neuronal protection against oxidative stress and inflammation. Their ability to enhance cell viability, modulate key antioxidant enzymes and regulate important transcription factors suggests that they could be useful due to their diverse neuroprotective functions and therapeutic potential in neurodegenerative diseases such as Alzheimer's, Parkinson's and amyotrophic lateral sclerosis (ALS).

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Acknowledgements:
We are pleased to Gobierno de Aragón for financial support (Phyto-Pharm Group B44_23R) and University San Jorge for giving financial support through Proyecto Interno 2223025.

P32

Antidiabetic and anti-glycation activity of apple peel extracts: revalorization of apple wastes and byproducts

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Type-2 diabetes and its metabolic complications are some of the more prevalent diseases nowadays, being oxidative stress some of the altered molecular pathways. Polyphenols are bioactive compounds that through different mechanisms and pleiotropic actions can help to prevent or treat a wide range of disorders [1]. Phenolic compounds can be found in peels giving an added value to these by-products and wastes [2].

Six extracts were obtained from local and commercial apple peel cultivars by ultrasonication using methanol as solvent [3]. The first assay consisted of determining the Total Phenolic Content (TPC) using the Folin-Ciocalteu assay. Subsequently, the capacity to inhibit *in vitro* α-glucosidase and pancreatic α-amylase and lipase as well as advanced glycation end products (AGEs) was quantified to determine the antidiabetic activity. Antioxidant activity was studied using the 2,2-diphenyl-1-picrylhydracyl (DPPH) free radical. Cellular viability was determined performing MTT assay in Caco-2 cell lines in order to discard cytotoxicity at physiological concentrations.

All apple peel extracts had great phenolic content and showed antioxidant and anti-glycation activity in the DPPH and AGEs assays; additionally, the extracts obtained from local samples showed better activity against α-glucosidase than the commercial samples known as Verde doncella or Pinova. Nevertheless, none of the extracts showed activity against pancreatic lipase. The extract obtained from the local cultivar "Amarilla the Octubre" was the best sample in terms of IC_{50} values for the α-glucosidase/ α-amylase enzymes, phenolic content and AGEs/DPPH assays. For these reasons, this extract was superior in terms of bioactivity. Regarding the viability measured in Caco-2 cells, none of the samples were toxic at physiological concentrations.

Apple peel extracts can be considered a rich source of bioactive phenolic compounds with interesting pharmacological properties with potential therapeutical applications, especially in metabolic diseases, like type-2 diabetes.

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Acknowledgements: Teva Pharma S.L.U for the research grant and for the Aragon Government for the funding of Phyto-pharm group *(ref. B44_23R);* project *APPLEDIV (PID2022-141847OR-C33) funded by the Research State Agency in the 2022 call for Projects of Generation Knowledge oriented towards societal challenges*.

P33

Effects of some olive fruits derived products on cardiovascular biomarkers in experimental diabetes mellitus

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In recent years, the effect of polyphenols and their association to protect against various aspects of the complications of diabetes mellitus, mainly cardiovascular risk, has been studied (1, 2, 3). We therefore set out to evaluate in this experimental model two olive derivatives in comparison with extra virgin olive oil (EVOO): olive seed oil and olive oil from pitted and dehydrated olives. The aim of this study was to assess the possible effect of olive seed oil (OSO) and pitted and dehydrated olive oil (PDOO), in comparison with EVOO, on some cardiovascular biomarkers in an experimental model of diabetes mellitus. Diabetic animals showed evident alterations in biomarkers involved in the evolution of diabetic vasculopathy, quantifying increases in those that favor vascular damage between 1.5 and 5 times the values of non-diabetic animals, and a lower amount of those that protect against such damage (25-75% less than in healthy controls). The three oils administered decreased the concentration of biomarkers of vascular damage (35-45% in serum lipid profile, 15-40% in early biomarkers of vascular inflammation, 20-60% in platelet aggregation and in thromboxane/prostacyclin imbalance). The greatest effect was antioxidant, both in the inhibition of lipid peroxidation and in the increase of glutathione. PDOO showed a significantly greater effect on oxidative stress and on thromboxane/prostacyclin imbalance than that shown by OSO and EVOO. This greater effect may possibly be explained by its higher triterpenoid content (913 mg/kg, compared to 113 mg/kg in OSO and 75 mg/kg in EVOO). We conclude, in the light of the results of this study, that these oils meet two basic conditions: they could improve the yield of the olive industry and they equal, and may even increase, the beneficial effects of EVOO on cardiovascular processes.

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Acknowledgements: Funding project: PROYECTO OPERATIVO FEDER ANDALUCÍA 2014-2020 UMA-20-FEDERJA-054. Consejería de Transformación Económica, Industria, Conocimiento y Universidades. Dirección General de Investigación y Transferencia del Conocimiento.

P34

Immunomodulatory activity of S-954 from *Wasabia japonica* in an *ex vivo* murine model of peritoneal macrophages stimulated by LPS

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Considering current limitations of pharmaceuticals and nutritional therapy, including dietary modifications as well as the use of nutritional supplements in recent years, the research on the therapeutic properties of secondary metabolites from plants has experienced a spectacular increase, mainly due to the possible applicability as functional and phytotherapeutic foodstuffs. An example of this fact is *Wasabia japonica*, a semi-aquatic herbaceous plant of Japanese origin that is not only used as a condiment in typical Japanese dishes, but it has also been showed interesting cosmetic and therapeutic properties[1-3]. The main objective of the present study was to assess the immunomodulatory activity of S-954, a compound from *W. japonica*, in the LPS-stimulated murine peritoneal macrophage model.
Peritoneal macrophages were isolated from 20-25g female CD1 mice, which were injected intraperitoneally with 1 mL of sodium thioglycollate (3.8% w/v). The immune cells were pretreated with S-954 (12.5 or 6.25 µM) in the presence of bacterial lipopolysaccharide (LPS) (5 µg/mL) for 18 hours. The cytotoxicity was determined by using the sulforhodamine B (SRB) assay. The production of pro-inflammatory cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-17) was quantified by enzyme-linked immunosorbent assay (ELISA). Protein expression levels were analysed by Western Blotting. The intracellular production of reactive oxygen species (ROS) was quantified using a 2',7'-dichlorofluorescein diacetate (DCFDA) assay kit and the release of nitric oxide (NO) was indirectly determined using a Griess assay.

S-954 exhibited significant reduction in oxidative and nitrosative stress by lowering NO_2^- and intracellular ROS levels, possibly due to the activation of Nrf2/HO-1 antioxidant system and inhibition of the pro-inflammatory enzymes iNOS, COX-2 and mPGES-1. In the same way, it decreased the production of proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL- 17 and IL-18) probably by modulating canonical and non-canonical signaling pathways of the inflammasome, JAK2/STAT3 and p38, JNK and ERK MAPKs.
Regardless of these interesting results, further *in vivo* studies would be required to investigate the immunomodulatory effects of S-954, which may be a promising nutraceutical molecule for the treatment of a diverse range of immunoinflammatory diseases.

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Acknowledgements:
The authors gratefully acknowledge the assistance of the Center for Technology and Innovation Research, University of Seville (CITIUS). M. Alcarranza gratefully acknowledges support from FPU fellowship and financial sponsorship from the Spanish Ministerio de Universidades. Project. PID2019-104767RB-I00 funded by MCIN/ AEI /10.13039/501100011033 and P20_01171 US/JUNTA/FEDER, UE.

P35

Obacunone mitigates solar simulated radiation-induced molecular alterations in primary keratinocytes and full-thickness human skin

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Background: Solar radiation can cause damage to the skin, leading to various adverse effects such as sunburn, reactive oxygen species production, inflammation, DNA damage, and photoaging. To study the potential of photoprotective agents, full-thickness skin models are increasingly being used as *in vitro* tools. One promising approach to photoprotection involves targeting the redox-sensitive transcription factor Nrf2, which is responsible for regulating various cellular defense mechanisms, including the antioxidant response, inflammatory signaling, and DNA repair. Obacunone, a natural triterpenoid, has been identified as a potent Nrf2 agonist. The present study aims to explore the potential photoprotective effects of obacunone on full thickness skin models and human keratinocytes. **Material and methods**: Phenion® full-thickness skin models and keratinocytes were incubated with increasing concentrations of Obacunone and irradiated with solar-simulated radiation. Various photodamage markers were evaluated, including histological integrity, oxidative stress, apoptosis and photoaging and photocarcinogenesis-related markers. **Results**: Solar-simulated radiation was found to modulate various biomarkers related to sun damage. However, Obacunone attenuated cytotoxicity, oxidative stress, sunburn reaction, photoaging, and photocarcinogenesis in both keratinocytes and full thickness skin models. **Conclusions:** These results suggest that Obacunone may have potential as a photoprotective agent for preventing the harmful effects of solar radiation on the skin.

P36

Natural compounds enhance skin photoprotection promoting antioxidants pathways

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The skin is the main body's barrier against external damage, such as environmental pollution, pathogens or ultraviolet (UV) radiation, which is a major risk factor for skin pathologies, causing redness, swelling, skin inflammation and DNA damage. Topical application of sunscreen is one of the most important practices to protect our skin from damage caused by solar radiation, both in the short and long term. Thus, there is a trend to evaluate natural products such as polyphenols and carotenoids, which have beneficial effects due to their antioxidant and anti-inflammatory activities, to improve sunscreen formulas[1] as well as protect the environment[2]. The present study aims to analyse the effect of a combination of two polyphenols (MAG46 and MAG48) and a carotenoid (F-48) against the damage produced in human HaCaT keratinocytes exposed to UVB radiation, to determine its potential application as boosters in photoprotection by modulating antioxidant natural defence. First, to evaluate cell viability, HaCaT keratinocytes were pre-treated with different concentrations of the compounds (0.31, 0.625, 1.25, 2.5 and 5 □M) for 24 h and the resazurin assay was carried out. Furthermore, HaCaT cells were pre-treated with the combinations (F48-MAG46 and F48-MAG48) at the doses of 1.25, 2.5 and 5 □M for 4 h and then irradiated with UVB radiation. Antioxidant activity was measured by H2DCFDA technique. Interleukin-6 (IL-6) levels were measured by an ELISA kit. Malondialdehyde (MDA) intracellular levels were analysed to determine the lipid peroxidation. Nrf-2/HO-1 signalling pathway and COX-2 levels were measured by Western-blot and apoptosis was analysed by the annexin-V kit. Results showed that both combinations significantly reduced ROS and IL-6 levels in comparison to irradiated cells. In addition, tested compounds markedly supressed UVB-induced MDA levels. Furthermore, pre-treated cells showed a positive modulation of the Nrf-2/HO-1 signalling pathway, and COX-2 levels were down-regulated in comparison to UVB irradiated keratinocytes. The treatment with the combinations allowed a reduction of the percentage of late apoptotic cells and an increase of viable cells in comparison to irradiated control cells. These results demonstrated that both combinations present photoprotective effects and could be an interesting natural strategy to enhance photoprotection and prevent skin damage by promoting antioxidant defence.

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Acknowledgements:
We thanks to the "VII Plan Propio de Investigación y Transferencia" of The University of Seville for the funding for this project.

P37

Potential anticancer mechanism of action of Euphorbiaceae isolated compounds

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The Euphorbiaceae family of plants, is a huge family of flowering plants, including a wide variety of useful plants, from toxic to edible plants. These plants have very different shapes, from cactus-like forms to small herbaceous plants and enormous trees. Several authors have reported the cytotoxic activity of these plants in different cancer cell lines. This cytotoxicity could be due to different mechanisms of action. The extracts and compounds from these plants can activate the extrinsic and the intrinsic pathway of apoptosis. In this work we presented the structures and proposed mechanisms of action of 8,9-seco-ent-kaurane, Ebracteolatain A, Ebracteolatain B, Euphorbia Factor L2, Jatrogrossidione, Latilagascene B, Methyltrewiasine and N-methyltreflorine. and, Trigothysoid N, isolated from Euphorbiaceae plants.

P38

Antibacterial and anti-biofilm activity of commercial essential oils traditionally used for infectious diseases

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The World Health Organization (WHO) has designated antibiotic-resistance as a priority, being particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa* high-priority pathogens in the latest update of the WHO Bacterial Priority Pathogens List (1). Additionally, these bacteria are commonly found in the biofilm form, indicating they can aggregate and form multicellular communities, characterized by a high level of organization, which provides the constituent bacteria with protection against adverse conditions, as well as increased tolerance to antimicrobials and the immune system itself. The challenges presented by biofilm infections and their treatment make it imperative to explore new antimicrobial sources (2). In this context, essential oils (EO), natural volatile products obtained by steam or hydro distillation from different parts of the plants, may constitute an interesting alternative compatible with the ONE-HEALTH strategy.

The aim of this study is to determine the anti-biofilm properties of four commercial and chemically characterized EO, studying their potential against both *S. aureus* and *P. aeruginosa*. The following EO were provided and chemically characterized by Pranarom for this study: palmarosa (*Cymbopogon martini*), cinnamon (*Cinnamomum cassia*), oregano (*Origanum vulgare*) and tea tree (*Melaleuca alternifolia*). Bacterial viability in both planktonic and biofilm states was assessed using the resazurin assay, while the total biomass of the biofilm was quantified using the crystal violet assay. Additionally, the toxicity of the essential oil (EO) was evaluated in hepatic and alveolar cell lines, HepG2 and A549.

All the EO herein studied reduced *S. aureus* viability in the biofilms, in both the pre-exposure and the post-exposure model whereas vancomycin (positive control) activity was considerably reduced in the post-exposure. However, for *P. aeruginosa* only *C. cassia* proved to be active against the bacteria within the biofilm. Regarding cytotoxicity, the EO exhibited toxicity against the cells at higher concentrations, being palmarosa the safest oil in terms of selectivity (IC_{50} = 0.973 mg/ml) whereas cinnamon proved to be the most toxic (IC_{50} = 0.491 mg/ml).

The results exhibit encouraging outcomes, proving the significant activity of EO against relevant bacteria and biofilms.

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Acknowledgements: DGA grants for predoctoral research personnel in training 2023-2027, to Pranarom for enabling the study's financing, and to the Government of Aragon for funding the recognized Phyto-Pharm group (ref. B44_23R).

INFLAMMATION AND IMMUNOMODULATION PHARMACOLOGY

P39

Pharmacological characterization of the mechanism of action of reference ligands active at chemokine receptor CX_3CR1

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G protein-coupled receptors (GPCRs) are the largest family of membrane receptors and play a crucial role in physiopathology. Chemokine receptor CX_3CR1, a Gi-coupled GPCR, is the sole human receptor for the chemokine fractalkine (CX_3CL1). The CX_3CL1/CX_3CR1 axis is a key player in inflammation/immunity as well as in neuron-microglia communication. CX_3CR1 emerges as a potential drug target in immune/inflammatory conditions, metastasis, and psychiatric/neurological diseases among other areas. Nevertheless, the validation of CX_3CR1 as drug target and its exploitation with therapeutic purposes are inhered by the poorly known receptor pharmacology and the scarcity of selective ligands.

We aim at characterizing the affinity, potency, and efficacy of commercially available selective CX_3CR1 ligands E6130, AZD8797, and JMS-17-2, together with the endogenous ligand fractalkine, in different *in vitro* and cell-based assay set-ups. Whereas E6130 behaved as a moderate potency, full agonist in BRET-based beta-arrestin 2 recruitment assays in transient transfected HEK293T/17 cells, AZD8797 showed a profile more compatible with a non-competitive ligand, potentiating at low concentrations the fractalkine efficacy while antagonizing at higher concentrations the response of the orthosteric ligand.

Our results validate diverse mechanisms of action of structurally unrelated chemotypes targeting CX_3CR1. A fully understanding of the mode of action of reference CX_3CR1 ligands might aid in the search for novel paths of pharmacological modulation of this emerging drug target for unmet clinical needs.

Acknowledgements: This work was supported by Spanish Ministry of Economy and Competitiveness (MINECO) (Grant Number PID2020-119754GB-I00/AEI/10.13039/501100011033 to M.C.). A. Paz-Castro acknowledges funding from Deputación da Coruña, and Fundación José Otero-Carmela Martínez in collaboration with Universidade de Santiago de Compostela.

P40

Search for new immune mediators in Abdominal Aortic Aneurysm development

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Background: Abdominal aortic aneurysm (AAA) is a localized dilation of the abdominal aorta, resulting in an increase of its normal diameter by 50% or more [1]. It predominantly affects men over 65, and currently, the clinical therapeutic approach to AAA is limited to surgical repair. Nowadays, the development of AAA is considered to have a clear inflammatory component. Due to the high mortality rate associated with AAA, it is important identify new effective therapeutic strategies to prevent the progression of disease. Therefore, since inflammation appears to be the crucial force driving onset and progression of AAA, a better understanding of the inflammatory response that is involved in this process, as well as the role of the different immune players, is required to discover new therapeutic targets to either inhibit or prevent AAA development.

Material and Methods: Eight-week-old male apolipoprotein E-deficient (apoE$^{-/-}$) mice were used. An osmotic minipump (Alzet, Model 2004, Charles River) implanted subcutaneously was used to infuse Ang-II (n=5) at a rate of 1000 ng/kg/min or saline (n=5) for 28 days. Lesion formation, macrophage, T lymphocyte, eosinophil and pSTAT-6+ cell infiltration as well as eotaxin-1/CCL11 and IL-4 expression were determined within the lesion through histological and immunohistochemical techniques. Statistical significance was determined using a Student's t-test.

Results: Ang-II-infused apoE$^{-/-}$ mice had a higher incidence of AAA than those with saline. The maximum diameter of the adrenal region in the animals infused with Ang II was 2.19±0.16 mm, while control (saline) animals was 1.10±0.02 mm. Increased diameter was also observed in both the aortic arch and the thoracic region of the aorta. Moreover, enhanced macrophage (CD68+), CD3+ lymphocyte, eosinophil and pSTAT6+ cell infiltration and neovascularization were observed compared to saline-infused animals, which was accompanied by greater eotaxin-1/CCL11 and IL-4 expression within the lesion area.

Conclusion: Additional studies must be conducted to understand the role of eotaxin-1/CCL11 in AAA development.

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Acknowledgements: This work was supported by the Spanish Ministry of Science and Innovation: [grant number PID2020-120336RB-I00]; the Carlos III Health Institute (ISCIII) and the European Regional Development Fund (FEDER) [grant number PI21-00220]; the Generalitat Valenciana [grant numbers APOTIP/2020/011, CIPROM/2022/45].

P41

Glucocorticoid receptor deletion leads to a pro-inflammatory transcriptomic profile in intestinal organoids

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Background: Glucocorticoids (GC) are important immunosuppressive and anti-inflammatory agents in the management of inflammatory bowel disease and many other conditions. However, GC-induced deleterious effects on the intestinal barrier function limit their clinical benefit. In this regard, mice with dextran sodium sulphate (DSS)-induced colitis show a worse general animal status (weight loss, rectal bleeding, death) when treated with GC, despite amelioration of colitis [1], associated to antiproliferative actions on the intestinal epithelium. Besides, previous studies of our research group revealed that mice carrying a conditional deletion of the glucocorticoid receptor (encoded by the *Nr3c1* gene) in intestinal epithelial cells are protected against experimental colitis, despite showing a transitory pro-inflammatory status shortly after deletion [2]. In this study, our aim is to study at the transcriptomic level the role of glucocorticoid receptor in intestinal epithelial cells using intestinal organoids.

Material and methods: Intestinal organoids were obtained by jejunal crypt isolation from wild type (WT) and *Nr3c1*$^{flox/flox}$ Villin-Cre-ERT2 mice. Crypts were seeded in Corning-Matrigel® (Thermo Scientific) and Intesticult® (StemCell). 24 hours after organoid passage, GR deletion was induced by tamoxifen 1 µM addition to culture media. Cells were collected 5 days after for RNA extraction. RNA-seq analysis was carried out in four replicate samples for each genotype. The software R studio was used to evaluate the transcriptomic profile. Additionally, RNA-seq results were verified by RT-qPCR.

Results: 532 genes were significantly modulated (451 upregulated) by glucocorticoid receptor deletion, considering a False Discovery Rate (FDR) < 0.05. These organoids exhibit an evident pro-inflammatory profile, based on the upregulation of genes related to defense response to bacteria (*Lyz1, Reg3g, Tlr2, Tlr4, Cd14, Nos2, Ptgs2*), interferon signalling (*Aim2, Irf5, Oas1a, Sting1*) and 22 defensins. These results were confirmed by RT-qPCR on jejunal organoids but also in colonic organoids. Besides, knock out organoids show a modulation on glucose transporters (such as *Slc5a11*, *Slc5a12* and *Slc2a7*) without modification on sodium chloride nor water carriers. Surprisingly, knock out organoids show a higher expression of some glucocorticoid-targeted genes, such as *Esr1*, *Sgk1* and *Sgk2*.

Conclusions: Our results show that glucocorticoid receptor deletion leads to a clear pro-inflammatory profile, which correlates to our observations in vivo. However, intestinal barrier function and proliferation related genes were not modulated. The phenotype will be further characterized in future experiments.

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P42

Different phenotypes of glial cell during retinal neuroinflammation

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Background

Retinal neurodegenerative diseases such as retinitis pigmentosa (RP) or central areolar choroidal dystrophy (CACD) are hereditary retinal dystrophies that curse with photoreceptor cell death and neuroinflammation. The exact role of glial cells and inflammation is still controversial, and it is not fully understood whether it is beneficial or harmful, which makes difficult to choose the best therapeutic strategy. It has been described that two different phenotypes of glial cells are involved in brain inflammation, one neuroprotectant and the other neuroinflammatory. The activation of the complement system has been related with neurodegeneration, in fact the protein C3 has been identified as a marker of the neurotoxic phenotypes of microglia and astrocytes in brain. We aim to study the different inflammatory profiles of glial cells involved in retinal neuroinflammation present in neurodegenerative diseases.

Materials and Methods

Two animal models of retinal degeneration were used in this study, rd10 mice, a model of RP, and $Prph2^{KI/KI}$ mice, a model of CACD. C57Bl/6 mice were used as healthy controls. We used antibodies against CD11b, GFAP, CRALBP and complement factor C3 for the expression analysis of these proteins by flow cytometry and immunohistochemistry, using samples at different stages of retinal degeneration in each model of study.

Results

Our results show that all populations of glial cells, including microglia, Müller cells and astrocytes, display two phenotypes with different C3 expression, and that this expression increases as the degeneration evolves. Moreover, the profile of C3 expression varies among glial cell types, pointing to a different contribution of each of them to the degenerative process. At least three different subpopulations of Müller cells were present according to the expression of GFAP and C3 markers.

Conclusions

During the course of retinal degeneration, different phenotypes of glial cells involved in inflammation can be identified. These results can help to better understand both the function and role of these cells on neuroinflammation.

P43

PGE_2 as modulator of proliferative and inflammatory response in skin

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Dermal fibroblasts have been described to behave like mesenchymal stem cells reducing leucocyte recruitment and dendritic cell activation through PGE_2 release (1). Thus, a loss of homeostasis due to a deficient PGE_2 synthesis could lead to the exacerbation of chronic inflammatory skin diseases, such as psoriasis (2). In the present study, we sought to determine the effect of PGE_2 on human keratinocyte by analyzing cell proliferation, wound scratch assay and release of psoriatic pro-inflammatory mediators. In addition, we analyzed the regulatory role of PGE_2 on human monocyte response.

Keratinocytes were isolated from foreskins of adult healthy donors, after dispase II treatment and epidermal digestion with trypsin. Cells were grown in KGM media in a serum-free low- Ca^{2+} and proliferation assay was performed by MTT test in 24-well culture plates (10^5 cells/well) after 48h incubation in the presence or absence of TNF α (10 ng/ml). Previously, cells were treated with PGE_2 at the concentration released by stimulated fibroblasts in previous studies (50 ng/ml), with the non-selective COX inhibitor indomethacin (10 µM), or the combination of both. Supernatants were also used to determine the release of IL-8, IL-6 and human β-defensin (HBD-2) by ELISA. Scratch wound assay was performed in keratinocytes seeded into a 12-well culture plate ($2x10^5$ cells/well) after 48h of incubation with the same treatments indicated above. Human monocytes isolated from peripheral blood were also incubated with PGE_2, indomethacin or their combination previous the stimulation with IL-1β (2.5 g/ml) during 24h. The levels of IL-10 and TNF-α were measured in the supernatants. Results showed that inhibition of endogenous cyclooxygenase products by indomethacin significantly reduced keratinocyte proliferation (15.8 %) and wound closure (47.2%) without affecting cell viability. These effects were reverted by PGE_2 treatment, suggesting a beneficial role of this eicosanoid as an autocrine modulator of keratinocyte growth. Interestingly, the release of IL-8 (663 ±19.37 pg/ml), IL-6 (297.2± 33.11 pg/ml) and HBD-2 (22.66 ± 1.11 pg/ml) induced by TNFα in keratinocytes was also significantly reduced after PGE_2 treatment (317.0±21.9 pg/ml, 134±8.2 pg/ml and 9.85±1.5 pg/ml, respectively). Furthemore, in human monocytes stimulated with IL-1β, PGE_2 treatment reduced the release of TNF-α (170.80 ± 100.50 *vs* 269.40 ± 81.46 in control cells) and increased the release of IL-10 (92.76 ± 14.37 *vs*.15.65 ± 40.93) indicating that this eicosanoid favored the polarization of stimulated monocytes towards a resolutive phenotype. These results are consistent with a possible role of dermal fibroblasts-derived PGE_2 in the regulation of the inflammatory response skin.

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Research was funded by Spanish Ministry of Science and Innovation (PID2021-124890OB-I00). CD is recipient of the ERASMUS+ program.

VASCULAR PHARMACOLOGY

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Erythrocyte-derived extracellular vesicles induce endothelial dysfunction through arginase-1 and oxidative stress in type 2 diabetes

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Background: Red blood cells (RBCs) from individuals with type 2 diabetes (T2D-RBCs) induce endothelial dysfunction. However, the mechanism by which RBCs communicate with the vasculature is unknown.

Purpose: This study aimed to test the hypothesis that extracellular vesicles (EVs) secreted by RBCs act as mediators of endothelial dysfunction in T2D.

Methods: EVs released from T2D-RBCs (T2D RBC-EVs) and RBCs from age-matched healthy controls (H RBC-EVs) were isolated and co-incubated with mouse aortas to evaluate endothelium-dependent relaxation. The number of EVs produced, their uptake by endothelial cells, and their arginase-1 content were determined. Functional involvement of EV uptake, arginase, and oxidative stress were investigated using pharmacological interventions and expression analyses.

Results: Despite a lower production of T2D RBC-EVs, their uptake by endothelial cells was greater compared to H RBC-EVs. T2D RBC-EVs significantly impaired endothelium-dependent relaxation, an effect that was attenuated following inhibition of arginase in EVs. Additionally, inhibition of vascular arginase or oxidative stress improved endothelium-dependent relaxation. Arginase-1 was detected in RBC-derived EVs, and levels of arginase-1 and oxidative stress increased in the vasculature following co-incubation with T2D RBC-EVs. These EVs also increased levels of arginase-1 and NADPH oxidase 4 in endothelial cells. An increase in arginase-1 protein was observed even after mRNA silencing.

Conclusions: T2D-RBCs induce endothelial dysfunction through the increased uptake of EVs that transfer arginase-1 from RBCs to the vascular endothelium in T2D, leading to oxidative stress and endothelial dysfunction. These results shed important light on the mechanism underlying vascular injury mediated by RBCs in T2D.

Acknowledgements: Foundation - Swedish Heart-Lung Foundation (20190341, 20200326, 20220264 and 20190266). Novo Nordisk postdoctoral fellowship run in partnership with Karolinska Institute.

P45

SARS-CoV-2 spike protein triggers NLRP3 inflammasome activation and coagulation factors production in endothelial cells

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COVID-19 can lead to hyperinflammation, hypercoagulation, and endothelial injury. The SARS-CoV-2 spike (S) protein, a key component of the viral crown and the main product of mRNA COVID-19 vaccines, has been found in human tissue reservoirs. This study investigates whether the S protein alone can induce inflammatory and pro-coagulant responses in primary cultures of endothelial cells.

Primary cultures of human umbilical vein endothelial cells (HUVEC) were stimulated with increasing concentrations of the SARS-CoV-2 S protein. The components of NF-κB and the NLRP3 inflammasome were analyzed using Western Blot and indirect immunofluorescence. Additionally, levels of pro-coagulant factors and the release of von Willebrand factor (vWF) were assessed using Western Blot and ELISA, respectively.

The S protein alone activated NF-κB and triggered the priming and activation of the NLRP3 inflammasome system starting at a concentration of 35 nM. Furthermore, coagulation factors such as vWF, factor VIII, and tissue factor (TF) were induced in response to the S protein. However, the S protein did not increase levels of the anti-coagulant protein ADAMTS-13, a key counter-regulator of the pro-coagulant activity of vWF.

Overall, the isolated SARS-CoV-2 S protein acts as both a pro-inflammatory and pro-coagulant stimulus in human endothelial cells. These effects could contribute to the vascular complications associated with both acute and long COVID-19.

Acknowledgements: Supported by funds from REACT-EU-Comunidad de Madrid and the European Regional Development Fund (SPACE2-CV-COVID-CM) to C. Peiró and Ó. Lorenzo and Plan Nacional I + D (PID2020-115590RB-100/AEI/) to C. Peiró and C.F. Sánchez-Ferrer.

P46

Canagliflozin works as an anti-inflammatory and anti-senescent drug on human vascular smooth muscle cells and mitigates vascular dysfunction in mesenteric microvessels of STZ-induced diabetic mice

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Diabetes Mellitus (DM) is a disease considered a highly prevalent cardiovascular risk factor, where inflammation and endothelial dysfunction stand out as early pathophysiological mechanisms. Canagliflozin, an SGLT2 transporter inhibitor drug, has been introduced in the treatment of Type II DM and, in addition to regulating blood glucose levels, induces cardiovascular benefits through mechanisms yet to be determined. This study aims to determine whether canagliflozin could have anti-inflammatory and anti-senescent effects on human aortic vascular smooth muscle cells (VSMCs) and whether it could reverse endothelial dysfunction present in mesenteric microvessels from streptozotocin (STZ)-induced diabetic mice.

Human VSMCs were stimulated for 18 hours with IL-1β (10 ng/mL), resulting in a significant increase in nuclear factor-kappa B (NF-κB) transcription factor expression, as well as components of the NLRP3 inflammasome and pathways related to cellular senescence, such as p53 and p21 proteins and phosphorylated histone H2AX (γ-H2AX). All these effects were reduced in the presence of 1 µM canagliflozin. In experiments conducted with a metabolic analyser, it was observed that IL-1β (10 ng/mL) significantly altered the metabolic profile of VSMCs, increasing the glycolytic pathway at the expense of oxidative phosphorylation. However, this effect was not modified in the presence of canagliflozin. Finally, endothelium- dependent relaxation was analysed in mesenteric microvessels from C57BL/6J mice with 2 weeks of STZ-induced diabetes. Endothelial dysfunction significantly improved in vessels preincubated with 1 µM canagliflozin, although it did not alter vasodilatory responses in microvessels from control animals.

In conclusion, our results indicate that canagliflozin reduces inflammatory and senescent responses induced by IL-1β in human VSMCs, while not affecting the inflammatory metabolic profile produced by this cytokine. Additionally, canagliflozin improves endothelial dysfunction associated with diabetes in mouse microvessels. These mechanisms may help to clarify the beneficial cardiovascular effects associated with canagliflozin treatment in diabetic patients.

Acknowledgements: This work is part of the research project titled "Identification of Pharmacological Targets for Preventing Inflammatory Vascular Aging of Cardiometabolic Origin." The project is funded by the National R&D Plan (PID2020-115590RB-100/AEI) and is being conducted at the Universidad Autónoma de Madrid.

P47

Targeting immune metabolism as a strategy for the treatment of vascular dysfunction and hypertension in a genetic mouse model of systemic lupus erythematosus

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Hypertension is a significant cardiovascular risk factor commonly found in patients with systemic lupus erythematosus (SLE) (Wolf VL, Ryan MJ. *Curr Hypertens Rep* 2019;21:10). This study investigates whether pharmacological interventions aimed at inhibiting glycolysis and mitochondrial dysfunction in immune cells can provide vascular protection in a genetic mouse model of SLE. Female NZBWF1 lupus mice, 29 weeks old, were treated for 4 weeks with either a vehicle (SLE group), a combination of 2-deoxy-D-glucose (2DG), a glycolysis inhibitor, and metformin (Met), which inhibits complex 1 of the mitochondrial electron transport chain, or rapamycin, which inhibit the mammalian target of rapamycin (mTOR). NZW/LacJ mice were used as controls. Blood pressure was measured using tail-cuff plethysmography, and endothelial function was assessed by myography of the aorta. Flow cytometry was used to analyze B and T cells. The treatment with 2DG + Met prevented the onset of hypertension and improved autoimmunity, kidney injury, endothelial dysfunction, and the proinflammatory and oxidative status of the vasculature. Additionally, it reduced aortic T helper (Th)17 cell infiltration in SLE mice. This treatment also normalized splenic metabolism by increasing AMP-activated protein kinase (AMPK) activity and inhibiting mammalian target of rapamycin (mTOR) activity, resulting in fewer B cells, activated Th cells, and Th17 cells. Furthermore, the activation of AMPK in the vascular wall contributed to improved endothelial function. Similarly, rapamycin inhibited mTOR, reducing splenic Th17 differentiation and aortic Th17 infiltration, which improved vascular oxidative stress and endothelial dysfunction.
In conclusion, immunomodulatory drugs that target glycolysis and mitochondrial metabolism can improve autoimmunity and vascular abnormalities in SLE mice. The enhancement of endothelial function is mediated by increased nitric oxide (NO) bioavailability, due to reduced NO inactivation by reactive oxygen species via the Th17/IL17/Rho kinase/NADPH oxidase pathway.

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

P48

Exploring the impact of SARS-Cov-2 S protein on endothelial cells senescence and metabolic profile: the role of cellular anti-oxidant protective system and the NLRP3 inflammasome

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Abstract

Premature vascular aging and endothelial cell senescence are major risk factors for cardiovascular diseases and atherothrombotic disturbances, which are prominent complications of both acute and long COVID-19[1,2]. The spike (S) protein of SARS-CoV-2, which forms the coronavirus's crown-like structure, is able to induce endothelial cells inflammation and it has been found as an isolated element in the circulation and in human tissues reservoirs months after infection[3]. In this study, we explored the direct effects of the S protein on endothelial cell senescence and identified some underlying mechanisms.

In primary cultures of human umbilical vein endothelial cells (HUVEC), SARS-CoV-2 S protein enhanced in a concentration-dependent manner the cellular content of senescence and DNA damage response markers (senescence-associated-β galactosidase, γH2AX), as well as growth-arrest effectors (p53, p21, p16). Moreover, S protein altered endothelial cells bioenergetics and mitochondrial function leading to a shift towards a higher glycolytic state. In parallel, the S protein reduced the availability of cytoprotective proteins, such as the anti-aging protein klotho, Nrf2 or heme oxygenase-1, and caused functional harm by impairing ex vivo endothelial-dependent vasorelaxation in murine microvessels. These effects were prevented by the pharmacological inhibition of the NLRP3 inflammasome with MCC950. Furthermore, the supplementation with either recombinant klotho or angiotensin-(1-7), equally protected against the pro-senescence, pro-inflammatory and pro-oxidant action of the S protein.

Globally, this study proposes novel mechanisms of disease in the context of COVID-19 and its vascular sequelae and provides pharmacological clues in order to prevent such complications.

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Acknowledgements:
This work was supported by grants from REACT-EU-Comunidad de Madrid and the European Regional Development Fund (SPACE2-CV-COVID-CM) to CP and OL, Plan Nacional I+D (PID2020-115590RB-100/AEI/https://doi.org/10.13039/501100011033) to CP and CFSF. Fondo de Investigación Sanitaria-FIS Carlos III (PI20/00923) to OL. Plan Nacional I+D (PID2022-137373OB-I00 granted by MICIU/ AEI / 10.13039/501100011033 / FEDER, UE to ISP. Plan Nacional I+D (PID2021-126274OB-I00) to FC. L.S. is the recipient of FPI Universidad Autónoma de Madrid (SFPI / 2020-00053). IV is the recipient of a Sara Borrell postdoctoral grant (CD22/00101). SF was supported by Comunidad de Madrid grants PEJ-2021-TL/BMD-22441.

NOVEL APPROACHES TO *IN VIVO* PHARMACOLOGY

P49

Towards brain bioluminolysis of an adenosine a2a receptor photodrug

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Photopharmacology is an emerging approach that allows the spatiotemporal control of receptor function using photodrugs. Compared to conventional pharmacology, this discipline offers superior tissue specificity and raises the opportunity for reduced off-target effects. In previous studies, we successfully demonstrated light-dependent activation of the photocaged compound MRS7145 (a derivative of the selective A_{2A} receptor ($A_{2A}R$) antagonist SCH442416) after local irradiation through optical fiber implantation in mice striatum [1]. In this follow-up study, we investigate the use of bioluminescence resonance energy transfer (BRET) to effectively photouncage MRS7145. This involves the use of light generated by the interaction of the nanoluciferase (NL) enzyme with its substrate coelenterazine (CTZ), a process known as biolumilolysis [2]. Recently, we uncovered the initial proof of concept for autaptic $A_{2A}R$ blockade, produced by the photorelease of SCH442416 from MRS7145 in HEK-293 expressing $A_{2A}R$ tagged with NL at the N-terminus ($A_{2A}R^{NL}$). Bioluminolysis-mediated $A_{2A}R$ blockade was assessed by measuring cAMP accumulation after activation of the A_{2A} agonist CGS21680. Furthermore, we use an adeno-associated virus (AAV) encoding a functional $A_{2A}R$ coupled with a NL enzyme (AAV- $A_{2A}R^{NL}$) to transition to *in vivo* experimentation. The expected expression and functionality of $A_{2A}R^{NL}$ in the mouse striatum was confirmed by *ex vivo* luminescence recordings and immunohistochemical detection. Ultimately, this experimental strategy aims to validate the feasibility of uncaging a MRS7145 using bioluminolysis produced within the brain parenchyma, independent of external light sources (i.e. lasers, LED). Thus, our method will offer the potential to improve animal behavior studies by eliminating the requirement of restraining cables or implantation of optic fiber.

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Acknowledgments:
Programa d'ajuts Margarita Salas, financiat per l'Unió Europea – NextGenerationEU» (Ministerio de Universidades)

P50

Photopharmacology for targeted treatment of psoriasis: Development and evaluation of a diazocine-based A3R agonist

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Photopharmacology uses light to transform a drug molecule from an inactive to an active state at specific body sites, enabling precise spatial and temporal control of drug activity. This approach aims to enhance therapeutic specificity and minimize off-target effects. In this study, we introduce MRS7787, a diazocine-based derivative of the selective adenosine A3 receptor (A3R) agonist piclidenoson (IB-MECA), as a new photopharmacological agent for the treatment of psoriasis.
Psoriasis, a chronic inflammatory disorder, has been linked to A3R's role in modulating inflammatory responses. Current treatments, while effective, are not curative and often fall short for some patients, underscoring the need for innovative therapies. Photopharmacology presents a promising solution that allows localized activation of A3R in psoriatic lesions to minimize systemic exposure and potential side effects. Importantly, this study marks the first use of a diazocine-based photopharmaceutical in vivo. Unlike photoswitches previously described, which are typically active in their thermally stable configuration and become inactive upon exposure to 420 nm light, MRS7787 is designed to function in the opposite manner, making this reversal pharmacologically advantageous. MRS7787 was synthesized by incorporating a diazocine group into IB-MECA, enabling reversible light-induced Z-E isomerization. Photochemical characterization revealed efficient Z-E and E-Z photoisomerization upon irradiation with 420 nm and 520 nm light, respectively, with robust photostability over multiple cycles. Interestingly, biological assays demonstrated that E-MRS7787 selectively activated A3R by inhibiting cAMP accumulation, while Z-MRS7787 remained pharmacologically inactive.
Notably, in a mouse model of psoriasis induced by IL-23 intradermal ear injection, MRS7787, in its E-state (activated by 420 nm light), significantly reduced ear inflammation and epidermal thickening, indicating effective A3R-mediated anti-inflammatory activity. These findings suggest that MRS7787 can be photoactivated in vivo to exert therapeutic effects, representing a potential breakthrough in the treatment of psoriasis.
This study highlights the potential of combining diazocine-based photopharmacology with conventional phototherapy to improve therapeutic results for patients with psoriasis, paving the way for more precise and effective treatments in the future.

Acknowledgements:
We thank Centres de Recerca de Catalunya (CERCA) Pro-gramme/Generalitat de Catalunya for IDIBELL institutional support and Maria de Maeztu MDM-2017-0729 to Institut de Neurociencies, Universitat de Barcelona. We thank Esther Castaño and Benjamín Torrejón from the Scientific and Technical Services (SCT) group at the Bellvitge Campus of the University of Barcelona for their technical assistance.

P51

Insights of schizophrenia-associated dopamine D2 receptor variants: effects on antipsychotic-mediated modulation of receptor heteromerization

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Schizophrenia has a prevalence rate of approximately 1 in 300 people worldwide. The hypothesis of simultaneous dysregulation of neurotransmission systems is considered the most widely accepted by the scientific community. However, emerging evidence strongly suggests the involvement of non-canonical neuromodulatory systems, especially the adenosinergic system. Interestingly, adenosinergic control of striatal dopaminergic signaling is highly dependent on the molecular and functional interaction between the adenosine A_{2A} receptor ($A_{2A}R$) and dopamine D_2 receptor (D_2R). Consequently, a hypoadenosinergic state, characterized primarily by a decrease in extracellular adenosine concentration (or a relative down-regulation of $A_{2A}R$ versus D_2R expression), would reduce the well-established tonic inhibition of D_2R function mediated by $A_{2A}R$ within the context of the $A_{2A}R$-D_2R heteromer. Therefore, it has been postulated that increasing allosteric inhibition mediated by this heteromer within the striatopalidal GABA pathway could represent a promising strategy for the treatment of schizophrenia. In the presented work, our main objective is to evaluate the impact of well-established schizophrenia-associated D_2R genetic variants, namely S311C, K327E, R360H, R150H, and R220H mutants, on its molecular ability to heteromerize with $A_{2A}R$. Accordingly, a Schematic representation of the different mutants was developed, followed by immunofluorescence and immunoblotting detection of the different D_2R variants. Additionally, NanoBit and cAMP assays were conducted to elucidate the molecular dynamics of these variants. Interestingly, Bonferroni's post-hoc test revealed a significant ($P = 0.0048$) increase in D_2R density in cells treated with haloperidol for 16 h. Consequently, we then assessed the impact of antipsychotics on the temporal dynamics of $A_{2A}R$ and D_2R heteromer formation. Thus, concentration-response curves were constructed with increasing concentrations of main antipsychotic drugs (i.e. haloperidol, clozapine, and aripiprazole) for a total of 2 or 16 h. Interestingly, a 2 h incubation with haloperidol or aripiprazole did not alter $A_{2A}R$-D_2R heteromer content, while the same treatment with clozapine significantly reduced the amount of heteromer formation in a concentration dependent manner ($pEC50=6.1\pm0.3$). On the contrary, 16 h exposure to clozapine did not affect the density of the $A_{2A}R$-D_2R heteromer, while haloperidol or aripiprazole caused a significant concentration-dependent increase in the formation of the $A_{2A}R$-D_2R heteromer formation ($pEC50=7.8\pm0.4$ and $pEC50=6.8\pm0.4$, respectively). In conclusion, different D_2R variants exhibited differential responses to the total exposure time of antipsychotics. Thus, modulating the $A_{2A}R$-D_2R heteromerization. This variability could probably be due to molecular structural disturbances that are able to alter the $A_{2A}R$-D_2R interface (or the interaction) with scaffold proteins.

Acknowledgments: Supported by FEDER/Ministerio de Ciencia, Innovación y Universidades–Agencia Estatal de Investigación (PID2020-118511RB-I00) and the Catalan government (2021 SGR 00698).

CARDIOVASCULAR PHARMACOLOGY

P52

Circulating endothelial damage biomarkers predicts susceptibility to positively react to Mirabegron treatment in pulmonary hypertension

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Pulmonary Hypertension (PH) is a debilitating disease of the pulmonary vessels that involves progressive narrowing and occlusion of distal arterioles resulting in increased pulmonary pressures and vascular resistance. The origin of certain forms of PH is not well understood, however it is well known that endothelial damage and dysfunction followed by inflammation and vascular remodelling are critical. Despite important advances in the field, none of the current therapies are curative[1]. This lack of effectiveness could come by various reasons including the lack of a precision medicine strategy to tackle specific characteristics on each patient considering PH subtypes as unique and different entities rather than a common one. For this endeavour, it is necessary to have biomarkers that allow us to better discriminate between patients. Recently, Mirabegron, a β3-adrenergic agonist approved by drug agencies in the treatment of overactive bladder, has been proposed by us and other for its use in advanced stages of heart failure and PH acting on the endothelial cells[2-4]. In this sense, the recent clinical trial SPHERE-PH was carried out by our group at H.U. Clinic de Barcelona with neutral results[2]. We believe that a better molecular characterization of these patients could help us to predict the responsiveness of Mirabegron and will allow us to design a personalized therapeutic strategy. Therefore, the purpose of the current work is to search potential prognostic biomarkers related to endothelial health, inflammation and vascular remodelling that allow us to select patients that will be susceptible to be successfully treated with Mirabegron. For this purpose, we have investigated the levels of 24 circulating markers by using Luminex XMAP technology to determine the exact concentration of these analytes on plasma from 85 patients enrolled at SPHERE-PH clinical trial either before and after 16 weeks under treatment, and comparing the effectiveness of mirabegron depending on previous expression of biomarkers. Normal markers levels were obtained from 18 healthy donors. Our results show the potential use of endothelial damage biomarkers to predict a positive effect of Mirabegron over the pulmonary vascular resistance values, therefore allowing us to detect susceptible patients for a positive precision medicine strategy. These results will also help us to better understanding Mirabegron mechanism of action and validating as a therapy against PH.

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Acknowledgements:

This research was funded by Ministerio de Ciencia, Innovación y Universidades/Agencia Estatal de Investigación MCIN/AEI/10.13039/501100011033 (PID2021-123167OB-I00), by CSIC Talent Attraction program (20222AT010) and by Proyectos de Investigacion en Salud of Instituto de Salud Carlos III (PI21/01690). LdlBC is beneficiary of a predoctoral fellowship granted by Comunidad de Madrid (PIPF-2022/SAL-GL-24824). CNIC is a Severo Ochoa Center of Excellence (CEX2020-001041-S) funded by MCIN/AEI.

P53

Rapamycin reduces senescence-associated secretory phenotype in human endothelial cells

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Background: In a previous study, the effect of rapamycin treatment on the replicative senescence of human umbilical vein endothelial cells (HUVEC) was evaluated, showing a significant increase in culture lifespan and an improvement in cellular senescence markers. The rapamycin treatment also preserved the endothelial phenotype and functional capacities of HUVEC, such as new capillary formation and wound closure [1].

Objective: We aim to investigate the effect of rapamycin on the senescence-associated secretory phenotype (SASP). Understanding how rapamycin modulates SASP in senescent endothelial cells could provide valuable insights into its potential mechanisms for cardiovascular disease prevention and lifespan extension in humans.

Methods: HUVEC from healthy donors were cultured and passaged until replication ceased. Rapamycin (10 nM) or vehicle was added to the culture media, with weekly 24-hour treatments. Population doubling levels were calculated, and assays were performed at passages 4, 10, 15, 20, and 25. These assays included PCR for evaluating interleukin-6 (IL-6), Western blot for measuring ICAM1 and VCAM1 adhesion proteins expression, and flow cytometry for measuring reactive oxygen species (ROS) generation. Additionally, a study of HUVEC adhesion to THP-1 monocytes was performed. Finally, THP-1 were treated with 48-hour conditioned medium from the HUVECs and tested for ROS generation and phosphorylated STAT3 (pSTAT3) VCAM and ICAM expression by Western blot.

Results: pSTAT3, ICAM1, IL-6 and ROS levels increased in HUVEC with cell passages, an effect significantly reduced by rapamycin (10 nM, 24 hours/week) treatment. In contrast, no significant changes in monocytic adhesion or VCAM1 expression were observed with either cell passages or rapamycin. In THP-1 monocytes exposed to conditioned medium from HUVEC, ROS generation, pSTAT3, and ICAM1 expression increased parallel to the HUVEC passage number, an effect reversed when conditioned medium originated from rapamycin-treated HUVEC.

Conclusion: Rapamycin treatment attenuated SASP in HUVEC, suggesting a potential role for this drug in preserving endothelial function and mitigating pro-inflammatory responses associated with endothelial senescence, implicating its therapeutic potential in cardiovascular health and aging-related disorders.

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Acknowledgements: Este proyecto fue financiado por el Ministerio de Ciencia e Innovación (PID2020-119178GB-I00) y 'Xunta de Galicia: Ayudas para la consolidación y estructuración de unidades de investigación competitivas del SUG, 2023-2026' (EDT431C 2023/22. Research group GPC GI-1862). Aitor Picos agradece de forma personal al proyecto "V Contratos de Iniciación a la Investigación" (PG062 06/2021) por el apoyo económico.

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Age and sex-related modulation of miR-125b-5p expression in experimental myocardial infarction

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Background: The influence of age and sex differences in the prevalence and presentation of cardiovascular diseases is understudied. miR-125 family participates in the development of cardiovascular diseases, although the specific role of miR-125b-5p in the sex-differential response to ischemic cardiac injury remains unclear. We studied miR-125b-5p expression and its predicted target genes in an experimental model of AMI in male and female Senescence-Accelerated Mice (SAM) and their dependence on estrogens in cultured endothelial cells.

Material and methods: AMI was surgically induced by coronary artery ligation in six-month-old SAM-Resistance (SAMR1, n=24) and SAM-Prone (SAMP8, n=24) mice (2020/VSC/PEA/0128, University of Valencia). Sham-operated mice were used as control. Mice were euthanized four hours after surgery and hearts were collected. Human Umbilical Vein Endothelial Cells (HUVEC, Lonza) were treated with physiological concentrations of 17β-estradiol (0.1 - 10 nM) for 24 h. RNA expression was evaluated by RT-qPCR using snRNAU6, RNU48 and GAPDH as endogen controls. miRNA targets were predicted by bioinformatic tools (TargetScan, microT-CDS and miRDB databases), followed by functional enrichment analysis using DAVID web server (National Institutes of Health).

Results: Ischemic injury down-regulated cardiac miR-125b-5p in male and female SAMR1 mice ($p<0.001$ and $p<0.01$ respectively) and male SAMP8 mice ($p<0.05$), whereas the miRNA expression was unaltered in senescent female mice. Predicted target genes CDH5 (VE-cadherin) and ITGA9 (integrin-α9), which are related with angiogenesis and hypertrophic cardiomyopathy respectively, were conversely overexpressed in injured cardiac tissue from all mice groups ($p<0.05$) except in female SAMP8 mice. Estradiol treatment in endothelial cells reduced miR-125b-5p expression from 0.1 nM ($p<0.05$) and increased ITGA9 and CDH5 mRNA levels ($p<0.05$).

***Conclusions*:** Dysregulation of miR-125b-5p signaling after ischemic injury points to its possible involvement in this pathological process. Moreover, results obtained in senescence-accelerated mice and in cultured endothelial cells, demonstrate the influence of age- and sex-related miR-125b-5p regulation after AMI, highlighting the importance of disaggregating data to avoid sex bias.

Acknowledgements:
Funded by the Spanish Ministry of Science and Innovation (ISCIII) PI22/1083 co-financed by the European Regional Development Fund (ERDF), and by the Generalitat Valenciana (CIAICO 2021/211; CIGE/2021/158). BDB is a predoctoral researcher (CIACIF/2022/331) from the Generalitat Valenciana.

RESPIRATORY, GASTROINTESTINAL, AND PAIN PHARMACOLOGY

P55

Development of a model of tobacco smoke-induced cellular senescence for the study of pharmacological therapies against premalignant oral lesions

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Cellular senescence is a response to various stressors that drive proliferating cells into a terminal state, which can be prematurely induced by certain environmental conditions [1, 2]. Repeated exposure to tobacco smoke extract has been shown to induce senescence in cell cultures [3]. Senescent cells exhibit increased expression of biomarkers such as p16 and p21, and decreased expression of lamin B. These biomarker changes are often observed in premalignant oral lesions, highlighting a potential link between senescence and the initial stages of oral carcinogenesis [4]. Therefore, targeting cellular senescence may offer a strategy to prevent the progression of premalignant oral lesions to oral cancer.

Oral mucosa samples were obtained from two groups of patients: smokers (n=15) and non-smokers (n=15). RNA was extracted and the gene expression of p16, p21 and lamin B was analyzed by qPCR. Additionally, Primary Human Oral Keratinocytes (POHK) and Primary Human Oral Fibroblasts (PHOF) were cultured and exposed to different concentrations of cigarette smoke extract (CSE) (from 1% to 5%). RNA was extracted and gene expression was subsequently analyzed by qPCR.

A significant increase in p16 and p21, and a decrease in lamin B, were observed in the oral mucosa of smoking patients compared to non-smokers. Similarly, POHK and POHF cultures treated with CSE exhibited significant changes in these biomarkers, mirroring the senescent state induced by tobacco smoke in patient samples.

The induction of cellular senescence in POHK and POHF cultures by CSE provides a viable model for studying the effects of tobacco smoke on oral cells. Given the association between cellular senescence and oral cancer, this model could be used to evaluate the efficacy of pharmacological agents in reversing senescence, potentially preventing the progression of premalignant lesions to oral cancer.

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Acknowledgements:
This study it is been supported by the "Cátedra ASISA – Universidad Europea"

P56

Characterization of JAK/STAT pathway activation in ulcerative colitis patients

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Ulcerative colitis (UC) is a condition characterized by inflammation and continuous ulcer formation along the colon, following a characteristic relapsing-remitting course. UC is associated with an alteration in intestinal homeostasis, leading to immune system overstimulation and chronic inflammation. Despite advancements in treatment, a significant proportion of patients remain non-responsive, resulting in a relatively high treatment failure rate. This failure is proposed to be due to the heterogeneity in the activation of inflammatory pathways involved in the pathophysiology of UC, such as the JAK/STAT pathway.

This study proposes a methodology to characterize the activation of the JAK/STAT pathway in UC patients and analyze its variability among individuals. The levels of phosphorylated proteins belonging to the JAK/STAT pathway in colon samples from each patient were determined using the Western Blot technique.

The analysis results indicate a significant increase in the phosphorylation levels of all studied JAK and STAT pathway proteins, with variation observed between specific proteins and the intensity of activation among different patients.

These findings pave the way for the differential stratification of patients in the selection of UC treatments.

P57

Pharmacological inhibition of mTOR/PI3K pathway improves bleomycin-induced cellular senescence in Idiopathic Pulmonary Fibrosis fibroblasts

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Idiopathic pulmonary fibrosis (IPF) is a rapidly progressive lung disease with poor prognosis, where current treatments have low clinical impact. Cellular senescence, a pathology driver in age-related diseases, has been described in IPF [1,2], with an overexpression of senescence biomarkers p21 and p16 and release of IL-6 and IL-8 [3]. Bleomycin induces senescence in lung cell cultures [4]. Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/AKT)/mammalian target of rapamycin (mTOR) signaling pathway can be considered as a master regulator for cellular senescence in IPF [1,5,6]. This study explored the effects of omipalisib (a dual PI3K/mTOR inhibitor), sapanisertib (a mTORC1/mTORC2 inhibitor), rapamycin (a mTORC1 inhibitor) and metformin (an AMP-activated protein kinase (AMPK) activator) on cellular senescence induced by bleomycin in cell cultures of Normal Human Lung Fibroblasts (NHLF) and Diseased Human Lung Fibroblasts from IPF (DHLF-IPF).

NHLF and DHLF-IPF were incubated with omipalisib (0.1-10 nM), sapanisertib (0.1-10 nM), rapamycin (0.1-100 nM) or metformin (2 mM) and exposed to bleomycin (40 µg/mL) over 72h. The mRNA expression of the cellular senescence markers p21 and p16 was measured by RT-qPCR. IL-6 and IL-8 release was measured by ELISA.

Bleomycin (40 µg/mL) increased gene expression of senescence markers p21 and p16 to a greater extent in DHLF-IPF than in NHLF. The same dose of bleomycin increased the release of senescence markers IL-6 and IL-8 in NHLF and DHLF-IPF. Specific doses of metformin, rapamycin, omipalisib and sapanisertib reduced significantly bleomycin-induced gene expression of p21 and p16, and the release of IL-6 and IL-8.

The inhibition of mTOR/PI3K pathway using metformin, rapamycin, omipalisib or sapanisertib improves bleomycin-induced cellular senescence state in lung fibroblasts from IPF patients, and can be considered as potential therapeutic strategy in clinical IPF treatment.

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Acknowledgements: This work was supported by a research grant (Reference: CIGE/2021/114) from the Regional Government "Consellería de Educación, Universidades y Empleo (Generalitat Valenciana).

NOVEL APPROACHES FOR THE DESIGN, DEVELOPMENT, AND DELIVERY OF DRUGS

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Bis-(hydroxymethyl) propionic acid-based dendrimers efficiently transfect siRNA and block key signaling pathways in glioblastoma cells

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Glioblastoma multiforme (GBM) stands as one of the most prevalent and aggressive malignant brain tumors. Its prognosis remains bleak, with a 5-year survival rate of about 2% [1]. The treatment options currently available include surgery, radiotherapy, and chemotherapy with the use of temozolomide (TMZ). However, the tumor relapses frequently and the second line of treatment is much less effective. In addition, the blood-brain barrier represents a major obstacle for achieving efficient drug concentrations reaching the tumor [2]. Consequently, there is a pressing need for novel therapies that will improve patients' outcome. In this context, nanotechnology emerges as a promising avenue for addressing diseases with low survival rates and challenging treatment, such as GBM [3].

We have synthesized several generations (G1-G3) of a novel family of hydrolytically stable amino functionalized 2,2-bis-(hydroxymethyl) propionic acid (bis-MPA) based polyester dendrimers and explored their interaction with siRNA. Subsequently, we studied the cytotoxicity induced by the dendrimers on various GBM cell lines, astrocytes and neurons using lactate dehydrogenase release assays. Finally, we used the Western Blot technique to evaluate the effect of specific siRNAs, transported into the cells by bis-MPA dendrimers, to knockdown different proteins (p42-MAPK, Rheb, MGMT) involved in proliferation and/or survival of GBM cells.

We found that G3 dendrimers, with 24 peripheral functionalities, bound siRNA and protected it from RNAses-mediated degradation, a key step for its use on living cells. These dendrimers were not toxic at the concentrations used for transfection (one µM) for either GBM cells, astrocytes or neurons. The dendrimers were able to transport specific siRNA into GBM cells and reduce the intracellular levels of the target proteins (p42-MAPK, Rheb, MGMT) to about 10 to 20 % of control values. This will likely interfere with the signaling pathways involved in proliferation and/or survival of GBM cells.

The results suggest that the studied bis-MPA dendrimers are excellent siRNA transfectant agents that could play a role in the developing of future effective therapies against GBM.

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P59

Exploring antipsychotic-induced changes in peripheral sensory neurons: Insights from the F11 cell line

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Antipsychotics have known effects on the development of neurons and in neurite length in the central nervous system (Frost et al., 2010). However, their effects on peripheral neurons are not well understood, and data on their impact on neuropathic pain are contradictory (Kim et al., 2022). The F11 cell line, an immortalized sensory neuron line, has been used to investigate the effect of antiviral and antitumor drugs on peripheral neurons as well as to explore new drugs and targets for the treatment of neuropathic pain (Martínez et al., 2024).

The hypothesis of this study is that F11 cells could be a useful model for studying the effect of typical and atypical antipsychotic drugs on sensory neurons. The objectives were to evaluate the effects of various antipsychotics on calcium response and neurite length in differentiated F11 cells.

The study utilized the F11 cell line to investigate the effects of typical (haloperidol) and atypical (quetiapine and risperidone) antipsychotics. Cells were stimulated with 30 mM KCl to measure calcium influx using fluorescence imaging techniques. Neurite length was assessed after treatment with different concentrations of the drugs. Both haloperidol and the atypical antipsychotics (quetiapine and risperidone) induced a significant increase in calcium influx in F11 cells in response to KCl stimulation ($p<0.05$, Dunnett's test). However, the effect on neurite length varied: haloperidol caused a dose-dependent reduction in neurite length, while atypical antipsychotics did not produce a significant reduction.

These findings demonstrate that F11 cells are an effective model for evaluating the effects of antipsychotics on sensory neurons. The distinct effects observed between typical and atypical antipsychotics could explain the variability in pain perception in patients undergoing antipsychotic treatments as reported in the literature, suggesting new avenues for studying the impact of antipsychotics on neuropathic pain.

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P60

Characterization of L-type voltage-gated calcium channels CaV1.2 and CaV1.3 in the differentiation of the immortalized F11 cell line

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Ion transients, including calcium, have shown to play a significant role on neuronal differentiation, a complex process through which immature neurons acquire the morphology of mature neurons and become excitable. We investigated the role of sodium transients in neuronal differentiation employing the immortalized DRG neuronal cell line F11 (1). This cell line, once differentiated, acquires a sensorial neuron phenotype, which allows the study of diseases like neuropathic pain (2).

Previously, we performed a transcriptomic study of the expression of calcium channels in the F11 cell line before and after differentiation by employing RT-qPCR. The results showed that the expression of L-type calcium channels $Ca_V1.2$ and $Ca_V1.3$ was increased after differentiation. Afterwards, we performed a pharmacological study to evaluate the impact of selective blockers of these channels during differentiation, which reveal a decrease in excitability and neurite outgrowth.

Our hypothesis is that $Ca_V1.2$ and $Ca_V1.3$ may play a role in neuronal differentiation. So, our aim is a futher characterization of these channels during differentiation and discern which of them is involved in this process.

We performed a transfection using $Ca_V1.2$ and $Ca_V1.3$ plasmids in F11 cell line to evaluate the impact in excitability and neurite outgrowth. Cells transfected with $Ca_V1.3$ plasmid exhibited an increase of 30% in the excitability compared to non transfected cells ($p<0.001$, ANOVA followed by Dunnett's post-hoc test). In neurite outgrowth, $Ca_V1.3$ transfected cells presented a increase of 20% in neurite length ($p<0.001$, ANOVA followed by Dunnett's post-hoc test).

These results support the hypothesis that $Ca_V1.3$ voltage-gated channel plays a significant role in the regulation of neuronal differentiation of F11 cell line, since overexpression of $Ca_V1.3$ led to the acquisition of neuronal phenotypic features.

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P61

Synthesis and preclinical evaluation of novel 1,3,5-triaril-pyrazole derivatives as Estrogen Receptor modulators

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The Estrogen Receptor α (ERα) is a ligand dependent transcription factor that belongs to the nuclear receptor superfamily of proteins that plays a critical role in physiological processes and several diseases both in female and male. It has been shown that ER binds an extensive variety of steroid and non-steroidal ligands and the diverse core structures of these ligands cover a broad range of synthetic accessibility. Among these, pyrazoles stand out as heterocyclic pharmacophores that, when modified with suitable substituents, yield high affinity and selective ERα ligands. This work introduces a unique 1,3,5-triaril-pyrazole-core based chemical library aimed at modulating ER dependent activity. Through molecular and induced fit docking studies on ERα structure, the influence of this pyrazole-core and their pyrazoline precursors was examined. Synthesis of several pyrazole isomer sets revealed the importance of phenolic hydroxyl groups in achieving favorable interactions with ERα. Docking studies confirmed that both the affinity for ERα and the orientation of pyrazoline precursors in the binding pocket were akin to their pyrazole counterparts. Thus, high-affinity ligands for hERα and hERβ were identified, with affinities ranging from 40 to 200 nM, and molecular docking values similar to PPT, a ERα selective agonist propylpyrazoletrisphenol. Moreover, these data underscored the critical role of hydroxyl groups on aromatic rings B and C in the structure of 1,3,5-triaryl pyrazole derivatives. Consequently, these compounds showed high potency (EC50 < 100 nM) to induce ERα-dependent transcription in breast cancer cells. However, the relative efficacy varied, ranging from 20% to 150% compared to E2, which suggests that these compounds can function as partial, pure or super-agonist ER ligands. Furthermore, the upregulated ERα-dependent transcription positively correlated with an augmented proliferation of ERα breast cancer cells. In summary, modification based on the 1,3,5-triaril-pyrazole-core represents a highly efficient chemical strategy for developing novel partial or pure agonist ligands capable of modulating ER-dependent transcriptional activity. Collectively, these findings demonstrate that a unique chemical library can facilitate the identification of small-molecules with potential therapeutic applications for treating diseases associated with deficient ER activity.

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Acknowledgements: This research was funded by the MICINN, (RTI2018-094356-B-C21) and MCIN-AEI (PID2022-136549B-100) and ACIISI (Pro ID 2017010071, Pro ID 2021010037). These projects are also co-funded by the FEDER. P.L.R. thanks the Spanish MINECO for a predoctoral FPU grant. Á.A. thanks the Cabildo de Tenerife for a postdoctoral grant Agustín de Betancourt. Finally, M.S.C is a recipient of a grant INVESTIGO 2023 from NEXT-GENERATION EU program.

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Development of a novel neuronal-like phenotypic in vitro model of familial Alzheimer´s using the human neuroblastoma-derived inmortalized cell line SHSY-5Y

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Alzheimer's disease is the neurodegenerative disorder with the highest prevalence globally with an expectancy of affecting quadruple the patients by 2050, presents a critical health, economic and social challenge in the present and future. Currently, Alzheimer´s disease remains without a cure, making the pathology a major focus for the pharmacological industry [1]. Taking all of the given difficulties underlying the disorder, we aimed in this study to obtain an in vitro model of familial Alzheimer´s disease by employing the neuroblastoma-derived immortalized cell line SH-SY5Y chemically transfected with two mutations in both amyloid precursor protein and Tau protein (APP V717I and MAPT P301L), which could be used in high-throughput screening assays for the identification of new potential pharmacological candidates for the pathology.

SH-SY5Y immortalized cell line was differentiated into a mature neuronal phenotype using two distinct protocols (Retinoic acid (RA) + Phorbol 12-myristate 13-acetate (PMA); RA + Glucagon like peptide 1 (GLP-1)) to test efficacy and reproducibility of the models. The delivery of the mutated material was introduced in the cells through chemical transfection. Analysis on the morphology of the differentiated cells was performed with an Operetta® high content microscope (Software Harmony 4.1.). Neuronal excitability was measured by the monitorization of intracellular Ca^{2+} by using the fluorescent probe *Calcium 6* upon cell exposure to 30mM KCl.

Differentiation of SHSY-5Y cell line employing 10 RA µM and 1 GLP-1 µM yielded cell cultures with a higher number of cells and arborization ($p<0.0001$) versus those differentiated with 10 RA µM + 10 nM PMA. Transfection with either MAPT P301L or APP V717I significantly decreased average cells per well ($p<0.0001$), average neurite length ($p<0.05$) and dendrite arborization ($p<0.01$). Double transfection with both mutations showed a significantly reduction in all the parameters assessed ($p<0.0001$). This reduction was significantly reverted by 1 µM Melatonin while 1 µM of resveratrol did not revert the decrease on arborization and average number of cells when cells were transfected with the V717I mutation. Differentiated cells showed a significatly higher increment in the Ca^{2+} signalling upon depolarization using KCl versus those differentiated and transfected with both mutations ($p<0.0001$). Both 1 µM of resveratrol and melatonin, significatly reverted the lowered signal that was registered in the mutated cultures ($p<0.0001$).

In this study, we developed a novel in vitro model based on the use of a neuroblastoma derived cell line differentiated to a mature neuron-like phenotype and transfected to express mutations on the MAPT and APP genes. The model was validated using melatonin and resveratrol at 1 µM which managed to significantly revert the changes observed as result of the transfection.

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New in vitro model of cognitive deficits associated with schizophrenia for High Throughput Screening Assays

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Schizophrenia is a chronic and debilitating mental disorder that affects up to 1% of the population worldwide. Patients suffering from this disorder present positive (hallucinations), negative (social withdrawal, anhedonia) and cognitive symptoms (memory and attention impairments). Current treatments address positive and negative symptoms quite effectively, but cognitive symptoms remain mostly untreated. There is also a lack of suitable predictive models of the disorder, which further hinders advances [1].
In this work, our aim was to develop a phenotypic neuronal model based on immortalised cell lines, mimicking cognitive deficits associated with schizophrenia, which could be employed in High Throughput Screening campaigns.
Differentiation of the human SH-SY5Y cell line was carried in two different ways: starting with a 5-day exposure to retinoic acid (RA), cells were exposed for 7 days to i) phorbol 12-myristate 13-acetate (PMA), or ii) glucagon-like peptide-1 (GLP-1). In concordance with previous studies [2], the obtained cells showed a neuronal phenotype, confirmed by the expression of tyrosine hydroxylase (TH) and/or the vesicular glutamate transporter type 2 (VGLUT2), employing an Operetta High Content System to study fluorescence. Characterisation of electrophysiological activity of the cells using Microelectrode Arrays (MEAs) was also assessed, obtaining no signal for the PMA-induced cells, but showing action potentials in GLP-1-induced cells. Interestingly, both models showed significant activity and neuronal firing in 3D cultures.
Induction of damage to simulate cognitive deficits on the cellular model was carried out incubating the cells with sub-chronic doses of different compounds dysregulated in schizophrenia: excess of dopamine and NMDAR antagonists.
After a 72-hour incubation at different doses and combinations of the tested insults, there was a significant increase in the membrane potential, which could be partially reverted by the atypical antipsychotic clozapine, but not with the typical antipsychotic haloperidol.
In conclusion, exposure to dopamine, glutamate, and MK-801 for 72 hours, modified the response of the cells, mimicking the phenotype observed in patients with cognitive deficits associated with schizophrenia. Our developed model will be useful for running screening campaigns to identify compounds which could revert these alterations.

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Acknowledgements: This work was supported by a predoctoral grant of the Xunta de Galicia (ED481A 2021/222), and by other grants of the Xunta de Galicia (ED431C 2022/20), Agencia Estatal de Investigación (PID2020-119428RB-I00), and by the Fondo de Desarrollo Regional Europeo (ERDF)

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Enhancing neuroprotection with repositioned drug combinations in neurodegenerative diseases

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During the second half of the 20th century, the one-disease-one-target-one-drug paradigm has been incredibly successful with numerous diseases. In complex chronic diseases, however, it has been the combination of drugs that, acting on two or more pathogenic targets, has provided better results in the last two decades; as in the case of cancer, heart failure, hypertension or cardiac insufficiency, hypertension or AIDS. In our laboratory, certain *in vitro* (Alejandro Romero et al., 2010) and *in vivo* (Mónica Sobrado et al., 2003) experiments support this hypothesis of neuroprotective synergy with the combination of melatonin and galantamine or with citicoline and nimodipine.

In our current work, we are looking for drugs that can enhance neuroprotection linked to pathogenic pathways involved in neurodegenerative processes. The search for candidates is performed on repositioned and selected drugs belonging to the FTH chemo library that have been shown to be Ca_VL1 calcium channel blockers or Orai1 calcium channels, and others that block the purinergic receptor P2X7, or induce the transcription factor Nrf2 that exhibits antioxidant effects. These drugs have been previously screened with computational chemistry techniques to obtain a theoretical prediction of their potential blockade on the targets included in our project.

The possible neuroprotective additivity or synergism is being studied in SH-SY5Y cells together with the addition of toxics capable of mimicking mechanisms associated with neurodegenerative pathologies under study (Parkinson's disease, Alzheimer's disease). We will present data on this protection afforded by some drugs currently under study.
It is concluded that: (1) we have found drugs in clinical use, with affinity for some of the study targets; (2) we have defined the conditions for the study of neurotoxicity, neuroprotection and neuroinflammation in these cell lines; (3) we have found some drugs with affinity for targets other than those implicated in their current clinical indication, with potential neuroprotective with a potential neuroprotective effect, either individually or in combination.

Acknowledgements:
Teófilo Hernando Foundation and University Autónoma de Madrid.

TEACHING INNOVATION IN PHARMACOLOGY

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Instructional value of audios in higher education: Student perspectives

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Background. In recent years we have been encouraged to use alternative methodologies to improve students' feedback and interest. Instructional audios have become popular among students, as they can be followed in informal learning environments. Thus, the study was designed to explore the effect of several audios as a learning mean to improve their knowledge and motivation.
Material and methods. The population of this study comprises the students enrolled in the subject Drugs & Sport (Degree in Physical Therapy) in the academic course 2022-23 (30 students). They were provided with seven audios related to different topics on doping and drugs over the semester. Perceived student satisfaction with the activity carried out and the academic performance achieved were evaluated.
Results. The majority of students participated in the innovative strategy carried out (90 %). Of those, 44.5 % were women. Downloads ranged from 27 to 11 depending on the audio considered, and gradually reduced throughout the semester. Academic scores varied between 7.4 and 9.9 points (out of 10) in partial examinations, and between 8.4 and 9.8 points in final marks. No improvement was shown in final marks when compared with those obtained in the previous academic year (2022-23) (no significant differences with Mann-Whitney U test, $p \geq 0.05$). The satisfaction survey was answered by 13 students (48.1% of the participants), and students' opinions were positive. Most of them pointed out that the number of audios provided was adequate (92.3 %), and not difficult to follow (84.6 %). All of them showed a high level of satisfaction with the innovative strategy developed.
Conclusions. The results provide evidence that instructional audios have been well received by students. They may be an effective and engaging resource, in order to support and reinforce essential concepts, and enable meaningful and active learning.

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A gamification case study of an interactive approach to teaching antibiotic use in veterinary pharmacy

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Inappropriate use of antibiotics is a major cause of the emergence and spread of resistant bacteria, posing a significant global challenge to modern medicine. Community pharmacists play a crucial role in dispensing antibiotics for both human and veterinary use. A notable example is the vital role community pharmacies play in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) network, where they must report annual sales data of antimicrobials for veterinary use. Hence, pharmacists need fundamental training in the use of antimicrobials in animals.

In this context, the Faculty of Pharmacy at the University of Santiago de Compostela introduced an elective course in 2011 called Veterinary Pharmacy for fifth-year students. This course aims to succinctly review the characteristics of drug use in animals, considering physiology, common infectious diseases, pharmaceutical technology, and pharmacology. As part of the pharmacology section, a seminar on the use of antimicrobials in common veterinary diseases was included.

The objective of this study was to apply a gamification tool to teach the seminar on antimicrobial use in veterinary pharmacy. Students were provided in the seminar with a table listing the main infectious diseases of bacterial aetiology, indicating the most frequent aetiological agents and the characteristics of the microorganisms, including cell envelope (Gram staining), oxygen requirement (aerobic, facultative anaerobic, and strict anaerobic), and whether they are intracellular or extracellular. Prior to the seminar, students had reviewed the action spectra of the main groups of antimicrobials.

During the seminar, students used the Kahoot platform, which presented fifteen questions where they had to choose among four antimicrobials to treat various bacterial infections. This experience was conducted over five academic years (2019/2020 to 2023/2024) with groups averaging 10 students. The students' correct response rate was 68% during the seminar. In the final exam, a question related to the seminar was included, and students who previously participated in the seminar achieved an 87% correct response rate.

Thus, using gamification platforms allows students to review the spectra of antimicrobials interactively and apply this knowledge to treating common infectious diseases in veterinary pharmacy.

Acknowledgements:
We want to express our gratitude to the students who participated in this experience. We would also like to acknowledge the Faculty of Pharmacy of the University of Santiago de Compostela. Also, to Professor Dolores Viña for providing the materials to develop the Pharmacology section of the subject.

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Implementation of combined physiology and pharmacology practices in the degree in human nutrition and dietetics

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During the teaching-learning process, it is essential that students know how to interrelate the knowledge acquired between the different subjects of the degree they are studying. The objective of this study was to relate the knowledge of physiology and pharmacology to increase the ability of human nutrition and dietetics students to solve problems in a real situation. The topic chosen to carry out this experience was the renal system. A practical activity was designed in two phases, the first focused on renal physiology and the second on the pharmacology of diuretic drugs. The practice of renal physiology was carried out in the second course of the degree and the practice of diuretic drugs was carried out in the third year of the degree. In both subjects, the software for physiology laboratory simulations PhysioEx 10.0 from Pearson was used. In both subjects, the activity "effect of hormones on urine formation" was carried out. In the subject of "physiology", the activity was carried out in two groups of practices, which was evaluated through a test-type knowledge questionnaire before and after performing the exercise with the PhysioEx software. In pharmacology the activity was carried out with 4 groups of practices. The activity with the PhysioEx software was carried out in 2 practice groups and the other 2 groups (control groups) did not perform this activity. To evaluate activity, a question about aldosterone antagonist diuretics was included in the final exam and the result was compared between the control groups and the groups that had used the computer tool. In the subject of physiology, the questionnaires given to the students prior to the simulation with the computer program had a success rate on the part of the students of 94% and 67%. Once the simulation was carried out, the percentage of correct answers in the same questions was 100% in both groups. However, in the subject of pharmacology, the data show that the percentage of success after using the PhysioEx program was 26.32% and 18.18%, while the students who did not use the computer software obtained a success percentage higher, 63.16% and 44%. With respect to the results obtained in the practice of pharmacology, it should be added that the average exam grade of the control groups was also higher than that of the groups that used the computer program. Therefore, we could conclude that multiple choice questions do not always reflect the skills acquired by students during the development of a practice. For future experiences, other evaluation methods will have to be considered to be able to assess the skills acquired in the practical part of a subject.

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Scientific poster presentations as oral evaluations: Bringing learning pharmacology closer to reality

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Background: Creating research posters is a key scientific skill. To help our Neuroscience Master students gain such experience, and in the context of the course "Neuropharmacology and Neurotransmission Systems", we substituted our traditional 20-minute oral presentations on central nervous system pathologies and their pharmacological treatments for the design and presentation of a scientific poster in English. The goal of this approach was to offer a realistic research experience and enhanced their communication abilities [1,2], while promoting the integration of our international students and providing global perspectives for local students.

Materials and methods: The 15 students enrolled in the class first selected a topic for their poster among the pharmacological groups available to treat brain-related disorders. Then, students were given a poster template to help them understand what was expected of the activity in terms of content. After autonomous research, students received support from the instructors during a seminar session to answer any doubts about the activity. Finally, posters were hanged in panels across the classroom so each student could present their design to the rest of the class for 10 min, followed by a 5-min question period. Both instructors and classmates were given an evaluation rubric to assess the quality of the poster, focusing on originality, design, clarity, and question-handling. Students were also given a questionary to rate the activity.

Results: Students meticulously assessed the activity utilizing the evaluation rubrics provided. Their evaluations included various facets, which emphasized the appropriateness and motivational impact of the task. Results highlighted the efficacy of the activity for bridging learning closer to the professional scientific reality. However, students expressed reservations regarding the time investment required for preparation, citing a disproportionate commitment compared to other activities. Furthermore, the linguistic demands posed by English usage added an additional layer of complexity. Nevertheless, most of the feedback remained extremely positive, concluding the need to enhance such initiatives within Master's studies.

Conclusions: The evaluation by students yielded highly positive results, highlighting the activity's success in integrating academic and professional skills. Despite minor concerns regarding time and language challenges, the feedback underscored a pivotal role for this activity in nurturing essential competencies for future careers. These outcomes underscore the significance of incorporating such initiatives into educational settings.

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Acknowledgements: Institut de Recerca i Innovació Educativa (IRIE); PID232509.

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Pharmaceutical intervention of USJ students on the dispensing of medicinal plants: Protocols of the pharmaceutical indication service of the General Council of Pharmaceutical Associations

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Pharmacists are the health professionals most specialized in medicinal plants, due to their curricular training in pharmacognosy, botany and phytotherapy in their undergraduate studies. The General Council of Pharmaceutical Colleges (CGCOF) proposed the tools and relevant information for its application in this professional activity. Consequently, a series of "Pharmaceutical Indication Service Protocols" were developed, consisting of a collection of nine protocols that will allow pharmacists to decide which is the most optimal dispensation based on the pathologies presented by patients[1].

From the Phytopharmacy subject, the students of the Degree in Pharmacy at the San Jorge University (USJ) apply the Protocols of the Pharmaceutical Indication Service during their practices in the pharmacy office to identify presentations appropriate to the patients' pathologies.

After studying the documents established by the Council, pharmacy students carry out a search for medicinal plants and products suitable for the treatment of various pathologies (cardiovascular, immunological, digestive). Furthermore, thanks to the database on the fitoterapia.net website they can find extensive and more detailed information on pharmacological actions, indications and adverse effects as well as the presentations that are most suitable for each patient.

Once the dispensing has been completed, the pharmacist must speak to the patient to conclude the pharmaceutical care service provided. With this work methodology, students are able to decide which is the most appropriate pharmaceutical indication for each pathology according to the presentations available in the pharmacy office and the protocols of the General Council of Pharmaceutical Colleges.

Finally, Phytopharmacy students have demonstrated efficient application of the Pharmaceutical Indication Service Protocols during their practices in the pharmacy office, helping patients improve their pathologies.

1. Consejo General de Colegios Oficiales de Farmacéuticos. (2023) [Internet]. Disponible en www.Farnaceuticos.com

Acknowledgements:
Universidad San Jorge is thanked for giving financial support.

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Could clinical simulation in an interactive structured objective clinical examination scenario improve undergraduate students' learning about personalized therapy?

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Background and Aim. Practical training in pharmacology based on Clinical Simulation in a Structured Objective Clinical Evaluation scenario is a strategy to improve the quality of learning for students of Medicine, Podiatry and other Health Sciences disciplines. If it is also implemented with possible evolutions and different responses of the patient (real or simulated) based on their interaction with the students, it can bring the student closer to the scenario and needs of personalized medicine and therapy. ***Aim***: To evaluate the impact of clinical simulation in an interactive OSCE (SC-OSCE) scenario with different characteristics and evolution of patients based on patient interaction with the students in communication, diagnosis, learning of medications and therapeutic skills development in students of Medicine and Podiatry Degrees.

Methods. Two-year prospective study in which students of the Medicine Degree (Anaesthesia and Psychopharmacology subjects) and the Podiatry Degree (Pharmacology subject) were invited to voluntarily form groups (10 students maximum). Each group must design and solve their SC-OSCE (5-10 min) showing a clinical situation in which medications use was necessary. Each group, based on the common initial data of a patient named "Josefa", had to interact with the patient (questions and answers about medical history, symptoms, tests, treatments and evolution, search for evidence) and solve the SC-OSCE in a personalized way based on the interaction developed with the patient. Medical history, mobile phone video, photographs, actors, simulators, use of bibliographic bases and CIMA were allowed. Students were invited to present their work to the rest of the class so that each group could compare how different interactions with the same patient could condition different treatments and responses. Students were encouraged to ask questions and to voluntarily responded a satisfaction survey.

Results. 745 students were enrolled, 65.4% women, 20±3.7 years old. 82 SC-OSCEs showing a clinical situation or clinical problem were performed and resolved. The average time spent by students completing the SC-OSCE was 3.56±0.3 h. This activity increased their success rate in the final evaluation (+20.1%). The percentage of students who were satisfied with this activity was 95.6%. All students were able to compare how different interactions can lead to different therapeutic approaches and patient outcomes.

Conclusion. Clinical Simulations in an interactive Structured Objective Clinical Evaluation scenario designed and solved by undergraduate students improved their communication skills, their results in the final evaluation and their understanding of what personalized medicine is.

Acknowledgements: Subvencionado por el Grupo Permanente de Innovación Educativa (GpIE) PIE22-038-GpIE en Simulación y ECOEs (SimEco) convocatoria INNOVA22 de la Universidad de Málaga.

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ChatGPT use during practical lessons of Pharmacology

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Artificial intelligence (IA) is nowadays part of the life of our students, who daily make use of AI systems to solve tasks set in class. Although helpful, these systems may generate incorrect information, which should be noted by the users, who must be aware and supervise the solutions offered by this IA. It is important that the students have a real understanding of the advantages and disadvantages of the use of the IA on a daily basis. Here we present a learning activity carried out with the students of "Pharmacology and principles of biochemistry", a compulsory subject included in the third year of the Degree in Biomedical Engineering. The activity was set during a computer practical session lasting two hours, and the students were distributed in groups of 2-3 people. First, the students were asked to choose between one of out of the three proposed projects: (1) Create a computer program to perform pharmacokinetic calculations; (2) design a chip that would be implanted in a patient for determining the values of a drug in a tissue; (3) design a device for controlled drug release that delivers a specific amount of substance when implanted in the patient. Next, the students were asked to discuss and choose, without consulting any source of information, the parameters that are considered essential to carry out this project. Then, they were allowed to complete this information by searching sources of information related to pharmacology. Finally, the students were asked to perform a search using ChatGPT, a free-to-use AI system, and critically assess the suitability of the parameters reported by this application, analyzing their usefulness for the development of the previously chosen project. The students' perception of the activity was evaluated by conducting anonymous surveys, who mostly chose projects 2 and 3. The search in databases related to pharmacology allowed the undergraduates to complete the pharmacological parameters they had chosen according to their previous knowledge. The students stated that the activity was useful for them in order to acquire the skills of the subject. They considered that artificial intelligence is useful to develop projects, but the researcher's critical thinking judgment is required to select the most relevant.

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Escape room activity for teaching Pharmacology in Biomedical Engineering

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Curiosity and competitivity can be used as formative tools to increase the interest of the students for the subject. One of these teaching resources can be an "escape room" activity, based on games that propose the participants to solve some questions or puzzles in order to escape from a closed room. This activity allows the students to interact with their pairs and to reinforce the knowledge acquired during the previous lectures in a relaxed atmosphere, which can be inspiring and motivating.
In this work we show the results of an "escape room" exercise, that was carried out with the students of "Pharmacology and Principles of Biochemistry", a compulsory subject of the third year of the Degree in Biomedical Engineering. The activity took place during the last class of the year, a two-hour session. We prepared four questionnaires with 9 yes-no questions which were based on all the pharmacological knowledge taught during the previous lectures. Each "yes"-response counted for one point and each "no"-response counted for 0 points. The sum of values of the questions included in each questionnaire gave one of the digits, which were necessary to obtain the correct code that opens a four-numbers lock. The students, working in groups of four people, were not allowed to consult any additional source of information during the first thirty minutes of the activity. After the first half an hour, they were able to search for any kind of information, either on the internet or class notes. No clues were given to the students at any time of the test, including the number of digits that were correct in their attempts. The first group that answered correctly all the questions and opened the lock won the exercise. The students' perception of the activity was evaluated through an anonymous online survey.
A total of 52 students participated in the "escape-room" exercise. After the first half an hour, one group of students have solved three of the four-number combination. The lock was opened after 50 attempts in approximately one hour. The students enjoyed the activity and valuated it very positively. They appreciated the dynamics of the exercise and stated that the activity had been useful for them to improve their knowledge in the subject. The teachers involved in the activity also found it useful to reach the teaching objectives of the subject.
We consider that "escape room" activities are a good formative game-based approaches to reinforce the knowledge on a subject.

OTHERS

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Furosemide and omeprazole are the most prevalent drugs involved in pharmacological interactions in polymedicated elderly patients of rural pharmacies of the province of Valencia, Spain

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Drug interactions are considered one of the major preventable causes of harmful adverse effects, mainly in elderly patients under polypharmacy, who are at higher risk due to their age-related changes in pharmacodynamics and pharmacokinetics. Community pharmacists are the most accessible healthcare providers in rural areas and this study was aimed to identify the active ingredients that cause a greater number of Drug-Related Problems (DRP).

Using the software platform Revisem® from the Very Illustrious Official College of Pharmacists of Valencia (MICOF), a pilot observational, prospective study was carried out in 17 community pharmacies from rural municipalities (<5,000 inhabitants) of the province of Valencia, on the East coast of Spain, from August 1, 2023, to April 30, 2024. Inclusion criteria were patients aged 55 years or older and polymedicated (5 or more medications). The patient's pharmacotherapeutic history was registered and a type 1 medication review was automatically performed by Revisem®. DRP were identified following the Pharmaceutical Care Network Europe (PCNE) criteria. The program also generates automatic reports with the recommended pharmaceutical interventions.

The pharmacotherapeutic history of a total of 171 patients (64.9% women) was analysed, detecting a total of 1,390 DRP. The mean age per patient was 76.7±10.6 years, on active treatment with 9.6±4.3 drugs on average. Interactions were the most prevalent DRP (44.6%). The acceptance rate of pharmaceutical interventions was 45.2% and allowed to significantly reduce the incidence of DRP detected per patient from 9.7±6.9 to 8.8±6.9 ($p<0.05$). The active ingredients that appeared most frequently in the interactions studied were Furosemide (13.9%) and Omeprazole (11.1%). Despite being a well-recognized pharmacological interaction, 1.9% of the patients had both drugs prescribed concomitantly. The study of health problems showed that 13.3% of all patients suffered from bone pathologies, which could be aggravated by the interaction mentioned above. A significant correlation was identified between this interaction and the risk of needing supplemental calcium treatment.

These findings support the prominent role of community pharmacists to promote the rational use of medications given their strategic position to detect DRP, for which the computerized platform (Revisem®) has proved to be an excellent tool.

Acknowledgments:
Funded by Chair for the Rational Use of Medicines MICOF-UV.