XLII Annual Spanish Society of Pharmacology Meeting

2nd Meeting on Translational Pharmacology

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Plaza Virgen de la Paz, 3,
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Spain

BOOK OF ABSTRACTS

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WELCOME

On behalf of the **Spanish Society of Pharmacology**, we are delighted to welcome you to the **XLII Annual Spanish Society of Pharmacology Meeting / 2nd Meeting on Translational Pharmacology**, which will take place at **Espai ADEIT** in **Valencia**, Spain, from **September 10th to 12th, 2025**.

As in previous editions, the meeting is expected to bring together a large number of pharmacologists, along with scientists and professionals from related disciplines such as chemistry, pharmacy, and medicine. We have prepared a diverse and engaging scientific program that will cover cutting-edge topics in pharmacology and therapeutics, featuring renowned keynote speakers and insightful panel discussions. Recognizing that the future of science depends on the next generation of researchers, this year's conference will place a special emphasis on supporting early-career scientists. We encourage their participation through short presentations of their research and grants to help cover their expenses to attend the meeting.

Beyond the scientific exchange, Valencia, capital of the Valencian Community in the Mediterranean coast of Spain, offers a unique blend of history, innovation, and charm. From the Gothic beauty of La Lonja and the Cathedral in Ciutat Vella to the futuristic City of Arts and Sciences, the city captivates with its cultural richness. Visitors can enjoy authentic Valencian cuisine, explore the vibrant Central Market, or relax on golden beaches like Malvarrosa and Patacona. Just outside the city, Albufera Natural Park offers a peaceful retreat with boat rides and unforgettable sunsets.

For all these reasons, we look forward to meeting you in **Valencia at the XLII Annual Spanish Society of Pharmacology Meeting / 2nd Meeting on Translational Pharmacology.**

Mª Carmen Montesinos Mezquita

COMMITTEES

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María Jesús Sanz Ferrando
Antonio Vidal Puig
Dolores Viña Castelao
Francisco Zaragozá García

CONGRESS TIME-TABLE

Day 1 10/09/2025	*Room 1*	*Room 2*
8:30-9:00	Delivery of documentation	
9:00-10:30	**Session 1:** Neuropharmacology **Moderator:** Victoria Maneu (Universidad de Alicante) **Invited speaker:** Inés Erkizia (Universidad del País Vasco) "Preclinical insights into psilocybin: psychedelic mechanism and antidepressant effects". **Selected oral communications:** Leslye Paola Valarezo (Universidad de Santiago de Compostela) Rubén García-Cabrerizo (Universitat de les Illes Balears) Jaime Rubio (Universidad de Castilla-La Mancha) Patricio Iturriaga-Vasquez (Universidad de La Frontera, Chile)	**Session 2:** Young SEF Researchers Session "AI in pharmacological research". **Moderator**: Celia Llorente (Universidad Complutense de Madrid). **Invited speakers:** Óscar Pastor (Instituto Universitario Valenciano de Investigación en Inteligencia Artificial; Universidad Politécnica de Valencia); Susana Conde (Johnson & Johnson Innovative Medicine. Global Discovery Chemistry) Álvaro Serrano (Grupo de Bioinformática; Centro Nacional de Investigaciones Cardiovasculares)
10:30-11:00	Coffee-break	
11:00-11:30	Official Opening	
11:30-12:30	**Invited Lecture 1: "**Metabolic drivers of MASLD/MASH". **Moderator:** Mª Jesús Sanz (Universitat de València) **Invited speaker: Antonio Vidal Puig** (University of Cambridge, UK)	
12:30-13:30	**Round Table 1:** "El nuevo marco normativo de los medicamentos". **Moderator:** Francisco Zaragozá (Universidad de Alcalá de Henares) **Invited speakers:** Arantxa Sancho (Farmaindustria); Elena Casaus (Asociación Española de Medicamentos Genéricos); Jordi Dalmases (Consejo General de Colegios Farmacéuticos)	
13:30-15:00	Lunch	
15:00-16:30	**Session 3:** Pain and inflammation. **Moderator:** Antonio R. Artalejo (Universidad Complutense de Madrid) **Invited speaker:** María Paula Ávila (Asociación Colombiana de	**Session 4:** Natural products pharmacology **Moderator:** Nuria Cabedo (Universitat de València) **Invited speaker:** José Mª Prieto (Liverpool John Moores University,

	Farmacólogos, Bogotá DC, Colombia) "Targeting Inflammatory Pathways in Pain Management: Advances and perspectives" **Selected oral communications:** Cristian Bis-Humbert (Universitat Pompeu Fabra) Aràntzazu Alonso-Carrasco (Universitat de Barcelona) Nazaret Prieto (Universidad Cardenal Herrera CEU) María Carmen Carceller (Universitat de València	UK) "Natural cytotoxic products against melanoma: towards the genomic bases of their multitarget synergic activities" **Selected oral communications:** Manuel Alcarranza (Universidad de Sevilla) Carmen Ortiz-González (MolDrug AI Systems SL) Luis Rodríguez-Santos (Universidade de Santiago de Compostela)
16:30-17:30	**Session 5:** Pharmacogenetics and Precision Pharmacology **Moderator:** Luis Sendra (Universitat de València) **Invited speaker:** Mª José Herrero (Universitat de València) "Clinical implementation of Pharmacogenetics" **Selected oral communications:** Diego Robés (Universidade de Santiago de Compostela) Idoia Álvarez-Ajuria (MolDrug AI Systems, València)	**Session 6:** Teaching innovation in Pharmacology **Moderator:** Emilio J. Sanz (Universidad de La Laguna) **Invited speaker:** Manuela S. Rodrigues Morato (Universidade do Porto, Portugal) "Active learning through real world simulations: engaging, realizing and understanding" **Selected oral communications:** María Pilar D'Ocon (Universitat de València) Marina Sánchez-Hidalgo (Universidad de Sevilla)
17:30-19:00	**Poster Session 1** Sala 3	
20:30-22:00	**Welcome Cocktail** Jardí Botànic (C/ Quart, 80, València)	

Day 2 11/09/2025	***Room 1***	***Room 2***
8:30-9:00	Delivery of documentation	
9:00-10:30	**Session 7:** Cardiovascular Pharmacology I **Moderator:** Eduardo Oliver (Centro de Investigaciones Biológicas "Margarita Salas", CSIC, Madrid) **Invited speaker:** Miguel Romero (Universidad de Granada) "Gut Microbiota as a Therapeutic Target in Hypertension: Emerging Mechanisms and Pharmacological Perspectives"	**Session 8:** Neuropharmacology II **Moderator:** Mª Julia García Fuster (Universitat de les Illes Balears) **Invited speaker:** Luis Gandía (Universidad Autónoma de Madrid) "Is amyotrophic lateral sclerosis a "drug-resistant" pathology?" **Selected oral communications:** Raquel Lama (Universidade de Santiago de Compostela) Yaiza Trueba (Universidad

	Selected oral communications: Ricardo Caballero (Universidad Complutense de Madrid) Álvaro Gómez-Martín (Instituto de Investigaciones Sanitarias INCLIVA, València) Susana Novella (Universitat de València) Laura de la Bastida-Casero (Centro de Investigaciones Biológicas "Margarita Salas" (CIB)-CSIC, Madrid)	Complutense de Madrid) Susana García-Cerro (Universidad de Sevilla) Francisco Navarrete (Universidad Miguel Hernández)
10:30-11:00	Coffee-break	
11:00-12:00	**Invited Lecture 2:** "A small TAT-TrkB peptide prevents BDNF receptor cleavage and restores synaptic physiology in Alzheimer's disease". **Moderator:** Ricardo Borges (Universidad de La Laguna) **Invited speaker: Maria José de Oliveira Diógenes Nogueira** (Universidade de Lisboa, Portugal)	
12:00-13:30	**Round Table 2: "**Uso Racional del Medicamento: objetivo compartido" **Moderator:** Mª Luisa Ferrándiz (Universitat de València) **Invited speakers:** Carlos Fluixá Carrascosa (Médico de familia y expresidente de la SVMFiC); Marta Aparicio Cueva (Farmacéutica de Atención Primaria y miembro de la Junta de la SVFAP); Luis Salar Ibáñez (Farmacéutico comunitario y vocal de SEFAC-CV);Mónica Climente Martí (Hospital Universitario Dr. Peset Valencia); Juan Eduardo Megías Vericat (Oficina Autonómica de Medicina Predictiva, Personalizada y Terapias Avanzada (OMPTA) de la Comunitat Valenciana); José Antonio González Correa (Universidad de Málaga y miembro del Comité de Medicamentos de Uso Humano de la AEMPS)	
13:30-15:00	Lunch	
15:00-16:30	**Session 9:** New approaches to drug design, development and administration **Moderator:** Mª Carmen Carceller (Universitat de València) **Invited speaker:** Mª Jesús Vicent (Centro de Investigación Príncipe Felipe, València) "Polypeptide-based Nanomedicines: enhancing tropism and overcoming biological barriers" **Selected oral communications:** Xabier Rovira (Institut de Química Avançada de Catalunya, CSIC, Barcelona) Irene Rodríguez-Clemente (Universidad de Castilla-La Mancha) Ana Jarén (Universitat de València)	**Session 10:** Cardiovascular pharmacology II **Moderator:** Laura Piqueras (Universitat de València) **Invited speaker:** Eduardo Oliver (Centro de Investigaciones Biológicas "Margarita Salas", CSIC, Madrid) "Exploring New Therapeutic Applications of Beta-Adrenergic drugs: From Lab Discovery to Clinical Impact" **Selected oral communications:** Diego Berlanas (Universidad Autónoma de Madrid) Víctor Collado-Díaz (Universitat de València)

	Fernando Yáñez-Gómez (Universitat de les Illes Balears)	Anaïs Clara Terol-Úbeda (Universidad de Salamanca) Dolores Viña (Universidade de Santiago de Compostela)
16:30-17:30	SEF Assembly	
17:30-18:30	**Poster Session 2** Sala 3	
21:00	**SEF Congress Dinner (prior reservation required)** Restaurante Àtic en Palau Alameda (Carrer de Muñoz Seca, 2, El Pla del Real, València)	

Day 3 12/09/2025	***Room 1***	***Room 2***
9:00-9:30	Delivery of documentation	
9:30-10:30	**Session 11:** Medicina personalizada. Terapias avanzadas **Moderator:** Antonio R. Artalejo (Universidad Complutense de Madrid) **Invited speaker:** Andrea Romero (Konexio Biotech, Madrid)	**Session 12:** Digestive & Respiratory Pharmacology **Moderator:** Mª Carmen Montesinos (Universitat de València) **Invited speaker:** Raquel Abalo (Universidad Rey Juan Carlos) "Enteric neuropathies induced by antitumoral drugs" **Selected oral communications:** Clara Quintas (Universidade de Porto, Portugal) Carmen de la Fuente-Gómez (Universitat de València)
10:30-11:00	Coffe-break	
11:00-12:00	**EPHAR Lecture:** "Development of miRNA therapeutics for cardioprotection and other indications" **Moderator**: Eva Delpón (Universidad Complutense de Madrid) **Invited speaker**: Péter Ferdinandy (Semmelweis University, Hungary)	
12:00-12:45	**SEF Young Investigator Award and Lifetime Achievement Award in Pharmacology**	
12:45-13:00	Proyecto "Mapa de capacidades en investigación de la SEF"	
13:00-13:45	**Closing ceremony and remarks**	

ABSTRACTS

INVITED LECTURES

Metabolic drivers of MASLD/MASH

Antonio Vidal-Puig

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The link between obesity and type 2 diabetes is clear on an epidemiological level, however the mechanism linking these two common disorders is not well defined. One hypothesis linking obesity to type 2 diabetes is the adipose tissue expandability hypothesis. The adipose tissue expandability hypothesis states that a failure in the capacity for adipose tissue expansion, rather than obesity per se is the key factor linking positive energy balance and type 2 diabetes. All individuals possess a maximum capacity for adipose expansion which is determined by both genetic and environmental factors. Once the adipose tissue expansion limit is reached, adipose tissue ceases to store energy efficiently and lipids begin to accumulate in other tissues. Ectopic lipid accumulation in non-adipocyte cells causes lipotoxic insults including insulin resistance, apoptosis and inflammation. In this lecture, we will address the mechanisms of adipose tissue expandability and briefly address the antilipotoxic role of BAT and the relevance of qualitative aspects of lipotoxicity in MASLD.

A small TAT-TrkB peptide prevents BDNF receptor cleavage and restores synaptic physiology in Alzheimer's disease

João Fonseca-Gomes[1,2], Tiago Costa-Coelho[1,2,3], Mafalda Ferreira-Manso[1,2,3,4], Sara Inteiro-Oliveira[1,2], Sandra H Vaz[1,2], Nuno Alemãn-Serrano[1,2], Henrique Atalaia-Barbacena[1,2], Leonor Ribeiro-Rodrigues[1,2], Rita M Ramalho[1,2], Rui Pinto[5,6], Hugo Vicente Miranda[7], Sara R Tanqueiro[1,2], Carolina de Almeida-Borlido[1,2], Maria João Ramalho[8,9], Catarina Miranda-Lourenço[1,2], Rita F Belo[1,2], Catarina B Ferreira[1,2], Vera Neves[2], Diogo M Rombo[1,2], Ricardo Viais[1,2], Juzoh Umemori[10], Ivo C Martins[2], André Jerónimo-Santos[1,2], António Caetano[1,2], Nuno Manso[1], Petra Mäkinen[11], Mikael Marttinen[11,12], Mari Takalo[11], Michael Bremang[13], Ian Pike[12], Annakaisa Haapasalo[14], Joana A Loureiro[8,9], Maria Carmo Pereira[8,9], Nuno C Santos2, Tiago F Outeiro[15,16,17,18], Miguel A R B Castanho[2], Adelaide Fernandes[3,4], Mikko Hiltunen[11], Carlos B Duarte[19], Eero Castrén[20], Alexandre de Mendonça1, Ana M Sebastião[1,2], Tiago M Rodrigues[1,2,18], Maria José Diógenes[1,2,19]

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Alzheimer's disease (AD) is characterized by cognitive decline linked to amyloid-β (Aβ) and tau pathology, synaptic dysfunction, and impaired neurotrophic signaling. One mechanism contributing to this impairment is the Aβ-induced cleavage of the brain-derived neurotrophic factor (BDNF) receptor TrkB-FL, which disrupts canonical BDNF signaling and promotes formation of a neurotoxic intracellular fragment (TrkB-ICD). We demonstrate that TrkB-FL cleavage is an early and progressive hallmark of AD pathology in human cerebrospinal fluid and postmortem brain samples, correlating with Aβ levels. Overexpression of TrkB-ICD in cultured neurons induced dendritic spine loss, hyperexcitability, and dysregulation of synapse-related genes. To counteract the loss of BDNF function and the gain of toxicity caused by the formation of TrkB-ICD, we designed and TAT-fused peptides spanning the TrkB cleavage site. A TAT-TrkB peptide with a lysine-lysine linker exhibited high structural stability, membrane permeability, and blood-brain barrier penetration. This peptide effectively prevented TrkB-FL cleavage in vitro, in neuronal cultures, and in vivo, reduced Aβ-induced synaptic dysfunction, preserved BDNF-mediated synaptic plasticity, and rescued long-term potentiation and glutamate release under Aβ-toxic conditions. In the 5XFAD mouse model of AD, chronic systemic administration of TAT-TrkB improved hippocampal-dependent learning and memory, restored synaptic protein levels, prevented dendritic spine loss, and reduced tau hyperphosphorylation and Aβ plaque burden, without detectable toxicity to liver or kidneys. Our findings establish TrkB-FL cleavage as a key pathogenic mechanism and validate the TAT-TrkB peptide as a promising disease-modifying therapeutic strategy to restore synaptic function and mitigate cognitive deficits in AD.

EPHAR Lecture:

Development of miRNA therapeutics for cardioprotection and other indications

Péter Ferdinandy, MD, PhD, MBA

Center for Pharmacology and Drug Research & Development, Semmelweis University; Dept. Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary; Pharmahungary Group, Szeged, Hungary

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Despite intensive research in the last almost 4 decades, drug treatment of myocardial infarction and the consequent post-infarction heart failure is still an unmet clinical need, possibly due to its complex molecular mechanism [1]. Therefore, targeting a molecular network rather than a single molecular drug target may be the adequate strategy for cardioprotective therapy. Unbiased, comprehensive analysis of gene expression pattern in normal and protected ischemic myocardium may lead to exploration of the transcriptomic and proteomic molecular network to identify novel molecular targets for cardioprotection [2]. The non-coding oligonucleotides microRNAs (miRNA) targeting multiple mRNAs form a transcriptomic dynamic molecular network. Targeted perturbation of the molecular network by miRNA oligonucleotide compounds is a promising approach for treatment of diseases of complex molecular mechanisms [3]. By a systematic unbiased analysis of the transcriptomic network of healthy, infarcted, as well as protected by ischemic conditioning heart samples, we have discovered endogenous cardioprotective miRNAs (e.g. miR125b-3p, miR-450a-3p) and validated them in vitro and in vivo and patented the nucleotide sequences of around these miRNAs [4, 5]. We termed these miRNAs protectomiRs. We are developing a miRNA discovery platform consists of bioinformatic software packages (see *miRNAtarget.com*) to build the transcriptomic and proteomic molecular network and identify potential oligonucleotide drug candidates to treat myocardial infarction and other diseases with unmet clinical need. We improve the performance of these algorithms by machine learning to reach an effective drug discovery enabling platform.

In conclusion, miRNA therapy may represent a breakthrough in the treatment of diseases with complex molecular mechanisms such as myocardial infarction. Here we developed a miRNA discovery enabling platform and currently developing miRNA compound pipeline for several cardiac and other indications.

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1.Ferdinandy P, et al., *Interaction of Cardiovascular Nonmodifiable Risk Factors, Comorbidities and Comedications With Ischemia/Reperfusion Injury and Cardioprotection by Pharmacological Treatments and Ischemic Conditioning.* Pharmacol Rev, 2023, **75**: 159-216.

2. Perrino, C., et al., *Epigenomic and transcriptomic approaches in the post-genomic era: path to novel targets for diagnosis and therapy of the ischaemic heart? Position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart.* Cardiovasc Res, 2017, **113**:725-736.

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5. Szabados, T., et al., *Pharmacokinetics and cardioprotective efficacy of intravenous miR-125b* microRNA mimic in a mouse model of acute myocardial infarction.* Br J Pharmacol, 2025. **182**(2): p. 432-450.

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ROUND TABLES

Round Table 1: El nuevo marco normativo de los medicamentos

Moderator: Francisco Zaragozá (Universidad de Alcalá de Henares)

Invited speakers:

Arantxa Sancho (Farmaindustria)

Elena Casaus (Asociación Española de Medicamentos Genéricos)

Jesús Aguilar (Consejo General de Colegios de Farmacéuticos)

Round Table 2: Uso Racional del Medicamento: objetivo compartido

Sponsored by the Chair for the Rational Use of Medicines MICOF-UV

Moderator: Mª Luisa Ferrándiz (Universidad de Valencia)

Invited speakers:

Carlos Fluixá Carrascosa (Médico de familia y expresidente de la SVMFiC)

Marta Aparicio Cueva (Farmacéutica de Atención Primaria y miembro de la Junta de la SVFAP)

Luis Salar Ibáñez (Farmacéutico comunitario y vocal de SEFAC-CV)

Mónica Climente Martí (Hospital Universitario Dr. Peset Valencia)

Juan Eduardo Megías Vericat (Oficina Autonómica de Medicina Predictiva, Personalizada y Terapias Avanzada (OMPTA) de la Comunitat Valenciana)

José Antonio González Correa (Universidad de Málaga y miembro del Comité de Medicamentos de Uso Humano de la AEMPS)

SESSIONS

Session 1: Neuropharmacology I

Moderator: Victoria Maneu (Universidad de Alicante)

Invited speaker:

9:00-9:30 Inés Erkizia (Universidad del País Vasco)
Preclinical insights into psilocybin: psychedelic mechanism and antidepressant effects

Oral communications:

9:30-9:45 Leslye Paola Valarezo Riascos (Universidad Santiago de Compostela)
Study and identification of G protein-coupled receptor interactions in an in vitro phenotypic model of schizophrenia on the effect of antipsychotic drugs

9:45-10:00 Rubén García-Cabrerizo (Universitat de les Illes Balears)
Psilocybin modulates social behavior in mice: sex-specific lasting effects

10:00:10:15 Jaime Rubio Sanz (Universidad de Castilla La Mancha)
Crosslink between cofilin pathway and ER stress in neuron-like dopaminergic cells

10:15-10:30 Patricio Iturriaga-Vásquez (Universidad de La Frontera, Chile)
Zebrafish as a tool for drug discovery: the role of nicotinic antagonist in nicotine and ethanol addiction

Preclinical insights into psilocybin: psychedelic mechanism and antidepressant effects

Ines Erkizia-Santamaría[1], Nerea Martínez-Álvarez[1], Roser Alles-Pascual[1], Leyre Salinas-Novoa[1], Igor Horrillo[1,2,3], J. Javier Meana[1,2,3], Jorge E. Ortega[1,2,3]

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Major depressive disorder (MDD) is a common illness that severely limits psychosocial functioning and diminishes quality of life. MDD is characterized by phenotypic heterogeneity and high incidence of comorbidities, such as anxiety. Currently available antidepressants demonstrate severe limitations, and there is an urgent need for improved effective treatments. In the search for novel rapid-acting antidepressants, the psychedelic psilocybin has emerged as a promising therapeutic in several clinical trials. However, the role of psychedelic-induced subjective effects in therapeutic outcomes is still subject to debate. Thus, preclinical evaluation of psilocybin treatment in translational animal models of disease is imperative.

The *in vitro* pharmacodynamic profile of psilocin, the *in vivo* acute mechanism of action of psilocybin and the potential antidepressant and anxiolytic effects were addressed in this work.

Competition radioligand binding studies revealed an analogous pharmacodynamic profile of psilocin for human and mouse serotonin 2A (5HT2AR), 2C (5HT2CR) and 1A (5HT1AR) receptors. In the head-twitch response (HTR), the most translational assay to characterize psychedelic-like effects in rodents, psilocybin exhibited an inverted U-shaped dose-response curve, with a maximal dose of 1 mg/kg. Psilocybin-induced HTR was blocked by 5HT2AR antagonism, and modulated by 5HT2CR antagonism and 5HT1AR agonism. Moreover, changes in endogenous levels of serotonin (5-HT) modulated psilocybin-induced HTR, and a significant inverse correlation between cortical 5-HT and HTR was observed. Additionally, the therapeutic potential of psilocybin was studied in a stress-based mouse model. Chronic unpredictable mild stress (CUMS) model was treated with two doses of psilocybin (1 mg/kg, i.p.) and a wide behavioural evaluation was conducted. Psilocybin reversed impairments in anhedonia and behavioural despair dimensions of depressive phenotype but not in apathy- related behaviour. Anxiety-like phenotype was also improved by the drug. Physiological alterations caused by chronic stress, indicative of a hyperactive hypothalamic-pituitary-adrenal axis, were not reversed by psilocybin. When neuroplasticity-related proteins were assessed in cerebral cortex, brain-derived neurotrophic factor (BDNF) was found to be decreased in stressed animals, and treatment did not reverse such impairment. Psilocybin administration increased expression and function of 5HT2AR in brain cortex of control and CUMS groups. On the other hand, psilocybin treatment caused a selective increase in the expression of glucocorticoid receptor (GR) in brain cortex of CUMS mice.

This work revealed important mechanistic aspects of the psychedelic effects of psilocybin. Altogether, these data provide new knowledge on the behavioural actions of psilocybin and contribute to the understanding of the therapeutic mechanism of action of psychedelics. These results may provide a novel insight concerning therapeutic targets of psychedelics for future pre-clinical and clinical studies.

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Study and identification of G protein-coupled receptor interactions in an *in vitro* phenotypic model of schizophrenia on the effect of antipsychotic drugs

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Schizophrenia is a chronic psychotic disorder with heterogeneous (positive, negative and cognitive) symptoms[1]. Although the etiopathogenesis has not been completely elucidated due to the complexity of the molecular mechanisms involved, dysregulation of the dopaminergic, glutamatergic and serotonergic neurotransmitter systems has been identified, involving ion channels of NMDA, several G protein-coupled receptors (GPCRs), such as D2, D3, 5-HT2A and mGlu2, which represent main targets of typical and atypical antipsychotics used in the clinic of this disease[2]. To find novel compounds acting on cognitive deficit of schizophrenia, we have previously developed and *in vitro* phenotypic model resembling these symptoms.[3] Thus, our aim here was to evaluate the serotoninergic, glutamatergic and dopaminergic signalling through D2 and NMDA receptors in the novel *in vitro* model.

We employed a SH-SY5Y cell line differentiated to a neuronal phenotype by treatment with retinoic acid, GLP-1 and different supplements. To evaluate the functionality of dopaminergic, serotonergic and glutamatergic receptors, as well as to analyze the possible reciprocal modulation (crosstalk) among them, second messenger measurement assays were employed by detecting intracellular cAMP levels (HTRF cAMP kits from Revvity) and calcium mobilization using calcium-6 fluorescent probe. The study of GPCRs evidenced a lack of response to 5-HT mediated by 5-HT2A recpetors. However, there was a dopamine response compatible with D2 receptor expression (EC50= 0.60 µM), this response was inhibited after administration of 0.1 µM haloperidol (Kb= 0.409 µM). On the other hand, glutamate induced an increase in intracellular calcium levels (EC50= 5.27 µM). When evaluating reciprocal modulation, it was found that NMDA receptor activation conditioned the effect of D2, increasing its potency (EC50= 0.090 µM; $p<0.05$, student's t-test), likewise, D2 activation also modulated the NMDA response, reducing its EC50 to 0.27 µM ($p<0.05$, student's t-test). Furthermore, it was observed that the dopaminergic response previously modulated by glutamate was inhibited after administration of the NMDA antagonist MK-801(1µM) (EC50= 1.327 µM), evidencing that the potentiating effect of glutamate on dopamine signalling is through NMDA receptors.

Accordingly, the presence of D2 and NMDA receptors was confirmed in our cellular model, validating it as a useful model for the study of mechanisms associated with schizophrenia. Likewise, a cross-modulation (crosstalk) between both receptors was evidenced, which could have significant implications for the development of new therapeutic strategies for this disease.

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Psilocybin Modulates Social Behavior in Mice: Sex-Specific Lasting Effects

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Social connection is a fundamental component of mental health, yet it is often disrupted in individuals suffering from stress-related disorders [1]. These impairments can lead to significant emotional and functional difficulties, and current treatments remain limited and often ineffective. This gap is partly due to an incomplete understanding of the neurobiological mechanisms supporting healthy social behaviour [2]. Psychedelics such as psilocybin have recently gain interest for their potential to enhance social functioning, potentially by modulating circuits involved in social cognition, reducing neuroinflammation, and promoting stress resilience [3]. In this study, we examined the immediate and long-lasting effects of psilocybin on social behaviour in adult male and female C57BL/6 mice. Mice received oral psilocybin (0.3 or 1 mg/kg; n = 11–8 per dose/sex) or vehicle (0.9% NaCl; n = 9–8 per sex) daily for seven days. Social behaviour was assessed using the reciprocal social interaction test with a novel conspecific matched for sex and age, analysing behaviours such as anogenital sniffing, nose-to-body, and nose-to-nose interactions [4] through two-way ANOVAs. A single psilocybin dose significantly increased direct social interactions 30 minutes post-administration (psilocybin effect: $F_{2,45} = 5.672$, $^{**}p=0.0064$; sex effect: $F_{1,45} = 9.559$, $^{**}p<0.0034$; interaction: $F_{2,45} = 3.226$, $^{*}p<0.0491$). *Post-hoc* analysis showed that this effect was restricted to male mice receiving the 1 mg/kg dose, with significant increases in anogenital sniffing ($^{*}p=0.03$) and nose-to-nose interactions ($^{**}p=0.013$) when compared to controls, indicating a lack of immediate prosocial response in females. Repeated dosing (1-day post-treatment) produced robust increases in direct social interactions across sexes (psilocybin effect: $F_{1,50} = 18.02$, $^{***}p<0.001$; sex effect: $F_{2,50} = 18.01$, $^{***}p<0.001$). *Post-hoc* comparisons revealed that, in males, the highest dose enhanced anogenital sniffing ($^{***}p=0.0005$) and nose-to-nose interactions ($^{***}p<0.001$), while in females it increased nose-to-body ($^{***}p<0.001$) and nose-to-nose interactions ($^{**}p<0.01$). At 7 days post-treatment, psilocybin's effects persisted selectively in males (psilocybin effect: $F_{2,50} = 6.293$, $^{**}p=0.0036$; sex effect: $F_{1,50} = 19.59$, $^{***}p<0.001$), with significant increases in anogenital sniffing ($^{**}p=0.0096$) and nose-to-nose interactions ($^{*}p=0.0440$) when compared to control mice. Contrarily, no significant differences were observed in females, suggesting a long-term loss of prosocial effect for this sex. These findings highlights that psilocybin promotes prosocial behavior in a dose- and sex-dependent manner, with rapid effects observed in males and more sustained improvements evident in both sexes after repeated exposure. Overall, this study supports the potential of psilocybin as a novel therapeutic strategy for ameliorating social deficits associated with stress-related disorders.

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Crosslink between cofilin pathway and ER stress in neuron-like dopaminergic cells

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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 and cell cycle control (2), but also in mediating neuronal apoptosis in response to excitotoxic stimulus (3).

The endoplasmic reticulum (ER) has been described as a potential sensor capable of triggering both adaptive and pathological signalling. Initially, ER stress leads to adaptations but, when persistent ER stress cannot be corrected, it triggers cell apoptosis (4).

In the present work we have studied the role of cofilin and ERstress 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 the mitochondrial and total reactive oxygen species (ROS) production by fluorescence microscopy; the activity of caspases -3,-9 and -12 by fluorometry; and the activation of ERstress and the status of cofilin phosphorylation, and its intracellular location by western blot. The interaction by different ERstress proteins with cofilin was determined by co-immunoprecipitation assays.

We found that Meth induced neuroblastoma cell-death in a time- and concentration-dependent manner, increased mitochondrial ROS production and activated the ERstress response. Moreover, cofilin was activated by dephosphorylation and translocated from mitochondrial membrane to cytosol. Pharmacological inhibition of both, cofilin and ERstress pathways showed a crosslink modulation between them suggesting that cofilin is a master regulator of Meth-induced neuroblastoma apoptosis.

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Zebrafish as a Tool for Drug Discovery: The Role of Nicotinic Antagonist in Nicotine and Ethanol Addiction

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Nicotinic acetylcholine receptors (nAChR) are part of the family of ion channels activated by ligands. There are several subtypes of nicotinic receptors, given their pentameric nature, among which the α4β2 and α7 subtypes appear to be the most abundant in the Central Nervous System (CNS). They are responsible for the permeability of the cell membrane to sodium and calcium ions, playing a crucial role in the central nervous system by modulating the release of various neurotransmitters, including dopamine and norepinephrine, among others[1]. Nicotinic acetylcholine receptors are associated with mood disorders such as anxiety and abuse of addictive substances such as nicotine and ethanol[2]. Our work has focused on the search for molecules of natural or synthetic origin that exhibit an affinity for nicotinic receptors, particularly the α4β2 nAChR subtype, acting as agonist or antagonist ligands. In recent years, we have ventured into in vivo models, finding that the zebrafish appears to be a helpful tool, given its versatility and the large number of experimental animals, for the search for substances with potential pharmacological activity[3]. This enables us to project these selected substances into a rat model, thereby extending our studies. Currently, we have used the Novel Tank Diving Test (NTT) paradigm as an anxiety or stress model and a Conditioning Place Preference model (CPP), like one used in rats, to generate a behaviour profile in zebrafish, which allowed us to discover new substances with a potential therapeutic effect against nicotine addiction and ethanol consumption. We have found that UFR2709, a synthetic nicotinic antagonist, can reverse the conditioning mediated by nicotine and ethanol on a CPP paradigm for zebrafish[4]. We also test a novel dual ligand that acts on nicotinic receptors and serotonin transporters, exhibiting an anxiolytic profile using the NTT paradigm, and can decrease ethanol consumption in a bibulous rat model[5]. Our results confirm that zebrafish are a valuable model for drug discovery. Additionally, we also confirm that a nicotinic antagonist can reverse the effects mediated by nicotine and ethanol in both zebrafish and rat behaviour.

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Session 2: AI in pharmacological research

Organizer: Association of SEF Young Researchers

Moderator: Celia Llorente Sáez (Universidad Complutense de Madrid)

Invited speakers:

Óscar Pastor López (Instituto Universitario Valenciano de Investigación en Inteligencia Artificial; Universidad Politécnica de Valencia)

Susana Conde Cedide (Johnson & Johnson Innovative Medicine. Global Discovery Chemistry)

Álvaro Serrano Navarro (Grupo de Bioinformática; Centro Nacional de Investigaciones Cardiovasculares)

Session 3: Pain and inflammation

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

Invited speaker:

15:00-15:30 María Paula Ávila (Fundación Universitaria de Ciencias de la Salud, Bogotá DC, Colombia)
Targeting inflammatory pathways in pain management: advances and perspectives

Oral communications:

15:30-15:45 Cristian Bis-Humbert (Universitat Pompeu Fabra)
Cannabidiol as a therapeutic agent for peripartum depression: behavioral and molecular effects in dams and offspring in a mouse model

15:45-16:00 Aràntzazu Alonso-Carrasco (Universitat de Barcelona)
Molecular insights into buprenorphine and morphine: binding pocket characteristics and pharmacological implications

16:00-16:15 Nazaret Prieto Aranda (Universidad Cardenal Herrera CEU)
Effects of synovial fluid from osteoarthritic patients on osteoclast bone resorption activity

16:15-16:30 María Carmen Carceller (Universitat de València)
Genetic susceptibility and inflammatory pathways in psoriatic arthritis: implications for pharmacological biomarker development

Targeting Inflammatory Pathways in Pain Management: Advances and Perspectives

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Inflammatory pain remains one of the primary causes of suffering and disability worldwide. The molecular pathways involved in inflammation including those mediated by prostaglandins, pro-inflammatory cytokines, ion channels, and innate immune receptors have gained considerable attention as potential therapeutic targets for pain management. This offers a critical review of the pathophysiological mechanisms linking inflammation with nociceptive sensitization, and highlights recent developments in targeted anti-inflammatory therapies. We will explore advancements in selective COX-2 inhibitors, IL-1β antagonists, TNF-α receptor blockers, and TLR4 modulators. In addition, novel agents such as resolvins and specialized pro-resolving mediators (SPMs) are discussed for their ability to actively resolve inflammation while preserving host immune defense. These approaches aim to overcome the limitations of traditional analgesics such as NSAIDs and opioids by reducing adverse effects and the risk of dependency. From a translational viewpoint, the presentation will address challenges in preclinical modeling, biomarker validation, and the clinical translation of these therapies. The future of inflammatory pain treatment also involves the integration of personalized medicine and artificial intelligence for mechanistic drug development. Additionally, real-world data from Latin American clinical settings particularly studies conducted by Ávila and colleagues underscore critical gaps between pharmacological guidelines and everyday hospital practice. Their work on analgesic prescription, adverse drug reactions, and patients' perceptions of postoperative pain offers important contextual insights for designing more effective and contextually relevant pain management protocols. This integrative approach aims to redefine pain management from a mechanism-based and patient-centered perspective, with the potential to transform clinical practice and significantly improve patient quality of life.

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Molecular insights into buprenorphine and morphine: binding pocket characteristics and pharmacological implications

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Chronic pain is a major health issue in Europe, further exacerbated by the opioid crisis due to the widespread use of opioids for severe pain management. Among available options, buprenorphine is an opioid with a unique pharmacological profile. Although structurally similar to the more problematic morphine, buprenorphine exhibits a distinct mode of action characterized by cell-type specificity and receptor density dependence[1]. It is classified as an agonist at mu-opioid receptor (MOR) and an antagonist at delta- and kappa-opioid receptors (DOR and KOR), yet displays strong analgesic efficacy with a markedly reduced risk of respiratory depression[1].

Buprenorphine's reduced side effect profile, particularly compared to morphine, underlies its dual utility both as an analgesic and as a maintenance therapy for opioid use disorder, where it minimizes the risk of overdose. Despite their pharmacological divergence, buprenorphine and morphine share a common cyclic scaffold, differing only by a few substituents. To investigate the molecular basis of their contrasting actions, molecular docking and long-timescale molecular dynamics simulations were performed to characterize their binding behavior at the MOR binding site. These analyses revealed key interactions and conformational changes that may account for buprenorphine's partial agonism and safer clinical profile.

Complementary functional assays, such as cAMP accumulation studies, revealed that morphine is more potent than buprenorphine in MOR-expressing systems, highlighting a potential signaling bias. Altogether, these insights provide a mechanistic understanding of buprenorphine's unique therapeutic window and offer a foundation for the rational design of next-generation opioids—aimed at maximizing analgesia while minimizing addiction liability and life-threatening side effects.

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Effects of synovial fluid from osteoarthritic patients on osteoclast bone resorption activity

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Osteoarthritis is a chronic degenerative joint condition with an inflammatory component. The development and progression of the disease involves an imbalance in bone resorption, mediated by osteoclasts, which are the cells responsible for this process. This imbalance is aggravated by the inflammatory environment associated with the disease. Synovial fluid, found in the affected area, contains inflammatory factors that may be linked to the severity of the condition [1,2].

To investigate the resorptive activity of osteoclasts derived from monocytes of healthy individuals in the presence of synovial fluid from patients with osteoarthritis.

Monocytes ($CD14^+$) were isolated from peripheral blood samples of healthy donors obtained from the Valencian Community Blood Transfusion Center by using magnetic bead isolation. Osteoclast differentiation was induced from monocytes using RANKL (50 ng/ml) and MCSF (25 ng/ml) cytokines over 10 days. The formed osteoclasts were then transferred to bone slices and cultured for 7 days with or without 20% synovial fluid obtained from osteoarthritic patients of the Clinical Hospital of Valencia. Toluidine-stained bone slices were analyzed for bone resorption activity using an inverted microscope (12 images per field at 20x magnification) and Image J software. Data analysis was performed with GraphPad 9.0.

Quantitative image analysis demonstrated that osteoclasts derived from healthy donors and exposed to osteoarthritic synovial fluid showed a 2.4-fold increase in resorbed areas compared to untreated osteoclasts ($2.5x10^5$ $\mu m^2 \pm 8.2x10^4$ *vs* $1.05x10^5$ $\mu m^2 \pm 2.1x10^4$; $p< 0.01$). No significant differences were found in the number of osteoclasts in the two experimental conditions (TRAP activity).

This study demonstrated a significant increase in resorption activity in osteoclasts from healthy donors stimulated with synovial fluid from osteoarthritic patients. This finding suggests that synovial fluid is one of the modulatory factors of osteoclast biological activity in joint pathologies.

Keywords: Osteoarthritis, osteoclast, resorption.

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Cannabidiol as a therapeutic agent for peripartum depression: behavioral and molecular effects in dams and offspring in a mouse model

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Peripartum depression (PPD) is a subtype of major depressive disorder affecting about 20% of women during pregnancy or in the year after giving birth[1] each year. Among symptomatology, a deficient maternal care is a key symptom of PPD, with the potential to disrupt mother-infant bonding and adversely affect the child's development. The recent adoption of allopregnanolone-based treatments has underscored the critical role of reproductive hormones and GABAergic transmission in the pathophysiology of postpartum depression[2]. Despite extensive research identifying numerous factors involved in the neurobiology of depressive disorders, the endocannabinoid system (ECS) has emerged as a key modulator of stress and anxiety responses[3]. In this context, the present study investigates the therapeutic potential of cannabidiol (CBD) to alleviate depressive-like symptoms associated with PPD by targeting the ECS. To do so, a well-established PPD mouse model combining 15 days of maternal separation and early weaning (MSEW) at postnatal day 17, was implemented and compared to a standard nest (SN) condition. Briefly, litters selected to MSEW were daily separated from their mothers for 4 h between postnatal day (PND) 2-5 and for 8 h between PND 6-16. Then, the offspring were prematurely weaned on PND 17, while the standard nest group remained undisturbed with their dams until PND 21. Dams from MSEW or SN groups were treated with CBD (10 mg/Kg) or vehicle from PND 2-16. After weaning, affective-like behaviour was assessed in both dams and offspring. Subsequently, RNA expression assessment (OpenArray) in order to evaluate the effects of this compound on our model. Behavioural results showed a negative affect state in vehicle-treated MSEW dams compared to the SN group, which was absent in MSEW CBD-treated mice. Accordingly, OpenArray analyses conducted on limbic and cortical areas revealed differential expression of genes related to mood, behaviour, and reward processing in vehicle-treated MSEW mice compared to the SN group. These results suggest CBD's involvement in restoring the SN phenotype in the MSEW group both in dams and the offspring. Altogether, these findings support the importance of the ECS in the neurobiology of PPD, opening up new possibilities for its pharmacological management.

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Genetic Susceptibility and Inflammatory Pathways in Psoriatic Arthritis: Implications for Pharmacological Biomarker Development

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Psoriatic arthritis (PsA) is a chronic autoimmune disease characterized by joint and skin inflammation, frequently emerging in patients previously diagnosed with cutaneous psoriasis (PsC). While PsA and PsC share several genetic traits, differences in their pathogenesis are largely explained by genetic heterogeneity (1). This study aimed to identify susceptibility-related allelic variants associated with PsA and PsC and to characterize the immunological pathways differentially involved in each condition.

Using curated data from genome-wide association studies (GWAS), we compiled a database of single nucleotide polymorphisms (SNPs) significantly associated with either PsA or PsC. Pathway enrichment analysis revealed a higher presence of PsA-specific variants in the Tumour Necrosis Factor (TNF) and NF-κB signaling pathways, alongside Interleukin (IL)12B and IL23-mediated pathways. These findings point to a distinct pro-inflammatory axis in PsA, suggesting that modulation of these pathways could inform future pharmacological strategies.

Despite the growing availability of targeted biologics (such as TNF inhibitors, IL-17A antagonists, and JAK/STAT modulators) early detection of PsA remains challenging. Most PsA patients exhibit cutaneous symptoms before joint involvement. Hence, identifying predictive genetic biomarkers in PsC patients could significantly improve early diagnosis and therapeutic outcomes.

This research contributes to a better understanding of the differential immunopathogenesis of PsA, reinforcing the need for validated genetic biomarkers to support precision medicine in inflammatory diseases.

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Session 4: Natural Products Pharmacology

Moderator: Nuria Cabedo (Universitat de València)

Invited speaker:

15:00-15:45 José Mª Prieto (Liverpool John Moores University, UK)
Natural cytotoxic products against melanoma: towards the genomic bases of their multitarget synergic activities

Oral communications:

15:45-16:00 Manuel Alcarranza (Universidad de Sevilla)
Dietary Supplementation with the Natural Compound AG-544 Ameliorates Collagen-Induced Arthritis in DBA/1J Mice

16:00-16:15 Carmen Ortiz-González (MolDrug AI Systems SL)
Unveiling the molecular mechanism of Pannexin1 inhibition by silymarin derivatives with anti-inflammatory potential through computational modelling

16:15-16:30 Luis Rodríguez-Santos (Universidad Santiago de Compostela)
A 28-Day Voluntary-Feeding Model of Diarrheic Shellfish Toxins Reveals Subclinical Intestinal Injury

Natural products against melanoma: towards the genomic bases of their multitarget synergic cytotoxic activities

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Natural products -botanical or mimics- make up for more than 65% of the current total chemotherapeutic armamentarium [1]. In the area of cancer, melanoma remains a challenging disease, compounded by often asymptomatic stage 4 patients [2]. As survival for early stages are near 100%, chemopreventative strategies are sought after, and natural botanical products -particularly terpenes such as ingenol and betulinic acid derivatives- are already in clinical use or trials [3,4]. One common characteristic of natural products is their multitarget bioactivities, making them "dirty molecules" from a drug discovery viewpoint [5] . Our group is actively exploring how to leverage their multitarget cytotoxic activities by using them either alone or in combination treatments [6,7]. Moreover, we are developing in silico tools (AI, gene network models) to predict the bioactivity of natural products in combination and their genomic effects on melanoma cells [8,9]. We here present an overview of our latest results with special focus on the combination of berberine and harmine, two alkaloids with cytotoxic activity on human melanoma cells.

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Dietary Supplementation with the Natural Compound AG-544 Ameliorates Collagen-Induced Arthritis in DBA/1J Mice

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Rheumatoid arthritis (RA) is a chronic, progressive, immune-mediated inflammatory disease (IMID) that involves both joint and extra-articular manifestations. Inadequate treatment can result in significant disability and increased mortality[1]. Although current pharmacological therapies are designed to control disease activity and prevent outbreaks, side effects often compromise patient adherence, underscoring the need for complementary approaches. Nutritional interventions are emerging as promising strategies in the management of IMIDs such as RA[2]. This study aimed to investigate the preventive and therapeutic potential of dietary supplementation with AG-544 in a murine model of collagen-induced arthritis (CIA). Male DBA/1J mice were administered an experimental diet containing AG-544 (0.025% w/w) from their fourth week of life until sacrifice, following two immunizations with type II collagen at weeks 11 and 14 to induce RA. The onset and progression of arthritis were monitored macroscopically, using the scoring system described by Devesa et al. (2005)[3], based on the appearance of redness and/or swelling in the phalanges or other paw regions. Joint inflammation and tissue damage were further evaluated by immunohistochemical analysis of MMP-9 and cathepsin K, key proteases involved in bone and cartilage degradation. Levels of inflammatory mediators (IL-1β, IL-6, IL-17, MMP-3, and PGE_2) were quantified by ELISA. Protein expression of TNF-α, iNOS, COX-2, pp65, Keap1, Nrf2, HO-1, as well as phosphorylated forms of MAPKs (pp38, pJNK) and the JAK/STAT pathway (pJAK3, pSTAT3), was analyzed by Western blot. Dietary AG-544 supplementation resulted in a significant reduction in both clinical and histopathological signs of arthritis, as evidenced by decreased joint inflammation and tissue destruction. This was accompanied by downregulation of MMP-9, MMP-3, and cathepsin K, and a marked decrease in the production of pro-inflammatory cytokines and enzymes, including IL-1β, IL-6, IL-17, PGE_2, TNF-α, COX-2, and iNOS. These protective effects could be attributed with modulation of the MAPKs (p38, JNK), JAK3/STAT3, and NF- κB signaling pathways, as well as upregulation of the antioxidant Keap1/Nrf2/HO-1 pathway. Consequently, this study proposes AG-544 as a potential nutraceutical compound for the treatment of IMIDs affecting the joints, such as RA, although nutritional intervention trials would be necessary to confirm the efficacy and safety in patients with RA.

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Acknowledgements

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Unveiling the molecular mechanism of Pannexin1 inhibition by silymarin derivatives with anti-inflammatory potential through computational modelling

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Pannexin 1 (Panx1) are heptameric channels involved in paracrine and autocrine signalling by modulating the passage of molecules, such as adenosine triphosphate (ATP)[1]. In presence of pathological stimuli, these channels may be activated, leading to the initiation of inflammatory pathways, such as the NLRP3 inflammasome[2]. In this study, *in vitro* and *in silico* testing approaches were combined to evaluate a potential alternative anti-inflammatory mechanism of plant-based natural derivatives from silymarin (SM) (silybin (SB), silychristin (SC) and silydianin (SD))[3], based on the inhibition of Panx1 channel.

The *in vitro* Panx1 channel inhibitory activity of the compounds was evaluated using an ATP release assay in three different cell lines (i.e. Dubca hPanx1, C6 rat glioma, and PMA- differentiated monocyte-derived macrophages from the THP-1 monocyte). Additionally, their impact on the NLRP3 inflammasome activation was assessed by measuring interleukin-1β (IL- 1β) and NLRP3 inflammasome-associated cell death through an ELISA and a LDH leakage assay, respectively. *In silico* approaches were performed to deeply understand the potential interaction between the compounds and Panx1 channel. Specifically, a molecular docking analysis was conducted to calculate the binding affinity between the testing compounds and Panx1, and to identify a potential binding site. The best docking poses were subsequently subjected to molecular dynamics simulation (MDS) for 100 nanoseconds to check the stability. *In vitro* testing demonstrated that SB, SC, and SD effectively inhibited Panx1 channel activity by reducing the extracellular ATP levels across all three cell lines. However, only SB significantly reduced IL-1β levels and attenuated NRLP3 inflammasome-associated cell death. The potential interaction between the candidate compounds and Panx1 channels was further studied with *in silico* approaches. Molecular docking showed that SB, SC and SD interact with Panx1 (docking scores around 8 kcal/mol). MDS trajectories analysis showed that the studied Panx1 complexes presented stability along time, with RMSD ranges from 2.5Å to 3.5Å, suggesting a similar binding to known inhibitors.

Based on the *in vitro* and *in silico* results, this study identified SB, SC, and SD as potential Panx1 channel inhibitors and suggested an alternative anti-inflammatory mechanism.

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A 28-Day Voluntary-Feeding Model of Diarrheic Shellfish Toxins Reveals Subclinical Intestinal Injury

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Okadaic acid (OA) and its analogues, dinophysistoxins 1 and 2 (DTX1 and DTX2), are natural marine biotoxins produced by dinoflagellates and accumulated in filter-feeding molluscs. Human exposure occurs through the consumption of contaminated shellfish causing Diarrheic Shellfish Poisoning (DSP) with gastrointestinal symptoms such as vomiting, abdominal pain and incapacitating diarrhea. Hence, these toxins are also referred as Diarrhetic Shellfish Toxins (DSTs). Although DSTs are the most abundant phycotoxins and a legal limit of 160 µg OA equivalents/kg shellfish meat has been set in Europe, the effects of chronic exposure at low doses remain poorly characterized.

To evaluate the potential long-term damage, a 28-day chronic study was performed in female and male Swiss mice that received 90 µg/kg OA, DTX1, or DTX2 by voluntary consumption. Toxins were incorporated into bread to avoid the invasiveness of oral gavage and reproduce a more natural feeding scenario. After a five-day period of habituation, the animals began to receive the toxic bread and relative body weight, food and water consumption, as well as fecal and urine production were monitored throughout the exposure period. In addition, the onset of both non-specific and specific symptoms was recorded. Episodes of diarrhea continued to be observed after 28 days.

Toxins were found in feces collected on the first day of the treatment and weekly thereafter. During necropsy, a macroscopic evaluation of the abdominal organs was performed, revealing alterations in the stomach and intestines, particularly in mice treated with DTX1. Additionally, proteins involved in inflammatory processes were detected in the intestinal contents collected during necropsies. These results provide important insights into the health risk associated with routine exposure to DSTs at doses equivalent to the regulatory limit.

Acknowledgements

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Session 5: Pharmacogenetics and Precision Pharmacology

Moderator: Luis Sendra (Universitat de València)

Invited speaker:

16:30-17:00 Mª José Herrero (Universitat de València)
Clinical implementation of Pharmacogenetics

Oral communications:

17:00-17:15 Diego Robés (Universidad Santiago de Compostela)
Steroid hormone profiling in H295R in vitro model for the identification of endocrine disruptors acting through the steroidogenesis pathway

17:15-17:30 Idoia Álvarez-Ajuria (MolDrug AI Systems, Valencia)
Metabolomics-based biomarkers for the development of diagnostic tools in drug induced cardiotoxicity

Clinical Implementation of Pharmacogenetics

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Pharmacogenetics is an essential component of Personalized Precision Medicine, which allows us to offer each patient the best treatment, maximizing its efficacy and minimizing its toxicity. This is of particular interest, especially since pharmacogenetic testing has been included in the common service portfolio of the Spanish public health system. Currently, we cannot address the rational use of many medications without taking into account the pharmacogenetic characteristics of patients. A confluence of events has led Spain to reach this important milestone and now become a European leader in the clinical implementation of pharmacogenetics. Of course, there are still obstacles to overcome, and implementation is not yet equal across all of Spain's autonomous communities, since the government competences are transferred to the communities in health matters. Finally, we must be aware that there is still much research to be done in this field, both at the basic level and in translational research, through multicenter projects that validate the usefulness of pharmacogenetic markers in the complex real world, with patients with multiple pathologies and multiple medications.

Steroid hormone profiling in H295R in vitro model for the identification of endocrine disruptors acting through the steroidogenesis pathway

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Endocrine disruptors (ED) are chemical substances capable of altering hormonal balance through various physiological mechanisms, such as steroidogenesis [1]. The aim of this study is to develop methodologies to identify these substances, with the ultimate goal of minimizing their use in both toxicological and drug development contexts.

To achieve this, we propose the development of phenotypic in vitro models using H295R cell line. This cell line produces all the steroid hormones and enzymes involved in human steroidogenesis, accurately simulating human physiology. Therefore, compounds that alter the hormone levels after exposure to the H295R model are labelled as compounds with potential to alter the production of steroids in humans and thus, identified as possible ED.

In this work, H295R cells were exposed to the studied compounds and the alterations on hormone concentration in the medium compared to non-treated cells were measured. In order to quantify the hormones concentration, an analytical method using ultra-high performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS) detection has been optimized to simultaneously evaluate the 17 steroid hormones involved in human steroidogenesis secreted by H295R cells and an internal standard, in the same sample and in a single injection, with detection and quantification limits obtained for each one.

Using this method, it was possible to carried out the assay following the OECD Test Guide 456 for Steroidogenesis Assays [2] that comprises two hormones, testosterone and estradiol, with an increased number of the measured hormones, with the goal to reach all the 17 secreted steroid hormones and achieve the complete hormone profiling. Assay performance was confirmed with reference compounds with already-known ED effect, as well as possible ED compounds present as environmental pollutants or used in the industry.

Additionally, the analytic method for hormone quantification has been adapted to a RapidFire 400 System, fully integrated with an Agilent MS System, which combines high-speed sampling, ultrafast automated solid-phase extraction (SPE), and powerful mass spectrometry data acquisition, reducing the sample processing time from 13,5 minutes to 11 seconds.

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Metabolomics-based biomarkers for the development of diagnostic tools in drug induced cardiotoxicity

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Cardiovascular disease is a leading cause of morbidity and mortality among cancer survivors (1), with evidence indicating that oncological treatments can triple the long-term cardiovascular risk (2). Current standard methods for monitoring cardiotoxicity, such as imaging and computed tomography (CT), provide prognostic insights primarily during the early treatment phases and are often costly. Consequently, there is a pressing need for personalized, cost-effective biomarkers to predict and detect cardiotoxicity across different stages of cancer treatment.

Here, we present the project BioCardioMetab that aims to explore the application of metabolomics to identify patient-specific biomarkers for early prediction and diagnosis of cardiotoxicity. Metabolomics is particularly suitable approach due to its high sensitivity to biochemical changes, its proximity to the phenotype, and its cost- and time-efficiency compared to other omics approaches.

Thus, we designed a prospective observational cohort study including adult patients with a first diagnosis of early-stage cancer requiring antineoplastic treatment. Blood samples were collected at two time points: prior to treatment initiation and after three months of therapy. At the latter time point, cardiotoxicity was assessed based on predefined clinical parameters.

Metabolic profiles were obtained for all plasma samples by nuclear magnetic resonance (NMR) spectroscopy following reproducible protocols. Then, multivariate statistical analyses of the metabolomic data in combination with clinical assessments were applied to identify potential prognostic biomarkers associated with cancer therapy-related cardiac injury.

In conclusion, our study provides relevant data to advance in the development of multidomain, personalized risk stratification tools that improve cardiovascular outcomes in cancer patients and facilitate the clinical translation of metabolomic biomarkers for cardiotoxicity monitoring in oncology care.

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Session 6: Teaching Innovation in Pharmacology

Moderator: Emilio J. Sanz (Universidad de La Laguna)

Invited speaker:

16:30-17:00 Manuela S. Rodrigues Morato (Universidade do Porto, Portugal)
Active learning through real world simulations: engaging, realizing and understanding

Oral communications:

17:00-17:15 María Pilar D'Ocon (Universitat de València)
REDFARMINN, a web-based meeting point for pharmacology teachers

17:15-17:30 Marina Sánchez-Hidalgo (Universidad de Sevilla)
Design of a digital escape room as a tool of gamification for the dynamisation of the practices of Pharmacology and Pharmacotherapy III and Clinical Pharmacy

Active learning through real world simulations: engaging, realizing and understanding

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The learning process is complex but can be supported by active pedagogical methodologies that are appealing for the students and involve them in the learning process. Besides, promoting simulations of real world practice increases consciousness on the programmatic contents, which is also relevant for deep understanding and long-lasting knowledge. Furthermore, guiding the students on metacognition consolidates lifelong learning capacities. The authors will share some pedagogic activities that have been implementing in the Pharmacology II course of the Integrated Master in Pharmaceutical Sciences of the Faculty of Pharmacy of the University of Porto, Portugal (4th year), a course with around 160 students per year. These activities have included: (1) analysis of a real medical prescription; (2) simulation of a real world situation regarding the urge for an unusual question/concern posed by a patient at a pharmacy; (3) development of soft skills by planning communication settings to specialists and non-specialists; (4) challenging the advantages and disadvantages of chat bots as a source of information; (5) self-assessment tests as a strategy of metacognition.

REDFARMINN, a web-based meeting point for pharmacology teachers

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Teacher networks have been emerging as a complementary approach to traditional teacher training based on courses led by experts. These networks not only promote learning but also encourage reflection on personal teaching experiences and foster communities for the exchange of educational tools and experiences.

The "Interuniversity Network of Teaching Innovation in Pharmacology" (REDFARMINN) was established in 2015, with the aim of achieving a common space to share resources and experiences of teaching innovation in the field of Pharmacology.

The objectives of the network are: i) To share and evaluate experiences previously implemented by network members through annual meetings. ii) To develop teaching innovation projects that involve the creation of online teaching materials tailored to specific educational needs. iii) To create a website including a repository of shared teaching resources º

The network's activities (published on its website) are planned to achieve these objectives and include annual meetings, innovative teaching projects and organization of a repository of teaching resources.

Annual meetings have been held in face-to-face (2016-2019), virtual (2020-2021), or hybrid (2022-present) formats.

Teaching innovation projects have served as collaborative spaces for the development of online teaching materials to be shared through the website. These projects, launched in the 2015–16 academic year, are conducted annually and have been funded-by the Office of the Vice-Principal for Lifelong Learning, Educational Transformation and Employability

The repository of shared teaching resources (available on the website) contains Creative Commons (BY-NC-SA)-licensed teaching materials contributed by network members and is divided into four sections:

a) Pharmacology program for active learning.
b) Clinical simulation cases based on real patients.
c) Specific teaching resources, based on previous experiences of teaching innovation applicable in specific areas (Nursing, Pharmacy, Medicine, Dentistry, Veterinary Medicine...)
d) Assessment resources, including multiple-choice questions and rubrics

The network currently comprises 194 members from 33 Spanish universities, and 6 Ibero-American universities (Portugal (1), Chile (3), Mexico (1), Colombia (1)). Participating professors teach in different Bachelor's Degrees (Nursing, Pharmacy, Medicine, Veterinary, Biomedical Sciences, Optics, Nutrition...) and Master's Degrees in Health Sciences. As a participation tool, a specific website has been generated that collects the activities of the network, as well as the repository of teaching material shared thanks to the support of the Office of the Vice-Principal for Lifelong Learning, Educational Transformation and Employability The website (www.uv.es/redfarminn) significantly enhances the network's visibility and social impact, and has been well received by Pharmacology faculty members affiliated with the Spanish Society of Pharmacology.

Design of a Digital Escape Room as a Tool of Gamification for the Dynamisation of the Practices of Pharmacology and Pharmacotherapy III and Clinical Pharmacy

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In recent years, active methodologies have gained prominence in the university environment as a response to the need to involve students more actively in their learning process [1]. Within this framework, the educational escape room is presented as an innovative and effective tool for assessing competences in a playful and active way while encouraging student motivation. Adapted to the academic context, these escape games transfer the thrill of challenge to a learning environment [2]. Furthermore, they have the capacity to function as an assessment instrument that transcends the limitations of conventional examinations, thereby promoting an assessment paradigm that prioritises the practical application of knowledge [3]. By transforming assessment into an immersive experience, wherein students are required to solve challenges that are aligned with the course content, a deep understanding of the subject matter is fostered, in addition to the development of skills such as critical thinking, problem solving, communication and teamwork. The objective of this project is to conceptualise a digital escape room for the subjects of Pharmacology and Pharmacotherapy III and Clinical Pharmacy. The primary goals of this initiative are twofold: firstly, to enhance the dynamism of the practicals, and secondly, to utilise the escape room as an evaluation tool. For this purpose, the Genially® platform has been utilised, resulting in the development of an escape game comprising four phases. The first section introduces general principles, while the subsequent three focus on case studies, wherein students are tasked with solving questions, puzzles and word searches. Participants will be divided into groups, and a ranking will be established based on the time spent and the number of correct answers. The attainment of this ranking will result in a reward of up to 1 point being allocated to the final mark of the internship. It is evident that each phase is associated with the theoretical content of the Pharmacology and Pharmacotherapy III and Clinical Pharmacy modules, including topics related to pharmacovigilance such as RAM, alerts, notification of RAM and additional monitoring. At the conclusion of each phase, the groups will be assigned a numerical identifier. The combination of these four numbers will form a 4-digit code that will unlock the final screen of the Escape Room. Furthermore, a satisfaction survey has been devised for students to complete upon completion of the gamification tool. The survey was conducted utilising the Microsoft Forms platform and comprised a total of ten questions, encompassing diverse domains such as the general experience, the materials employed, and the learning process.

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Session 7: Cardiovascular pharmacology I

Moderator: Eduardo Oliver (Centro de Investigaciones Biológicas "Margarita Salas", CSIC, Madrid)

Invited speaker:

9:00-9:30 Miguel Romero (Universidad de Granada)
Gut Microbiota as a therapeutic target in hypertension: emerging mechanisms and pharmacological perspectives

Oral communications:

9:30-9:45 Ricardo Caballero (Universidad Complutense de Madrid)
A new peptide, DECA11, exerts antiarrhythmic effects in a mouse model of heart failure by selectively increasing I_{Na} and I_{K1}

9:45-10:00 Álvaro Gómez-Martín (Instituto de Investigaciones Sanitarias INCLIVA, Valencia)
Tirzepatide, a novel dual agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors, attenuates the progression of abdominal aortic aneurysm

10:00-10:15 Susana Novella (Universitat de València)
Sex- and Age-Dependent Regulation of miR-423-5p Following Acute Myocardial Infarction in a Senescence-Accelerated Mouse Model

10:15-10:30 Laura de la Bastida-Casero (Centro de Investigaciones Biológicas «Margarita Salas» (CIB)-CSIC, Madrid)
β3-Adrenergic Receptor Agonists as Modulators of Endothelial Dysfunction and Inflammation in Pulmonary Hypertension

Gut Microbiota as a Therapeutic Target in Hypertension: Emerging Mechanisms and Pharmacological Perspectives

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Hypertension is a multifactorial disorder increasingly linked to alterations in gut microbiota composition and function. Emerging evidence from both preclinical and clinical studies highlights gut dysbiosis as a key contributor to elevated blood pressure through immune, metabolic, and neurogenic mechanisms. Disruption of the gut microbial balance promotes low-grade inflammation, increased sympathetic nervous system activity, and impaired intestinal barrier integrity. These alterations facilitate the translocation of microbial products into the bloodstream, triggering immune responses and contributing to endothelial dysfunction and vascular damage.

One key mechanism involves the production of short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate, generated through bacterial fermentation of dietary fibers. SCFAs exert antihypertensive effects by promoting vasodilation, reducing oxidative stress and modulating the renin–angiotensin–aldosterone system, as well as maintaining gut barrier integrity. These effects are mediated via G-protein-coupled receptor activation, nitric oxide signalling, anti-inflammatory pathways (e.g. TLR4 and NADPH oxidase), and immune pathways, including the balance between regulatory T cells and Th17 lymphocytes.

Interventions targeting the gut microbiota, including increased fiber intake and supplementation with probiotics (e.g., Lactobacillus, Bifidobacterium), have shown benefits for blood pressure regulation, vascular function, and inflammatory status. Faecal microbiota transplantation studies further support a causal relationship, as normotensive individuals receiving microbiota from hypertensive donors developed elevated blood pressure. The gut-brain axis also plays a key role in linking changes in the microbiota to central neuroimmune regulation and sympathetic activity. Several studies have demonstrated that restoring microbial homeostasis can reduce sympathetic nervous system overactivity and improve cardiovascular health. Some pharmacological approaches, such as mineralocorticoid receptor blockers, may help to restore the integrity of the gut-brain axis and the composition of the microbiota. Although clinical trials remain limited, the current evidence suggests that microbiota-directed strategies have therapeutic potential.

In conclusion, the gut microbiota is a promising and novel therapeutic target for hypertension. Microbiota-directed strategies, such as probiotics and dietary fibre, offer a promising non-pharmacological approach to managing hypertension. These approaches support the development of personalised medicine, opening up new strategies for improving cardiovascular outcomes.

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A new peptide, DECA11, exerts antiarrhythmic effects in a mouse model of heart failure by selectively increasing I_{Na} and I_{K1}

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Heart failure (HF) is a life-threatening condition affecting around 15 million Europeans. Mortality ranges from 50-67% within five years after diagnosis, mainly as a consequence of ventricular arrhythmias (VA) and fibrillation. Available antiarrhythmic drugs are contraindicated due to their pro-arrhythmic effects, which further increase mortality. New safe and effective advanced therapies are urgently needed to prevent premature death in HF patients. In the ventricles, the main voltage-gated cardiac Na^+ channel (Nav1.5) and the strong inward rectifier K^+ channel (Kir2.1) are critical in controlling cardiac excitability and action potential (AP) propagation. Nav1.5 channels generate the inward sodium current (I_{Na}) responsible for rapid depolarization during the AP upstroke. Kir2.1 channels generate the inward rectifier potassium current (I_{K1}) that controls the resting membrane potential (RMP), the approach to the AP threshold and the final phase of repolarization. Reduced membrane expression of these two essential channels in cardiomyocytes from HF patients impairs ventricular excitability and delays repolarization, contributing to life-threatening VAs. We have designed an eleven amino-acid peptide named DECA11 capable of increasing I_{Na} and I_{K1} (Patent ES2953301B2) in heterologous expression systems and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). DECA11 significantly hyperpolarized the RMP and increased the AP amplitude (P=0.01), but did not modify currents generated by other cardiac (Cav1.2, Kv4.3, hERG, and Kv7.1+minK) and neuronal (Nav1.1, Nav1.2, Nav1.7, and Nav1.8) channels. Both I_{Na} and I_{K1} densities significantly (P=0.0086) decreased as a consequence of electrical remodeling in cardiomyocytes from mice with transverse aortic constriction (TAC) – induced cardiac remodeling and reduced left ventricular ejection fraction (EF). Gene therapy with intravenous adeno-associated virus encoding DECA11 in TAC mice restored cardiomyocyte I_{Na} and I_{K1} densities to control values. Importantly, VA inducibility and duration were significantly reduced in TAC mice infected with DECA11 virus, compared with TAC animals infected with empty virus. Our results demonstrate that DECA11 is a novel and unique advanced therapy aimed at increasing instead of decreasing cardiac I_{Na} and I_{K1} to exert exciting antiarrhythmic effects hiPSC-CM and in a model of pressure overload-induced HF with reduced EF.

Tirzepatide, a novel dual agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors, attenuates the progression of abdominal aortic aneurysm

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Abdominal aortic aneurysm (AAA) is a pathological condition defined by a progressive dilation of the abdominal aorta, which significantly elevates the risk of rupture. Recent advances in the pharmacological management of metabolic disorders have introduced dual agonists of the glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) receptors as novel therapeutic agents for type 2 diabetes and obesity. However, their potential to directly modulate vascular pathophysiology, particularly in the context of aneurysmal disease, remains to be fully elucidated. This study aimed to assess the effects of tirzepatide, a dual GLP-1R/GIPR agonist, on endothelial dysfunction and AAA progression, and to delineate the molecular mechanisms underlying these effects through *in vivo* and *in vitro* experimental approaches.

The effect of tirzepatide treatment was investigated in a murine model of angiotensin II (Ang-II)-induced AAA in apoE($^{-/-}$) mice. The effects of dual GLP-1R/GIPR agonism on leukocyte-endothelial cell interactions induced by TNFα were also determined *in vitro* by a laminar flow chamber assay.

In apoE$^{-/-}$ mice infused with Ang-II, tirzepatide administration significantly attenuated the expansion of the maximal suprarenal aortic diameter. Histological examination revealed that tirzepatide markedly preserved aortic wall integrity by reducing elastin fragmentation, loss of medial smooth muscle cells, neovascularization, and macrophage infiltration within the aneurysmal lesions. Ang-II-induced upregulation of matrix-degrading enzymes (MMP-2, MMP-9) in the aortic wall was significantly suppressed by tirzepatide treatment. *In vitro* studies in human venous endothelial cells demonstrated that tirzepatide inhibited TNFα-stimulated leukocyte adhesion and downregulated the expression of vascular adhesion molecules VCAM-1 and ICAM-1. Moreover, immunofluorescence analysis showed that tirzepatide suppressed NF-κB signaling pathway activation in endothelial cells.

Tirzepatide confers vascular protection by attenuating endothelial inflammation and inhibiting the AAA progression.

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Sex- and Age-Dependent Regulation of miR-423-5p Following Acute Myocardial Infarction in a Senescence-Accelerated Mouse Model

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MicroRNAs are post-transcriptional regulators with critical roles in cardiovascular homeostasis. Among them, miR-423-5p is deregulated after acute myocardial infarction (AMI), yet its modulation by sex and aging remains unexplored. We previously characterized the Senescence-Accelerated Mice (SAM) as an adequate model to perform miRNA studies after an experimental myocardial infarction. This study aims to evaluate the impact of sex and aging on circulating and cardiac levels of miR-423-5p, and its involvement in signalling pathways following an AMI.

AMI was surgically induced by coronary artery permanent ligation in 6-month-old SAM-Prone (SAMP8) mice and their controls SAM-Resistance (SAMR1), both male and female (n=8/group; Ethics 2020/VSC/PEA/0128). Sham-operated mice served as control. Four hours after surgery, serum and cardiac tissue were collected. RNA was isolated and miR-423-5p was determined by qRT-PCR, using snRNAU6 as endogenous control. Functional pathway analysis were performed *in silico* using miRWalk and KEGG enrichment. Values were expressed as mean ± SEM and t-Test and one-way ANOVA methods were used to determine the difference between groups, when appropriate.

In young male SAMR1, circulating miR-423-5p was significantly downregulated after AMI (-53.3 ± 10.8%, $p < 0.05$) with no changes in females. In the aged SAMP8 mice both males and females, no significant changes were found in serum levels. However, cardiac miR-423-5p was downregulated only in SAMP8 males following AMI (-54.5 ± 10.4%, $p < 0.05$), indicating an age- and sex-specific pattern. Functional analysis of predicted target genes for miR-423-5p resulted included RAS, MAPK and PI3k-Akt signalling pathways, all critical for processes involved in cardiac repair and remodelling such as angiogenesis, cell survival, and cardiomyocyte proliferation.

miR-423-5p exhibits sex- and age-dependent regulation following AMI, particularly in aged male myocardium. Its involvement in key reparative pathways highlights its potential as a biomarker or therapeutic target for personalized cardiac recovery strategies.

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β3-Adrenergic Receptor Agonists as Modulators of Endothelial Dysfunction and Inflammation in Pulmonary Hypertension

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Pulmonary Hypertension (PH) is a progressive and life-threatening disorder characterized by increased pulmonary vascular resistance, leading to right ventricular (RV) hypertrophy and failure1. Endothelial dysfunction, often triggered by chronic inflammation or hypoxia, plays a key role in disease progression. Activated endothelial cells upregulate adhesion molecules such as E-selectin (CD62E) and vascular cell adhesion molecule 1 (VCAM-1), which facilitate leukocyte adhesion and contribute to inflammation and vascular remodelling processes through the induction of smooth muscle markers like α-SMA or GDF152. Our study investigates the effects of β3-adrenergic receptor (β3-AR) agonists, including Mirabegron and Vibegron—drugs currently used to treat overactive bladder—on hemodynamic, morphology, inflammatory and remodelling markers in PH models. Using a murine model of hypoxia+Sugen-induced PH, we demonstrated through western-blot and immunofluorescence that treatment with β3-AR agonists shows a tendency to reduce E-selectin, VCAM-1 and α-SMA in lung tissue. This was accompanied by a reduction of RV systolic pressure and hypertrophy with both drugs. These results were in concordance with previous works where we described a protective role of Mirabegron on the pulmonary endothelium3 and identified relevant diseases biomarkers in patients treated with this drug4. In addition, to molecular analyses, we performed positron emission tomography (PET) in treated mice, which showed marked improvements in pulmonary and myocardial metabolism. These findings suggest that β3-AR agonist may have beneficial effects not only on vascular inflammation and remodelling, but also on the overall cardiopulmonary metabolism and function. Our findings highlight the potential of β3-AR agonist as modulators of endothelial dysfunction and inflammation in PH. Biomarkers such as E-selectin or VCAM-1 not only reflect disease severity but may also guide future precision medicine strategies and support the repositioning of β3-AR agonist like Mirabegron or Vibegron as a novel therapeutic approach for PH.

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Session 8: Neuropharmacology II

Moderator: M. Julia García Fuster (Universidad de las Islas Baleares)

Invited speaker:

9:00-9:30 Luis Gandía (Universidad Autónoma de Madrid)
Is amyotrophic lateral sclerosis a «drug-resistant» pathology?

Oral communications:

9:30-9:45 Raquel Lama (Universidad Santiago de Compostela)
Development and characterization of an SH-SY5Y neurosphere model to investigate cognitive deficits in schizophrenia

9:45-10:00 Yaiza Trueba (Universidad Complutense de Madrid)
Attenuating effect of kynurenine on alcohol withdrawal-induced hyperalgesia

10:00-10:15 Susana García-Cerro (Universidad de Sevilla)
Clozapine Induces Perineuronal Net Remodelling in a Developmental Mouse Model of Schizophrenia

10:15-10:30 Lorena Martínez-Hostyn (Universidad Miguel Hernández-CSIC)
Effects of cannabidiol on acute spontaneous alprazolam withdrawal in adult male and female mice

Is amyotrophic lateral sclerosis a "drug-resistant" pathology?

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Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive neurodegenerative disease characterized by the selective loss of upper and lower motor neurons. Despite decades of intense research, available therapeutic options remain extremely limited, with only modest benefits in terms of survival or quality of life. This therapeutic stagnation persists despite a growing understanding of the complex, multifactorial nature of the disease.

In our laboratory, we have pursued a systems-level, multimodal approach to ALS therapy, guided by the notion that monotherapies targeting isolated mechanisms are unlikely to succeed against such a heterogeneous and dynamic pathology. Our strategy has focused on the rational repurposing of clinically approved drugs, assembled into triple-drug combinations designed to simultaneously modulate key pathogenic pathways implicated in ALS: neuroinflammation, excitotoxicity, oxidative stress and/or mitochondrial dysfunction. Drug combinations were selected based on mechanistic complementarity, and promising synergistic effects observed *in vitro* in cell line models under stress paradigms relevant to ALS pathology.

Encouraged by the *in vitro* efficacy of these combinations in reducing cell death, inflammatory signaling, and reactive oxygen species generation, we proceeded to test several of them *in vivo* using the $SOD1^{G93A}$ transgenic mouse model of ALS. In this talk, we will review the experimental trajectory and present unpublished data from our most recent studies. Disappointingly, none of the tested triads yielded significant improvements in disease onset, progression, motor function, or survival compared to untreated or vehicle-treated controls. Histological and biochemical analyses similarly failed to demonstrate robust neuroprotective effects at the spinal cord level.

We argue that the continued therapeutic failure in ALS cannot be explained solely by flawed models or poor drug choices. Instead, it may stem from core biological traits of the disease that require a fundamental shift in therapeutic thinking. Despite sound preclinical rationale and optimized dosing, repeated failures suggest an unsettling but plausible hypothesis: ALS may have inherent features that confer pharmacoresistance, limiting the effectiveness of standard small-molecule therapies. Potential mechanisms include: (1) reduced drug permeability due to blood–spinal cord barrier disruption and glial scarring; (2) persistent non-cell-autonomous degeneration involving microglia and astrocytes; (3) severe bioenergetic deficits hindering drug uptake and metabolism; and (4) depletion of endogenous compensatory systems before treatment begins—even at pre-symptomatic stages.

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Development and Characterization of an SH-SY5Y Neurosphere Model to Investigate Cognitive Deficits in Schizophrenia

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Cognitive impairment is a core feature of schizophrenia that remains poorly understood and largely unresponsive to current therapies. Conventional two-dimensional cell models fail to fully capture the structural and functional complexity of neural tissue. To address this gap, we developed a three-dimensional neurosphere model using SH-SY5Y neuroblastoma cells to study the molecular and functional alterations associated with cognitive deficits. We aimed to establish a physiologically relevant platform by generating differentiated neuronal phenotypes through the sequential application of retinoic acid (RA) followed by either phorbol 12-myristate 13-acetate (PMA) or glucagon-like peptide-1 (GLP-1). These assays lay the foundation for evaluating the response of neurospheres to cognitive deficit-related insults, such as MK-801 and dopamine, which mimic schizophrenia-associated dysfunctions.

Neurospheres were formed by culturing SH-SY5Y cells in individual wells on a methylcellulose-based matrix to promote spherical aggregation. After sphere formation, cells underwent a two-stage differentiation protocol: 5 days with 10 µM RA, followed by 7 days with either 80 nM PMA or 1 µM GLP-1. Morphological characterization included measurements of sphericity, border definition, and structural integrity. Functional analysis involved intracellular calcium mobilization assays using a Calcium 6 dye in response to depolarizing (KCl) and glutamatergic stimuli (glutamate).

Morphological evaluation revealed that neurospheres differentiated with PMA or GLP-1 formed compact, rounded structures with smooth, well-defined edges and high circularity (roundness index close to 1), indicating successful neuronal differentiation and structural maturation. In contrast, non-differentiated neurospheres displayed irregular shapes, poorly defined borders, and scattered peripheral cells, reflecting immature and unstable architecture. Functionally, calcium imaging showed that GLP-1-differentiated neurospheres exhibited significantly enhanced intracellular calcium mobilization upon KCl stimulation compared to both non-differentiated and PMA-differentiated spheroids. Moreover, only GLP-1-differentiated neurospheres responded robustly to glutamate stimulation, suggesting a more functionally mature glutamatergic phenotype that is particularly relevant for studying cognitive processes and synaptic plasticity.

We present a robust and reproducible 3D neurosphere model derived from SH-SY5Y cells that mimics key aspects of neuronal differentiation and function. Differentiation with GLP-1, in particular, generates a phenotype highly responsive to glutamatergic signaling, making it a valuable platform for investigating cognitive dysfunction in schizophrenia. The morphological and functional characterization of this model provides a solid basis for testing cellular responses to pharmacological insults such as MK-801 and dopamine, enabling mechanistic studies and therapeutic screening relevant to cognitive deficits in neuropsychiatric disorders.

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Attenuating effect of kynurenine on alcohol withdrawal-induced hyperalgesia

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Alcohol is the most widely consumed drug of abuse globally. It is now known that there is a strong association between chronic pain and alcohol use disorder (AUD). In fact, pain is an important risk factor for relapse during AUD treatment[1]. Ionotropic NMDA-type glutamate receptors play a role in both the development of chronic pain and the effects of alcohol, including withdrawal symptoms. Changes in signalling and expression of the 2B subunit of these receptors have been observed following ethanol exposure and in different models of neuropathic pain. Kynurenic acid, an antagonist of these receptors, can be increased by kynurenine (KYN) administration, which has been proposed as a possible treatment for pain.

In our study, we investigated the impact of KYN co-administered with probenecid (to prevent its clearance from the brain) on ethanol withdrawal-induced hyperalgesia. To explore the involvement of NMDA 2B subunits in this process, we also used ifenprodil, a selective antagonist targeting this subunit. We applied a chronic intermittent ethanol exposure (CIE) model[2], in which animals were exposed to ethanol vapour for four weeks to induce dependence. Mechanical hyperalgesia was assessed using the von Frey filament test during withdrawal in both treated and untreated groups.

Mechanical hyperalgesia was detected in both male and female subjects 24 hours after the final week of ethanol vapor exposure, and this sensitivity to pain persisted for at least 7 days into withdrawal. Treatment with KYN combined with probenecid reduced this withdrawal-associated hyperalgesia and did not result in tolerance, even with repeated daily administration. Additionally, treatment with ifenprodil also alleviated hyperalgesia during withdrawal, supporting the involvement of NMDA 2B subunits in this pain response.

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Clozapine Induces Perineuronal Net Remodelling in a Developmental Mouse Model of Schizophrenia

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Schizophrenia (SCZ) is a multifaceted neurodevelopmental disorder frequently associated with extensive dysregulation of extracellular matrix (ECM) components, both in cortical and subcortical regions, strongly linked to cognitive deficits [1, 2]. Among these ECM components, perineuronal nets (PNNs) are particularly affected, with significant changes observed in the prefrontal cortex (PFC) [3, 4]. Although it has been proposed that antipsychotic (AP) treatments impact on PNNs and ECM integrity in SCZ patients, these effects have not been confirmed yet [5, 6]. In this study, we employed a neurodevelopmental model of SCZ in mice, which involves perinatal NMDA receptor hypofunction induced by ketamine administration [7]. This model effectively mimics long-term alterations in the PFC associated with SCZ [8], providing insights into the impact on PNNs and interneuron populations, particularly parvalbumin (PV) neurons. While ketamine increased PNN compactness, VGLUT excitatory inputs, and c-Fos expression, clozapine (CLZ), an atypical AP [9], mitigated these changes, particularly restoring the structure of PNNs in the medial PFC. Moreover, CLZ treatment was associated with improved cognitive flexibility and social memory, functions strongly linked to PFC integrity. These findings support the role of CLZ in modulating ECM structure in the SCZ brain and highlight the need for further research on the effects of AP medications on PNN dynamics and ECM integrity.

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Effects of cannabidiol on acute spontaneous alprazolam withdrawal in adult male and female mice

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Benzodiazepine dependence can lead to a withdrawal syndrome, the management of which is currently a major public health problem. This study aims to evaluate cannabidiol (CBD) as a therapeutic strategy in a new animal model of acute spontaneous alprazolam (APZ) withdrawal, assessing possible sex differences.

Increasing doses of APZ (0.015-0.960 mg/kg/12 h, 3 weeks, p.o.) were administered to adult CD1 male and female mice. 24 h after cessation of APZ treatment, light- dark box and open field paradigms were used to evaluate the effects of an acute dose of CBD (20 mg/kg, i.p.). In addition, real time PCR experiments were performed in the amygdala (Amy) and the hippocampus (Hipp) to evaluate gene expression of GABAA receptor α2 (GABRA2) and ɣ2 (GABRG2) subunits, as well of cannabinoid receptors 1 (CNR1) and 2 (CNR2). Finally, blood plasma was isolated for quantification of the endocannabinoid ligands anandamide (AEA) and 2-arachidonoilglycerol (2-AG) by HPLC-mass spectrometry.

Spontaneous APZ withdrawal induced a significant anxiogenic effect in males and females, together with hyperactivity, increased number of rearings and rubbings, and reduced grooming behaviour in both sexes. Interestingly, CBD largely normalized these alterations. GABRA2 was significantly up-regulated in the Amy and Hipp of both males and females exposed to APZ withdrawal, whereas differential changes were observed between sexes in GABRG2. CB1r mRNA levels were higher in the Amy but lower in the Hipp of males under APZ withdrawal while no significant changes were detected in females. CB2r gene expression significantly increased in both sexes and regions. Treatment with CBD modulated the observed alterations in the GABAergic system. On the other hand, CBD significantly normalized the gene expression of CB1r in the Hipp of male mice, whereas CB2r was up-regulated in both brain regions of males and females. Finally, plasma levels of AEA were increased in males but decreased in females, whereas 2-AG was up-regulated independently of sex. Importantly, CBD significantly normalized all these alterations.

CBD showed therapeutic potential to ameliorate the behavioural and molecular alterations induced by spontaneous APZ withdrawal. Overall, this work suggests that CBD deserves attention as a new strategy for the management of APZ withdrawal syndrome.

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Session 9: New approaches to drug design, development and administration

Moderator: María del Carmen Carceller (Universitat de València)

Invited speaker:

15:00-15:30 Mª Jesús Vicent (Centro de Investigación Príncipe Felipe, Valencia)
Polypeptide-based Nanomedicines: enhancing tropism and overcoming biological barriers

Oral communications:

15:30-15:45 Xabier Rovira (Institut de Química Avançada de Catalunya, CSIC, Barcelona)
Light-regulated drugs for spatiotemporal regulation of β-adrenoceptors

15:45-16:00 Irene Rodríguez-Clemente (Universidad de Castilla La Mancha)
siRNA transfection: a new therapeutic approach to glioblastoma

16:00-16:15 Ana Jarén (Universitat de Valencia)
Targeted anti-MCL1 delivery in β-galactosidase positive cells reduces M1 macrophages and fibrosis in murine intestine

16:15-16:30 Fernando Yáñez-Gómez (Universitat de les Illes Balears)
Impact of incorporating active compounds into hydrogels functionality: controlled delivery of ketamine vs. fluoxetine in thermosensitive matrices

Polypeptide-Based Nanomedicines: Enhancing Tropism and Overcoming Biological Barriers

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Polypeptides play a pivotal role in various domains, particularly in nanomedicine[1]. The physicochemical properties and biological performance of polypeptide-conjugates are dictated by a complex interplay of structural factors, highlighting the critical need for detailed structure–activity relationship (SAR) studies to inform the design of hierarchical polypeptide systems. Notably, this structural complexity is advantageous, as even subtle molecular alterations can yield significant and unexpected biological effects[1].

In our research group, we have addressed conventional challenges in polypeptide synthesis through precise, controlled reactions, enabling the creation of well-defined architectures via N-carboxyanhydride (NCA) polymerization[2]. Post-polymerization modifications further extend functionality, introducing a diverse array of orthogonally reactive attachment points[1 3]. Utilizing this modular, bottom-up approach, we have engineered polypeptides with varied architectures—including diblock copolymers and star-shaped constructs—that self-assemble into supramolecular nanostructures. The scale up of such assemblies have been optimised by mean of microfluidics, to yield nanocarriers that have demonstrate promising traits, such as, tissue specificity[4], subcellular compartment targeting[5], and potential for brain delivery[6].

Coupling this structural strategy with rational crosslinking approaches and polymer–drug linker design[4–7] has enabled extensive in vitro and in vivo evaluation. These systems show minimal toxicity, enhanced cellular uptake, prolonged systemic circulation, and targeted accumulation in specific sites such as lymph nodes[4], mitochondria[5], and the brain[6]. These effects are modulated by structural parameters including stiffness, deformability, charge, size, or shape.

Collectively, these findings position our polypeptide-based nanosystems as promising candidates for use as nanocarriers in therapeutic and diagnostic applications[8].

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Light-regulated drugs for spatiotemporal regulation of β-adrenoceptors

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Recent studies have uncovered new functional features of G protein-coupled receptors (GPCR) related to their localization and processes kinetics. Indeed, GPCR signaling is currently recognized as a multidimensional process governed by molecular, spatial and temporal components. Uncovering the role of each of these dimensions is crucial to understand how different pathways give rise to cellular and physiological effects. Along these lines new methods are being developed to monitor the activity of GPCR, such as genetically encoded fluorescent sensors localized on specific cell types and subcellular regions. Some of these new methods have been used in alive animals to study GPCR in their native environment. However, to gain mechanistic insight on GPCR function, we also need molecular actuators. This is why, on the other side of the coin, exciting research is nowadays demonstrating the capability of light-regulated drugs to operate GPCR function in a time-resolved manner. This emerging field of research named Photopharmacology is based on the development and use of light-sensitive molecules to control a given target protein in native tissues. Using these molecules, both spatial and temporal regulation of the compound activity can be achieved in unprecedented manners compared to conventional pharmacology. Photopharmacology bears a great potential for the study of the interplay between the activation time and the receptor location during signaling processes in non-modified cells, tissues and whole organisms.

Our research is focused on the generation of light-controlled molecular tools for beta-adrenoceptors (β-AR), which are prototypical GPCR and important pharmacological targets for numerous diseases. In particular, we will present several compounds with promising pharmacological properties, which can be reversibly and irreversibly controlled by light. Some of these molecules are selective ligands for beta-1 and beta-2 adrenoceptors with low nanomolar activities, which enable a fine control of β-AR both in vitro and in their native environment. We believe that the results of these studies will certainly open the door to innovative research methods and procedures for studies targeting β-AR with spatiotemporal resolution.

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siRNA transfection: a new therapeutic approach to glioblastoma

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Glioblastomas (GBMs) are the most common type of primary brain tumor in adults [1]. The standard treatment for GBM includes maximal surgical resection followed by radiotherapy and concomitant temozolomide (TMZ)-based maintenance chemotherapy. Despite the standard treatments administered, the overall patient survival rate at 2 years from the time of the primary diagnosis is around 27% [2]. Hence, research on new treatments is required to further improve survival in patients with GBM.

Small interfering RNA (siRNA) is a synthetic double-stranded compound comprising 20 to 24 base pairs that mimics the endogenous RNAi system to interfere with various mechanisms involved in the genesis of several diseases. These molecules are highly specific and can rapidly and markedly decrease target protein levels. However, siRNAs are very labile and require binding to other molecules to prevent degradation and facilitate their transport to the cell interior.

We have used several families of nanoparticles: dendrimers, carbohydrate derivatives, and dihydropyridine derivatives to transfect siRNA into GBM cells from commercial sources, patients' tumors, and patient-derived organoids. We have found that all the nanoparticles bound siRNA and protected it from RNase-mediated degradation. The three families were able to transport siRNA into the cell interior and reduce the intracellular levels of the target proteins (p42MAPK, Rheb, and MGMT) involved in the proliferation and survival of GBM cells to about 10 to 20% of control levels.

Moreover, none of the nanoparticles were toxic to neurons or astrocytes *in vitro* or to animals *in vivo*. Biodistribution studies using either nanoparticles or siRNA labeled with a fluorescent probe indicated that some of the nanoparticles were able to reach the brain and the medulla. These findings demonstrate the potential of siRNAs as a new therapeutic approach to previously undruggable diseases and highlight the use of nanomedicine and nanoparticles as a novel therapeutic strategy for central nervous system diseases.

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Targeted anti-MCL1 delivery in β-galactosidase positive cells reduces M1 macrophages and fibrosis in murine intestine

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MCL1 immunostaining has been shown in fibroblasts of the fibrotic ileum of CD patients[1] suggesting that senescence of these cells may be a mechanism of fibrosis perpetuation. We aimed to analyse here the expression of senescence markers in murine intestinal fibrosis and to determine the effects, on this process, of galacto-oligosaccharide-functionalized Mesoporous Silica Nanoparticles (NPs) loaded with an anti-MCL1 drug, S63845 (MSN-S63845).

MCL1 and β-Galactosidase immunohistochemistry (IHQ) were performed in colons of mice underwent two DSS cycles, obtained from a previous samples collection. C57BL/6 mice (20–25 g) were drinking DSS at 2% during 2 cycles (a cycle consists in 7 days drinking water with 2% DSS, followed by ten days drinking water). After the first cycle, mice were administered intraperitoneally, 5 days a week, with MSN-S63845 (8 mg/kg), empty MSN, vehicle or S63845 (8 mg/kg), until the end of the experiment. Body weight was monitored daily, and mice were sacrificed 34 days after. A sample from the colon was paraffin embedded for the analysis of collagen deposition in Syrius Red-stained slides and CD68 immunohistochemistry which were both quantified by the QuPath software. Other sample was used for RT-PCR studies. Unless other mention, results are expressed as mean±sem.

Both MCL1 and β-galactosidase immunostaining were detected in leukocytes and fibroblasts of the colon of chronic DSS-treated mice. The effects of blocking MCL1, selectively in β-gal positive cells (MSN-S63845), or non-selectively (S63845) did not alter changes in body weight associated to DSS, but increased the colon length (cm) of mice receiving MSN-S63845 (5.7±0.2, P=0,08) or S63845 (6.2±0.3, P=0,04*) compared with the respective vehicles (5.1±0.1 or 5.2±0.2, respectively). Treatment of MSN-S63845 compared with empty MSN induced a significant diminution in: a) the number of CD68 positive cells (151.8±17.7 vs 255.8±40.1, P= 0,03*), b) the thickness of the submucosa (32.4±2.9 vs 51.6±5.9, P = 0,024*), and c) in the mRNA expression of inflammatory markers *il17* (2.6 (0.02-0.7) vs 1.2 (0.6-3.4), P=0.03$^{\#}$) and M1 macrophage marker *cd86* (0.6±0.2 vs 1.2±0.2, P=0.05*), while it failed to significantly modify the M2 macrophage marker, *cd206* (1.0 ±0.1 vs 0.9±0.1, P=0.88) and *il10* (0.8 ±0.1 vs 1.2±0.2, P=0.28). In contrast, treatment with S63845 compared with vehicle did not significantly alter any of these parameters. $^{\#}$Mann-Whitnney; *t-test

The selective blockade of MCL1 in β-galactosidase positive cells reduces the number of M1 macrophages, the expression of inflammatory cytokines and fibrosis in the colon of chronic DSS-treated mice.

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Impact of Incorporating Active Compounds into Hydrogels Functionality: Controlled Delivery of Ketamine vs. Fluoxetine in Thermosensitive Matrices

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Thermosensitive injectable hydrogels based on Pluronic F127 and hyaluronic acid (HA) offer a promising platform for subcutaneous drug delivery systems [1]. While ketamine has demonstrated effective controlled release using this matrix in preclinical studies, the adaptability of the same hydrogel matrix to other active compounds like fluoxetine—a widely prescribed SSRI [2]—remains unclear. Therefore, the present follow-up study aimed at comparing the physicochemical properties and release behavior of incorporating different active compounds into our hydrogels.

Hydrogels were formulated using Pluronic F127 (26% or 29% w/v) combined with HA (0.5% or 1% w/v), and loaded with ketamine or fluoxetine at equivalent preclinical doses (5 mg/kg per day). All formulations were evaluated for gelation capacity at 20 °C and 37 °C, structural integrity over 8 days, and drug release kinetics. Thermal behavior was analyzed via Differential Scanning Calorimetry (DSC), viscosity through rheometry, and network morphology by Scanning Electron Microscopy (SEM). *In vitro* release was quantified by UV-Vis spectrophotometry at fixed time intervals.

All hydrogels demonstrated effective *in-situ* gelation and structural stability. However, drug-dependent differences were observed in their functionality. Ketamine-loaded formulations, particularly 29/80/1 and 26/80/1, showed near-zero-order release profiles with minimal burst effect, favorable viscosity, and consistent gelation temperatures. In contrast, fluoxetine-loaded hydrogels exhibited a sharper initial burst release (up to 50% in the first 9 h), with irregular subsequent release, especially in 85/15 compositions. DSC analyses revealed slightly higher gelation temperatures for fluoxetine hydrogels, suggesting altered polymer-drug interactions. SEM imaging confirmed variations in pore architecture and density depending on the active compound.

The incorporated drug significantly influenced the functional performance of our thermosensitive hydrogel systems. While ketamine aligned well with the mechanical and release properties of the F127/HA matrix, fluoxetine disrupted controlled release dynamics, indicating the need for further optimization. These findings highlighted the critical role of drug-polymer compatibility in the design of versatile drug delivery platforms.

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Session 10: Cardiovascular pharmacology II

Moderator: Laura Piqueras (Universidad de Valencia)

Invited speaker:

15:00-15:30 Eduardo Oliver (Centro de Investigaciones Biológicas "Margarita Salas", CSIC, Madrid)
Exploring new therapeutic applications of Beta-adrenergic drugs: from lab discovery to clinical impact

Oral communications:

15:30-15:45 Diego Berlanas Vicente (Universidad Autónoma de Madrid)
Alamandine prevents IL-1β-induced inflammation and senescence in cultured human endothelial cells

15:45-16:00 Víctor Collado-Díaz (Universitat de València)
Integrase Inhibitors Modulate Cardiac Fibroblast Differentiation In Vitro

16:00-16:15 Anaïs Clara Terol-Úbeda (Universidad de Salamanca)
5-HT_{2A}-induced sympathoexcitatory action at vascular level is mediated by angiotensin ii in female rats

16:15-16:30 Dolores Viña (Universidad de Santiago de Compostela)
Effects of cAMP signalling activation on endothelial barrier disruption induced by hypoxia and hypoxia/reperfusion

Exploring new therapeutic applications of Beta-Adrenergic drugs: From lab discovery to clinical impact

Eduardo Oliver[1,2,3]

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Beta-adrenergic receptors (βARs) have long been established as pharmacological targets for cardiovascular diseases. Traditionally, β1ARs has been described as cardiac receptors while β3ARs has been deeply studied regarding fat metabolism (1). However, recent evidence by our group has revealed that selective modulation of β1 and β3ARs can exert profound anti- inflammatory and mitochondrial-protective effects, also acting over unexpected cells and tissues, opening new therapeutic avenues beyond hemodynamic control. As an example, the β1-selective antagonist metoprolol – a drug approved to treat hypertension, cardiac arrythmias and heart failure – displays unique immunomodulatory properties not shared by other β- blockers. In preclinical and clinical models of acute myocardial infarction, metoprolol administered during ischemia reduces infarct size, neutrophil infiltration, and ameliorates cardiac function (2,3). These effects extend to non-cardiac conditions: in stroke models, metoprolol attenuates neuroinflammation and neuronal death (4), while in patients with COVID-19–related ARDS, it improves lung inflammation and oxygenation (5). Mechanistic studies suggest these benefits stem from direct effects on neutrophil migration and phenotype, and emerging pharmacogenetic data highlight enhanced efficacy in individuals carrying the Arg389 variant of the β1AR, supporting a precision medicine approach (2,3). Concurrently, β3AR agonists such as mirabegron – an approved drug to treat overactive bladder –, have shown promise in preserving mitochondrial dynamics and endothelial function. In animal models of pulmonary arterial hypertension and aortic stenosis–induced heart failure, pharmacological stimulation or gene transfer targeting β3AR improves right and left ventricular performance respectively, reduces oxidative stress, and restores mitochondrial morphology (6,7). Additionally, cardiomyocyte-specific β3AR activation further protects against ischemia- reperfusion injury by sustaining mitochondrial structure and bioenergetics (8). Altogether, β1AR antagonism – particularly with metoprolol – and β3AR agonism represent promising and complementary strategies to modulate inflammation and mitochondrial dysfunction in cardiopulmonary diseases. These findings illustrate the translational potential of β-adrenergic drugs in novel clinical contexts and exemplify how mechanistic insights can guide rational drug repurposing having an impact on clinical guidelines and patients' quality of life.

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Alamandine prevents IL-1β-induced inflammation and senescence in cultured human endothelial cells

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Alamandine is a novel heptapeptide of the protective branch of the renin-angiotensin system (RAS), a key peptide hormone system for the maintenance of cardiovascular homeostasis. Indeed, in addition to the classical arm, that generates angiotensin II, the RAS also includes a counterregulatory axis, with alternative peptides and receptors, that activates multiple protectives signaling pathways. In the cardiovascular system, this protective branch mediates anti-inflammatory and antifibrotic effects opposing the potentially harmful actions of the classical axis. Here, we evaluated the effect of alamandine in the prevention of endothelial cell inflammation and senescence, two key components of premature vascular aging. We used human umbilical vein endothelial cells (HUVEC) treated with the proinflammatory cytokine interleukin-1β (IL1β) (2.5 ng/ml) as a model of prosenescent stress *in vitro*. Alamandine (100 nM) prevented the priming phase of the NLRP3 inflammasome (a multiprotein complex of the innate immune system that plays a central role in the development of vascular disease) by reducing the expression of: (1) NF-κB, a key transcriptional regulator; (2) NLRP3, essential component of the inflammasome; and (3) pro-IL1β, the precursor of mature IL1β. Alamandine also demonstrated the ability to attenuate the activation phase of the NLRP3 inflammasome by preventing increases of: (1) cleaved caspase-1, the enzyme responsible for the maturation of pro-IL1β; (2) cleaved IL-1β, the final product of the inflammasome; and (3) the formation of ASC (Apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain) specks by immunofluorescence, which is reflecting the activation of NLRP3 inflammasome itself. We also examined the anti-senescent potential of alamandine. This peptide prevented the increase of senescence-associated markers, such as SA-β-galactosidase activity, the marker of cell cycle arrest p16, and the expression of mTOR, a regulator of metabolism, proliferation, and differentiation. Furthermore, alamandine was able to prevent the acquisition of the senescence-associated secretory phenotype (SASP) by inhibiting the transcription of CCL2, IL-6, and IL-8. These effects were at least partially dependent on the Mas-related G protein-coupled receptor member D (MrgD) since the protective effects of alamandine were reduced by the specific receptor antagonist, D-Pro^{7}-Angiotensin-(1-7) (1 μM). These results suggest that alamandine can be a therapeutic alternative to prevent vascular inflammation and senescence, thus attenuating the progression of cardiovascular diseases associated with aging.

Integrase Inhibitors Modulate Cardiac Fibroblast Differentiation In Vitro

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Cardiac fibrosis is a scarring process triggered in response to tissue injury, characterized by the activation of pro-fibrotic mediators such as transforming growth factor-β1 (TGF-β1). These factors stimulate cardiac fibroblasts (CFs) to proliferate, migrate, and differentiate into myofibroblasts—cells that excessively deposit extracellular matrix proteins, including collagen I (Col1A), collagen III and overexpress α-smooth muscle actin (α-SMA) and N-cadherin, contributing to pathological remodelling of the heart. Integrase strand transfer inhibitors (INSTIs), an essential component of antiretroviral therapy, have been associated with an increased incidence of cardiovascular (CV) disease during the first two years of use[1]. While cardiac fibrosis has been observed in HIV-infected individuals [2,3], the specific effects of INSTIs on CF activation and differentiation remain unclear. Therefore, this study aims to investigate the impact of clinical concentrations of several INSTIs—bictegravir (BIC), dolutegravir (DTG), cabotegravir (CAB), and raltegravir (RAL)—and the non-nucleoside reverse transcriptase inhibitor (NNRTI) doravirine (DOR), which has not been linked to CV risk, on the differentiation of CF into myofibroblasts in vitro.

Primary cardiac fibroblasts were treated (24h-48h) with BIC (10 and 20 µM), DTG (10 and 20 µM), CAB (30 µM), RAL (10 µM), and DOR (5 µM). Myofibroblast differentiation was assessed by RT-qPCR (24 and 48 h) and flow cytometry (48 h), examining the expression of profibrotic and mesenchymal markers including Col1A, fibronectin (FN-1), α-SMA/ACTA2, PDGFR-α/β, vimentin and CD325.

RT-qPCR revealed that DTG significantly increased Col1A (Fold induction, 1.44±0.18 vs vehicle) and FN-1 (1.28±0.15 vs vehicle) expression after 24 hours. At 48 hours, both BIC (2.15±0.28 vs vehicle) and DTG (1.56±0.19 vs vehicle) elevated ACTA2 expression. Flow cytometry confirmed increased α-SMA (239.34±24.5 vs. 100% vehicle) and CD325 (263.69±15.21 vs. 100% vehicle) with BIC showing a more pronounced effect than DTG. Additionally, DTG upregulated PDGFR-α. At the same time, both BIC and DTG enhanced PDGFR-β, supporting a role in promoting CF differentiation into myofibroblasts.

These findings suggest that certain INSTIs, particularly BIC and DTG, promote CF to myofibroblast differentiation in vitro. This highlights a potential pro-fibrotic role of these agents, underlining the need for further investigation into their long-term CV effects in people living with HIV.

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5-HT$_{2A}$-induced sympathoexcitatory action at vascular level is mediated by angiotensin II in female rats

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Cardiovascular diseases (CVD) remain the leading cause of death in women worldwide, yet the understanding of sex-specific pathophysiological mechanisms is still limited. Thus, investigating the neurovascular and humoral regulatory pathways is crucial to find sex-related therapeutic strategies in CVD. Serotonergic system plays an important role in cardiovascular regulation. In fact, serotonin (5-hydroxytryptamine, 5-HT) exerts both inhibitory and excitatory effects on vascular sympathetic activity, depending not only on the receptor subtype and target tissue, but also on sex-related physiological differences [1-5]. We have recently shown that, in female rats, activation of 5-HT$_{2A}$ and 5-HT$_3$ receptors potentiates vascular noradrenergic outflow. These data differs from previous findings in males, where only 5-HT$_3$ receptors were involved in this sympatho-enhancing effect [5,6]. The aim of this study was to investigate the possible indirect mechanisms underlying this sympatho-excitatory action in female rats. To do so, female rats were anesthetized and pithed. An intravenous bolus injection of several pharmacological inhibitors—targeting prostanoids, endothelin, and the renin-angiotensin systems—were administered prior to the infusion of either TCB-2 (5-HT$_{2A}$ agonist; 1 µg/kg/min) or 1-PBG (5-HT$_3$ agonist; 10 µg/kg/min). Under these conditions, vascular sympathetic outflow was electrically stimulated (15 ± 3 V; 25 s; 0.1, 0.5, 1, and 5 Hz) to assess vasopressor responses [5]. The sympatho-potentiating effect of 1-PBG was not altered by any of the inhibitors tested, suggesting a direct action of 5-HT$_3$ receptors on sympathetic terminals, independent of endothelial or circulating mediators. In contrast, the sympatho-excitatory effect of TCB-2 was abolished by losartan (1 mg/kg, i.v.), an angiotensin II (Ang II) type 1 receptor (AT$_1$) antagonist. Furthermore, plasma levels of angiotensin II increased following TCB-2 infusion in female rats, suggesting that 5-HT$_{2A}$ receptor activation promotes local Ang II formation, which subsequently enhances sympathetic neurotransmission via AT$_1$ receptor stimulation. In summary, 5-HT$_3$ receptor-mediated sympatho-excitation occurs through a direct mechanism, whereas 5-HT$_{2A}$ receptor-induced sympatho-potentiation depends on Ang II. These findings provide novel insights into the sex-specific interplay between serotonergic and the renin-angiotensin systems in the modulation of vascular sympathetic tone, and opens new perspectives for exploring sex-specific therapeutic strategies in cardiovascular diseases.

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Effects of cAMP signalling activation on endothelial barrier disruption induced by hypoxia and hypoxia/reperfusion

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Cyclic AMP (cAMP) is a second messenger involved in various biological processes through the activation of two main effectors: protein kinase A (PKA) and exchange proteins directly activated by cAMP (Epac1). Among its functions, its role in the maintenance of endothelial function in health and disease is especially relevant. Numerous studies have linked dysfunctions in the brain endothelial cells, key components of the blood brain barrier (BBB), to the development of neurodegenerative disorders. Therefore, preserving BBB integrity is crucial for CNS homeostasis (Sweeney et al. *Nat Rev Neurol*. 2018, 14 (3): 133-150). Consequently, the activation of cAMP and its effectors PKA and Epac represents a promising strategy to improve endothelial function under disturbing stimuli (Viña et al. *Cells*. 2021, 10 (8): 1951).

The aim of this study was to evaluate the effects of forskolin and rolipram (cAMP-elevating agents), 8-pCPT-2'-O-Me-cAMP (Epac1 activator) and 6-Bnz-cAMP (PKA activator) against hypoxia (H) and hypoxia/reperfusion (H/R)-induced barrier dysfunction in brain murine microvascular endothelial cells (bEnd.3). This is the first study to directly compare the effects of Epac1 and PKA activation on BBB integrity under these conditions

H/R induced a loss of barrier integrity, significantly decreasing bEnd.3 trans-endothelial electrical resistance (TEER), without affecting cell viability. Claudin-5 protein levels were significantly decreased, and ZO-1 was internalized after H/R insult. Both H and H/R tended to increase reactive oxygen species production, while the expression of hypoxia-inducible factor 1-α (HIF-1-α) was increased after 6h H. Under these conditions, forskolin and rolipram, both cAMP-elevating agents, restored TEER and claudin-5 expression. Selective Epac1 activation with 8-pCPT-2'-O-Me-cAMP prevented H/R-induced loss of endothelial barrier integrity by increasing bEnd.3 TEER, while PKA activation with 6-Bnz-cAMP failed to improve barrier integrity. The roles of Epac1 and PKA in these effects were confirmed using selective Epac (ESI-09) and PKA (Rp-cAMPs) inhibitors.

In conclusion, cAMP/Epac1 signalling emerges as a promising target for protecting endothelial barrier integrity under hypoxic conditions. Given the strong link between BBB dysfunction, cerebrovascular disease, and neurodegeneration, modulating this pathway may offer therapeutic potential to reduce vascular contributions to cognitive decline.

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Session 11: Medicina personalizada. Terapias avanzadas

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

Invited speaker:

9:30-10:30: Andrea Romero (Konexio Biotech, Madrid)

Session 12: Digestive & Respiratory Pharmacology

Moderator: M. Carmen Montesinos (Universidad de Valencia)

Invited speaker:

9:30-10:00: Raquel Abalo Delgado (Universidad Rey Juan Carlos)
Enteric neuropathies induced by antitumoral drugs

Oral communications:

10:00-10:15 Clara Quintas (Universidade de Porto, Portugal)
RAAS enzyme signatures in feces: a novel biomarker approach in inflammatory bowel disease

10:15-10:30 Carmen de la Fuente-Gómez (Universitat de Valencia)
Therapeutic potential of fedratinib in reducing liver fibrosis in metabolic dysfunction-associated steatohepatitis

Enteric neuropathies induced by antitumoral drugs

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Cancer is a major global cause of morbidity and mortality. Importantly, survivorship is increasing, bringing new challenges. Cancer treatments, including chemotherapeutic drugs, immunotherapy, and radiotherapy, can have severe and impactful side effects occurring shortly after treatment (acute toxicity) or persisting for years after treatment ends (late/chronic toxicity or sequelae).

One of the main effects of antitumoral drugs is neurotoxicity, particularly affecting peripheral nerves, more exposed to their direct actions. Whereas peripheral neuropathy affecting somatic nerves has been extensively studied, that affecting the enteric nervous system is not so well known, yet it may have an important long-term impact on gastrointestinal functions, causing symptoms like diarrhoea, constipation or visceral pain.

Gastrointestinal functions are intrinsically controlled by the enteric nervous system, which encompasses both the submucous (Meissner) and the myenteric (Auerbach) plexi. Whereas the submucous plexus (located in the submucosa) controls functions such as absorption and secretion, the myenteric plexus (located between the two layers of smooth muscle in the muscularis propria) is the main controller of gastrointestinal motor patterns.

Using preclinical models, several laboratories, including ours, have described how different antitumoral drugs may cause relatively persistent alterations in the enteric nervous system, underlying gastrointestinal dysfunctions. The possible development of an enteric neuropathy in cancer patients after cancer chemotherapy (or radio-chemotherapy) has also been suggested in some reports, although evidence is more limited.

This talk will present an overview of the findings collected in both preclinical models and humans showing evidence of the development of chemotherapy-induced enteric neuropathy and will describe the mechanisms most likely involved in it, as the first step in looking for strategies that may contribute to reverse and, possibly, prevent its development, as well as its impact on gastrointestinal health in the increasing number of cancer survivors.

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RAAS Enzyme Signatures in Feces: A Novel Biomarker Approach in Inflammatory Bowel Disease

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Recent discoveries of a local renin-angiotensin-aldosterone system (RAAS) in the gastrointestinal tract and its role in inflammation and fibrosis prompt research of RAAS involvement in inflammatory bowel disease (IBD). The ACE/ACE2 balance is central to RAAS-mediated effects and may offer insight into IBD pathophysiology. Emerging data, primarily from retrospective studies, have explored systemic and tissue-level RAAS activity in IBD patients, focusing on the clinical outcomes of RAAS blocker use. However, the lack of reliable cost-effective fecal biomarkers of IBD highlights a critical gap in non-invasive disease monitoring. This study was approved by the Ethics Committee of Centro Hospitalar São João and of the Faculty of Pharmacy of the University of Porto (CE 147/21). Fecal and serum samples from 26 non-IBD controls and 46 IBD patients (17 Ulcerative colitis (UC) and 29 and Crohn's disease (CD)) were used. Fecal calprotectin levels were used to determine disease activity. ACE and ACE2 activities were measured by fluorimetric assays and total protein was quantified using the Bradford assay. ACE and ACE2 isoforms were identified by Western blotting.
Fecal ACE2 activity was significantly reduced ($p<0.05$) in CD (in nmol/min/mg of total proteins: active-CD - 236.3[124.2-111.4], remission-CD - 330.6[33.93-865.2]) and overall IBD patients (in nmol/min/mg of total proteins: active-IBD - 238.6[124.3-661.4], remission-IBD - 521.4[44.34-1293.0]) despite disease activity, compared to non-IBD controls (1276.0[397.4-2071.0] nmol/min/mg of total proteins). However, fecal ACE activity showed no major differences. In controls, fecal ACE activity was predominantly N-domain-related, unlike in IBD patients where both domains were equally active. Serum ACE activity was lower ($p<0.05$) in Ulcerative colitis, Crohn's disease and overall IBD patients, regardless of disease activity, (in nmol/min/mL: active-UC - 16.5[5.1-37.5], remission-UC - 15.1[6.7-24.9], active-CD - 14.2[7.6-33.7], remission-CD - 23.7[11.4-31.5], active-IBD - 16.1[7.0-34.3], remission-IBD - 15.1[11.4-23.7]) than in non-IBD controls (37.1[29.3-55.5] nmol/min/mL), while serum ACE2 activity remained similar across groups ($p>0.05$). No differences in enzyme activities were observed between active disease and remission stages. Additionally, 50 and 70 kDa ACE isoforms were found in the feces of IBD patients under conventional therapeutic approach but absent in the feces of non-IBD controls or IBD patients under therapy with biologics. Also, a 70 kDa isoform of ACE2 was found present in the feces of non-IBD controls, but absent in the feces of IBD patients, either under conventional therapeutic approach or therapy with biologics. These results are protected by Patent Cooperation Treaty (PCT/IB2025/055764). Our study raises the possibility of the presence of ACE and ACE2 isoforms in human feces, or a combination of both, could serve as a disease biomarker.

Therapeutic potential of fedratinib in reducing liver fibrosis in metabolic dysfunction-associated steatohepatitis

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Metabolic dysfunction-associated steatohepatitis (MASH) is characterized by hepatocellular ballooning, steatosis, and inflammation, often progressing to liver fibrosis, a condition for which effective pharmacological treatments are currently lacking. Fedratinib, a selective JAK2 kinase inhibitor approved by the FDA and EMA for the treatment of myelofibrosis, has also demonstrated antifibrotic effects in murine models of idiopathic pulmonary fibrosis. Given that the JAK2 signaling pathway seems to be implicated in fibrosis development, we evaluated the potential hepatic benefits of fedratinib in a murine model of MASH.

Male 6-week-old C57BL/6 mice were fed either a standard (control) diet or a MASH-inducing diet (choline-deficient high-fat diet) for 12 weeks. At 8 weeks, either a vehicle solution or fedratinib (5 mg/kg/day) was administered subcutaneously using osmotic pumps to the MASH group for 4 weeks. Then, blood samples were collected and liver sections were analyzed histologically using hematoxylin-eosin and picrosirius red staining to assess steatosis and fibrosis, respectively. Immunophenotyping of hepatic immune cells was performed by flow cytometry. Finally, biochemical parameters and plasma levels of relevant soluble mediators were assessed by colorimetric or ELISA assays.

While fedratinib had no effects on liver steatosis, it significantly reduced hepatic fibrosis by 40.3% in MASH mice. Moreover, treatment with fedratinib significantly reduced leukocyte infiltration in the liver, increased the presence of regulatory T lymphocytes, and shifted macrophage polarization towards an anti-inflammatory (M2-like) phenotype.

Our data support the potential use of fedratinib as a therapeutic approach to reduce liver fibrosis development in MASH, a disease for which pharmacological treatments are lacking. Nevertheless, further transcriptomic, proteomic, and histological analyses are needed to explore the underlying mechanisms and signaling pathways.

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POSTER SESSIONS

Neuropharmacology

P1

Perinatal Disruption of NMDA Receptors with Ketamine Induces Novel Long-Term Autism-Related Phenotypes

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Autism Spectrum Disorder (ASD) and schizophrenia (SCZ) are neurodevelopmental disorders with distinct clinical profiles yet substantial overlap in symptomatology, including social impairments, cognitive deficits, and abnormalities in sensory processing. A growing body of evidence suggests that an imbalance in excitation and inhibition (E/I) plays a crucial role in both disorders driven, at least partially, by NMDA receptor (NMDAr) hypofunction and dysfunction in GABAergic interneurons, particularly these parvalbumin-positive (PV^+) [1]. Additionally, perineuronal nets (PNNs), specialized extracellular matrix (ECM) structures involved in various forms of plasticity, are also implicated in these pathophysiological processes. While the NMDAr hypofunction theory has been extensively studied in SCZ, its role in ASD is less explored despite its potential to model core autistic traits [2]. Thus, this study examined the effects of NMDAr antagonism using a neurodevelopmental mouse model in which ketamine (30 mg/kg) was administered on postnatal days 7, 9, and 11 to conduct a brain- wide analysis of PV^+ interneurons and PNNs in key ASD-associated brain regions and explore their link to previously undescribed ASD-phenotypes. In adulthood, the model exhibited novel ASD-like traits, including deficits in social behavior, cognitive flexibility, and motor and sensory processing, as well as disruptions in PV^+ cells in the caudate-putamen, CA2 region, and somatosensory cortex, accompanied by alterations in PNNs. Notably, the cerebellum was also affected, displaying a severely disrupted E/I balance. Our findings support ketamine- induced NMDAr antagonism as a translational model for ASD, emphasizing the impact of early developmental disruptions in neurological disorders. Moreover, the study highlights the significance of underexplored brain structures in ASD, such as PNNs, offering novel insights into their potential roles in neurodevelopmental disorders and opening new research directions.

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P2

Spinocerebellar Ataxia type 3 affects early postnatal cerebellar development

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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, leading to a polyglutamine stretch that and the aggregation of the protein ataxin-3[1]. Being the most prevalent ataxia worldwide [2,3,] it causes severe disability, and normally ends with premature death of the patients. Although the onset of SCA3/MJD normally occurs during adulthood, the structure of the cerebellum is proved to be altered before the onset of the disease, as well as the existence of nonspecific symptoms several years before the clinical diagnosis[4]. Furthermore, there is no effective treatment, so research on unveiling when neural alterations exactly appear are of utmost importance 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 an 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. We have also characterized these progenitors electrophysiologically by patch clamp these and have concluded that SCA3/MJD cells show a slightly more mature neuron profile than their WT counterparts. In addition, we have combined these methods with immunohistochemical assays which suggest differences in postnatal cerebellar architecture, as well as gene and protein expresión assays or viability assays.

Furthermore, our research group has a long-lasting experience in the study of the purinergic system, which is known to have a key role in development, homeostasis and survival of neuronal populations of the cerebellum[6]. Consequently, we have analysed the expression and modulation of some components of this purinergic system, as a potential and promising strategy to treat SCA3/MJD.

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P3

Psilocybin modulates different stages of hippocampal neurogenesis in adolescent rats of both sexes

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There is an urgent need for novel, fast-acting antidepressants to treat resistant depression and prevent suicide, particularly in adolescents, as current treatment options are often limited and ineffective for this age group [1]. Psilocybin has recently emerged as a promising candidate in psychiatric research, especially for mood disorders, even in adolescence, as proved by our recent study [2]. However, the mechanisms behind its therapeutic effects remain unclear [3]. Since hippocampal neurogenesis is thought to play a role in mediating antidepressant effects, this study investigated how repeated oral psilocybin administration affected neurogenesis markers in adolescent male and female Sprague-Dawley rats. Animals were pre-treated with BrdU (2 × 50 mg/kg, i.p., 12 h apart for 3 consecutive days, postnatal days; PND 29-31) to label dividing cells. After 3 resting days, rats received psilocybin (0.1, 0.3, or 1 mg/kg) or vehicle (0.9% NaCl) orally (2 ml/kg) for 7 consecutive days (PND 36-42). Twenty-four hours after the last treatment dose, hippocampal tissue was analysed for markers of cell proliferation (Ki-67), early neuronal differentiation (NeuroD), and cell survival (BrdU) via immunohistochemistry. These markers were selected based on their relevance in distinct phases of the neurogenic process. Two-way ANOVAs revealed significant effects of psilocybin treatment across all markers, with no sex-related effects; therefore, data were pooled across sexes and analysed using one-way ANOVAs. The main results indicated that psilocybin significantly increased recent cell proliferation ($F_{3,42} = 4.114$; $p = 0.0120$), early neuronal differentiation ($F_{3,42} = 11.04$; $p < 0.0001$) and cell survival ($F_{3,42} = 7.917$; $p = 0.0003$). In particular, the doses of 0.3 and 1 mg/kg increased Ki-67 (33 ± 7% and 24 ± 7%) and NeuroD (60 ± 9% and 55 ± 9%) respectively as compared to controls. Finally, all doses tested of psilocybin increased BrdU (66 ± 11% at 0.1 mg/kg, 79 ± 15% at 0.3 mg/kg, and 92 ± 17% at 1 mg/kg) relative to controls. Overall, repeated oral administration of psilocybin modified several key markers of hippocampal neurogenesis in adolescent rats, regardless of sex. These data align with the antidepressant-like potential of psilocybin in adolescence [2], since the observed enhancement in neurogenesis supports the idea that psilocybin's antidepressant-like effects may be driven by its ability to promote neuroplasticity within the hippocampus.

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P4

Retinal glial cells phenotypes in a mouse model of retinitis pigmentosa

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Retinal neuroinflammation is a common mechanism in retinal neurodegenerative diseases as retinitis pigmentosa (RP). This process is initially triggered as a protective response; however, with persistent activation as occurs in retinal neurodegeneration, it evolves into chronic glial activation, resulting in detrimental effects on the tissue. Neuroinflammation is orchestrated mainly with the contribution of retinal glial cells, including microglia, Müller cells and astrocytes. It has been described that brain microglia and astrocytes have at least two main phenotypes each, one with a predominant proinflammatory and the other anti-inflammatory role. In contrast, little is known about the existence of different phenotypes of retinal glial cells in neuroinflammation context. Knowing the role of glial cell phenotypes in the retinal degeneration appears essential to fully know the molecular basis of the degeneration and to find new therapeutic approaches that could delay the onset or slow down the progression of the disease. To clarify the contribution of glial cell types to the neurodegenerative process, we have analyzed, by flow cytometry and immunohistochemistry, the expression levels of some of the main molecular actors in the neuroinflammation process; the complement factor C3, the calcium-binding protein S-100A10 and the C1 inhibitor, the serine-protease Serping1, in all retinal glial cells, that is Müller cells, astrocytes and microglia, in the rd10, a mouse model of retinitis pigmentosa. We have studied their expression at different ages, from postnatal age (P)17 until P40. As healthy controls we used retinas of C57BL6/J mice. We have observed different expression levels of C3, S100 and Serping1 proteins along all the glial cell populations starting as soon as P17, even before the signs of retinal degeneration are evident. These results point to the existence of different glial cells phenotypes, which suggests a differential contribution to the onset and the progression of retinal degeneration, what could be used to the finding of new therapeutic targets.

P5

Identification and validation of the I2 Imidazoline receptor as a target for Alzheimer's disease

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Neuroinflammation has emerged as a crucial process alongside the classical hallmarks of Alzheimer's disease (AD), namely amyloid and tau pathologies. Glial cells and neuroinflammatory responses are increasingly recognized as key players in potential therapeutic strategies. The I2 imidazoline receptor (I2-IR) has garnered attention as a promising biological target due to its analgesic, anti-inflammatory, and neuroprotective properties.

Our study aimed to achieve two primary objectives: first, to determine the nature of I2-IR, and second, to assess the neuroprotective potential of its ligands. To identify candidate proteins that may interact with I2-IR, we conducted Thermal Shift Assay (TSA) experiments on murine brain tissue treated with 2-BFI (5 mg/kg). This screening yielded 17 potential proteins, which were further refined to seven putative targets via molecular dynamics analysis. Thus far, three—MAO-B, α2A adrenergic receptors, and creatine kinase B—have been subjected to phenotypic assays in the presence of 2-BFI and Idazoxan. Our findings revealed a 9% inhibition of MAO-B enzymatic activity by 2-BFI and 1% by Idazoxan, with neither compound exhibiting a clear agonist or antagonist profile on α2A adrenergic receptors. To address our second objective, we utilized in vitro glial cultures and a 5XFAD murine model of AD. Microglia and astrocytes derived from primary cultures were treated with 2-BFI and Idazoxan, demonstrating neuroprotective effects regardless of LPS-induced inflammatory challenge. Additionally, both ligands enhanced calcium influx in glial cultures. However, cytokine expression analysis revealed distinct responses: 2-BFI (30 µM) upregulated IL-6, IL-1, IL-10, and TNF-α, while Idazoxan (30 µM) displayed differential effects on pro- and anti-inflammatory cytokines, reinforcing its antagonistic role in comparison to 2-BFI, as reported in previous literature.

In the 5XFAD model, both compounds improved short- and long-term working memory, further supporting their therapeutic potential. Overall, our results highlight that 2-BFI and Idazoxan elicit comparable neuroprotective effects within a proinflammatory environment, both in vitro and in vivo. The modulation of I2-IR could present a novel avenue for slowing or halting AD progression. As such, further characterization of I2-IR as a therapeutic target is warranted to enhance efforts in developing effective treatments, ultimately improving the quality of life for AD patients while reducing healthcare costs and familial burden.

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P6

Finding pathways related to cognitive déficits in an in vitro phenotypic model of schizophrenia through transcriptomic studies

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Schizophrenia is a chronic psychiatric disorder affecting 1% of the population, characterized by disruptions in glutamatergic and dopaminergic signaling. Positive symptoms are controlled by current antipsychotics, as well as some negative symptoms. However, there is a clinical need for novel drugs able to ameliorate cognitive symptoms of the disease. Target-based approaches had not been successful in finding novel drugs, thus to model schizophrenia- related cognitive deficits, we developed an in vitro pharmacological model. This model employed a human neuroblastoma cell line differentiated with retinoic acid and Glucagon-like peptide-1 (GLP-1), which showed to induce differentiation to glutamatergic and dopaminergic neurons.

In this work to aimed to induce a cognitive deficit-like phenotype and identify those pathways that are dysregulated under these conditions through transcriptomic studies.

Differentiated cells were treated with dopamine, the pore blocker of the NMDA receptor Dizocilpine (MK-801), or a combination of both. RNA-sequencing analysis revealed distinct gene expression profiles, with the combined treatment inducing a unique transcriptomic signature. GO analysis showed modifications in neurodevelopmental processes (e.g., axon guidance, neurogenesis), and KEGG analysis highlighted changes in the expression of pathways such as calcium signalling and retinol metabolism. In conclusion, this model recapitulates molecular features of cognitive impairment present in schizophrenia and identified pathways that can be useful targets for novel drugs ameliorating the cognitive deficits associated to schizophrenia.

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P7

A Preclinical Model of Premenstrual Dysphoric Disorder Induced an Anhedonia-like Phenotype in Female Sprague-Dawley Rats

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Premenstrual dysphoric disorder (PMDD) is a severe mood condition affecting 3–10% of reproductive-age women, characterized by emotional and physical symptoms that negatively impact daily life, including depression, irritability, anxiety, and physical discomfort. While PMDD is considered a pathological intensification of premenstrual symptoms during the luteal phase, its underlying mechanisms remain unclear. Current treatments often fail to relieve symptoms in a significant proportion of patients, highlighting the need to develop and validate preclinical models that can aid in the identification of novel therapeutic strategies. In this context, we aimed at validating a preclinical model of PMDD in female Sprague-Dawley rats in which to later evaluate potential treatment options. To do so, a total of 31 adult female Sprague-Dawley rats were treated intraperitoneally with progesterone (500 µg, 1 ml/kg) for either 5 (n=10) or 10 (n=12) consecutive days, or vehicle (1 ml/kg DMSO, n=9). After treatment, all animals underwent a 24-hour withdrawal period to mimic the abrupt decline in progesterone levels hypothesized to underlie PMDD symptomatology. Rats were evaluated using a battery of behavioral tests sensitive to affective-like disturbances: forced swim test (FST), open-field test (OFT), and sucrose preference test (SPT). The same procedure was carried out in a separate cohort of 27 ovariectomized rats (vehicle n=10; 5-day treatment n=8; and 10-day treatment n=9). To confirm that surgeries were performed correctly, pre and post ovariectomy blood analysis revealed a significant decrease in progesterone levels ($p<0.0001$) through an ELISA kit. Data analysis through one-way ANOVAs revealed a significant effect in the SPT ($F_{2,27} = 3.73$, $p = 0.037$), with Dunnett's post-hoc test indicating a significant reduction in sucrose preference in the 10-day progesterone group (–35 ± 13%, *$p = 0.020$), while the 5-day group showed a non-significant trend (–18 ± 13%, $p = 0.285$) when compared to vehicle-treated rats. These findings are indicative of an anhedonia-like response associated with the prolonged exposure to progesterone followed by an abrupt withdrawal. The same effects were observed in ovariectomized rats, with a 37 ± 12% decrease in sucrose preference after 10 days of progesterone treatment when compared to the vehicle group (**p = 0.0076). To better understand these effects, ongoing studies are assessing hormonal and neurochemical changes to further elucidate the mechanisms underlying the affective-like symptoms in this model. These findings confirm that the prolonged progesterone exposure and subsequent withdrawal is associated with anhedonia as measured by decreased sucrose preference, offering a robust and relevant preclinical model of PMDD. Moreover, this anhedonia-like phenotype is independent of circulating sexual hormones as it is observed both in sham and ovariectomized female rats. Thus, our PMDD preclinical paradigm is a valuable tool for assessing the efficacy of emerging therapeutic options targeting affective-like symptoms associated with the disorder.

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P8

Caenorhabditis elegans as a preclinical tool to study cognition

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Background: “Cognitive deficits” is the impairment of different domains of cognition. It is one of the most limiting symptoms that persist in many patients with different pathologies such as schizophrenia (SCZ) and Alzheimer's disease (AD), among others who respond to current therapies for other symptoms, but not for cognitive symptoms. Clinical findings have reconsidered studies and research with muscarinic acetylcholine receptor (mAChR) agonists as a new approach to potentially treat negative symptoms of SCZ and improve cognitive impairment in Alzheimer's patients1,2. Goals: Given these findings, we proposed the development of a model of cognitive evaluation in Caenorhabditis elegans, an alternative animal model to study pathways correlated with SCZ and AD. Methods: Strain Wild-type Bristol (N2) was obtained from the Caenorhabditis Genetics Center (CGC). To evaluate the effect of typical and atypical prototypic antipsychotics on cognition, we induced associative memory (butanone/Echerichia coli OP50) in adult worms of the N2 strain, previously treated with haloperidol (HAL, 160 µM), clozapine (CLZ, 160 µM), scopolamine (SCOP, 120 µM) as a negative reference drug or donepezil (DON, 10 µM) as a positive reference drug or vehicle for 24 hours, following protocols previously described3,4. The chemotaxis index (CI) = [(number of worms at test zone (butanone)) – (number of worms at control zone (ethanol))] / (total number of worms), and the learning index (LI) = [CI of trained animals] – [CI of naïve (untrained) animals] were evaluated at 0, 1, 2, 3 and 4 hours after conditioning of the worms. Data were analyzed by two-way ANOVA followed by Dunnett's multiple comparison test. Results are presented as mean ± standard error of the mean (SEM). Results: Conditioned untreated worms showed an increase in the learning index at times 0 and 1 h (0.55±0.054 and 0.50±0.057, respectively, $p<0.0001$) and 2 h (0.35±0.08, $p<0.001$), compared to the unconditioned vehicle group (-0.05±0.09) under starvation. After 3 and 4 h, the mentioned worms lose more than 50% of their previous performance. Conditioned worms treated with HAL or SCOP have impaired memory and learning at 0 h, compared to conditioned untreated worms (0.24±0.04 and 0.11±0.09 respectively, $p<0.05$), while worms treated with DON showed a significantly higher index of memory and learning compared to conditioned untreated worms after 3 and 4 hours (0.69±0.06, $p<0.01$ and 0.70±0.16, $p<0.05$, respectively). Worms treated with CLZ showed a reduction in the learning index at times 0, 1 and 2 h, without statistical significance. Conclusion: The Caenorhabditis elegans model has been shown to be useful in discerning the effects of drugs that can modulate cognitive capacity.

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P9

Assessing the in vitro and in vivo toxicity of TAT-TrkB: a potential therapeutic approach for Alzheimer's Disease

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In Alzheimer's Disease, the accumulation of amyloid plaques disrupts BDNF signalling [1-3], by promoting a calpain-mediated TrkB receptor cleavage [1,4]. TAT-TrkB was shown to re-establish in vitro BDNF signalling and improve memory and learning in vivo [1]. The BDNF/TrkB system is also expressed outside the central nervous system (CNS), namely in endothelial cells [5]. Here, we assessed the in vitro toxicity of TAT-TrkB in neurons and endothelial cells, as well as its in vivo toxicity in mice.

Neurons cultured using Wistar rat embryos (n=4) were exposed on DIV14 to 1, 5, 10 or 20mM of TAT-TrkB, 24h after which an MTT assay was performed. Similarly, human umbilical vein endothelial cells (HUVECs) were cultured until passage 2 at a density of 2.6 ×105 cells/mL in fast media (0.1% FBS) (n=4) and exposed to either control (media alone), BDNF (20ng/mL), TAT-TrkB at 3, 10 and 30mM, or both BDNF and TAT-TrkB, for 24h, and then processed for MTT assay. For the in vivo toxicity assays, either vehicle or TAT-TrkB (12.5mg/kg [i.v., oral], 25mg/kg [i.v., i.p.], 37.5mg/kg [oral] or 50mg/kg [oral]) were administered to C57BL/6J mice or 5xFAD mice, either single dose (24h) or multiple dose (5 days/week, 9 weeks), after which animals were sacrificed and blood samples and organs were collected and analysed.

No toxicity was observed in HUVECs under any of the conditions. Regarding neurons, a significant decrease in cell survival was seen with 20mM of TAT-TrkB. Concerning the in vivo studies, an increase in platelet count was observed at 12.5mg/kg (24h, oral), along with a decrease in leukocyte count at 12.5mg/kg (24h, i.v.), a decrease in red blood cells and haematocrit, and an increase in mean corpuscular volume and mean corpuscular haemoglobin at the 25mg/kg (9weeks, i.p.), all of which remained within normal range. All organs were normal, except 2 out of 4 animals in the 25mg/kg (24h, i.v.) group, which presented foci of acute liver necrosis.

In summary, data suggests that in vitro TAT-TrkB does not appear to exert toxic effects outside the CNS, even under conditions of pathway overstimulation with BDNF exposure, and only affects neuronal survival at the highest concentration. In vivo toxicity assays did not reveal any relevant differences, except for acute liver necrotic foci observed at 25mg/kg (24h, i.v.) Thus, overall, no relevant clinical toxicity is expected with either short- or long-term use of TAT-TrkB.

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P10

Anesthesia with propofol does not alter the antidepressant-like response induced by electroconvulsive seizures in male and female adolescent rats

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The induction of electroconvulsive seizures (ECS) is considered a safe option for treating adolescent patients with treatment-resistant depression. However, further preclinical research is necessary to better characterize the observed sex differences in treatment efficacy, and how it is impacted by general anesthesia. In prior studies, ECS was tested at different intensities (35, 45 or 95 mA) in male and female adolescent rats, proving sex-differences in the intensity needed to be effective; while 95 mA was effective in males, a lower intensity (35 mA) was needed to induce an antidepressant-like effect in females [1,2]. Subsequently, we assessed the behavioral effects of propofol and ketamine in adolescent male and female rats after daily administration for 5 days, since those are the most common anesthetic drugs used in pediatric patients. Neither anesthetic altered affective-like behaviors or long-term cognitive performance. The current follow-up study aimed to evaluate the behavioral outcomes resulting from the interaction between one of those anesthetics, propofol, and ECS. To do so, adolescent male and female Sprague-Dawley rats were pre-treated (i.p.) with propofol (50 mg/kg) or vehicle (saline, 1 ml/kg), and 5-min later followed by ECS treatment (95 mA for males and 35 mA for females), using the same stimulation parameters as previous studies (0.6 s, 100 Hz, 1 session/day, 5 days).

Control rats were subjected to SHAM treatment. The onset and duration of the loss of righting reflex (LORR) were recorded daily, observing the expected sex differences induced by propofol, with females showing a faster onset (4.8 vs. 6.02 min) and a longer duration (16.7 vs. 14.1 min) of LORR than males. Moreover, the characteristics of the seizures induced by ECS were also measured, proving anesthesia with propofol decreased the duration of the tonic and clonic phases induced by ECS in male rats, while showed no changes in females, since 35 mA rarely induced seizures at all. Then, the antidepressant-like response of ECS and its interaction with propofol was scored 1-day post-treatment under the stress of the forced-swim test. The results validated the antidepressant-like response induced by ECS in male (95 mA) and female (35 mA) adolescent rats, while proved that anesthesia through propofol did not alter that response. Interestingly, propofol changed the characteristics of the seizures induced by ECS without altering its antidepressant-like potential. Finally, short-term cognitive safety, which was evaluated in adolescence using the radial arm maze to assess working memory, showed no differences among experimental groups. Overall, these findings suggest that propofol is a suitable anesthetic for its use in conjunction with ECS in adolescence, as it did not diminish its antidepressant-like response. Nevertheless, further research is needed to explore the interaction of ECS with other anesthetic agents to identify the optimal combination that ensures both efficacy and safety during adolescence and into adulthood.

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P11

Impact of the COVID-19 pandemic on impulse control disorders associated with dopamine agonist drugs

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Impulse control disorders (ICD), including pathological gambling, compulsive shopping, hypersexuality, binge eating, etc., are known adverse drug reactions to dopaminergic therapy[1–4]. The impact of confinement with major lifestyle changes triggered by the COVID- 19 pandemic on the risk of dopamine agonist drug-induced ICD is unknown. This study aimed to assess the effect of the COVID-19 pandemic on the association between exposure to dopaminergic agonist drugs and the risk of developing ICD.

A study based on spontaneous reporting data from the European Pharmacovigilance Database (EudraVigilance) was performed. The following were compared between the two periods under study (11/03/2018/-10/03/2020 [P1] and 11/03/2020- 10/03/2022 [P2]): 1) demographic and clinical characteristics of individual cases, and 2) the disproportionality reporting of dopaminergic agonist drug-induced ICD (ROR, *reporting odds ratio* [IC 95 %]), through a case/non case analysis.

365 reports of suspected ICD caused by dopaminergic agonist drugs were analysed. There were no statistically significant differences in either the mean age (around 57 years in P1 and P2) or the sex of the patients. Following the onset of the pandemic, the mean ± SE number of ICDs per patient increased from 1.3 ± 0.04 in P1 (n= 244 patients) to 1.5 ± 0.1 in P2 (n= 121 patients). A disproportionality analysis of impulse control disorders in relation to dopaminergic agonist drugs revealed pramipexole and ropinirole to have the highest ROR (95% CI) values: pramipexole 130.78 (108.07-158.27) at P1 and 168.89 (130.14-219.19) at P2; ropinirole 84.92 (61.50-117.26) at P1 and 146.78 (100.82-213.71) at P2. This could be related to their binding affinity for the D3 receptor[4] and/or their mode of administration. Moreover, the increase in ROR at P2 in the disproportionality analysis of each ICD against all dopaminergic agonist drugs combined also indicated the impact of the pandemic that triggered them.

The outcomes of our study highlight of closely monitoring of patients treated with dopaminergic agonist drugs, as well as informing patients and their social circles about these possible adverse reactions, especially in stressful situations which may contribute to impulsive behaviours, such as during the pandemic.

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P12

Targeting G protein signaling in psychedelic-induced neuroplasticity: Exploring the role of Gq/11 and Gi/o

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In recent years, serotonergic psychedelics have attracted significant attention for their potential therapeutic effects on various mental disorders. These compounds primarily act on serotonin (5-HT) receptors, with particular emphasis on the 5-HT2A receptor, which is abundantly expressed in the brain. Although the 5-HT2A receptor preferentially couples with Gq/11 proteins, it has also been reported to couple with Gi/o proteins under certain conditions. The administration of serotonergic psychedelics has been shown to induce neuroplasticity; however, the intracellular signaling mechanisms underlying these morphological changes remain poorly understood.

We investigated the roles of Gq/11 and Gi/o signaling pathways in synaptogenesis, dendritogenesis, and the expression of the immediate early genes c-fos and egr-2, induced by serotonergic psychedelics. This was conducted using an in vitro cell line model composed of neurons and glial cells derived from differentiated murine fetal forebrain progenitors. The psychedelics examined included lysergic acid diethylamide (LSD), N,N-dimethyltryptamine (DMT), psilocin, and 2,5-dimethoxy-4-iodoamphetamine (DOI), as well as non-hallucinogenic derivatives: lisuride, 2-bromo-D-lysergic acid diethylamide (2-Br-LSD), and 1-(2,5-dimethoxy-4-methylphenyl)butan-2-amine (ARIADNE). All compounds were administered at a concentration of 10^{-5} M.

Dendritogenesis, synaptogenesis, and immediate early gene expression were examined under three distinct experimental conditions: (1) without G protein decoupling, (2) with selective decoupling of Gq/11 proteins achieved by pretreating cells with YM-254890 at a concentration of 10^{-7} M for two hours, and (3) with Gi/o protein decoupling accomplished by pretreatment with pertussis toxin at 100 ng/mL for 24 hours.

Our findings indicate that Gq/11 decoupling disrupts c-fos expression induced by all non-ergoline psychedelics, and egr-2 expression induced by all tested psychedelics except DOI. In contrast, Gi/o decoupling impairs the expression of both c-fos and egr-2 across all treatment conditions. The dendritogenic effects of serotonergic psychedelics are abolished when both G protein pathways are decoupled. Interestingly, synaptogenesis appears to be negatively regulated by both Gq/11 and Gi/o pathways under basal conditions, as their decoupling results in a significant increase in synaptogenesis. Notably, this synaptogenic enhancement induced by Gi/o decoupling can be reversed upon treatment with DOI and LSD.

These findings suggest that dendritogenesis and synaptogenesis are regulated through distinct and potentially independent signaling mechanisms. This underscores the importance of investigating diverse neuroplastic processes in detail to advance our understanding of the mechanisms underlying psychedelic-induced plasticity and to inform the development of psychedelic-based therapeutics.

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Temozolomide decreased neuronal differentiation without inducing a negative impact on affective-like behaviour in adult rats of both sexes

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In the context of cancer-depression comorbidity, the main chemotherapeutic drug used for the treatment of glioblastoma, temozolomide, is known to affect healthy brain cells by inhibiting adult hippocampal neurogenesis. Given that a decrease in this process is associated with depressive-like symptomatology, and since depression is often comorbid in many patients with brain cancer, this preclinical study aimed at evaluating how temozolomide might impact affective- and cognitive-like responses, as well as the changes taking place in the hippocampus (i.e., NeuroD, marker of neuronal differentiation; BDNF, marker of neuroplasticity), in adult rats of both sexes. To do so, adult Sprague-Dawley rats (n=39 females, n=40 males) were treated with temozolomide (2 cycles: 25 mg/kg, i.p., 5 days/cycle, 1 dose/day, 10 days total with 2 resting days in between cycles, n=9-11 per group/sex) or vehicle (DMSO, 1ml/kg, n=9-11 per group/sex). Groups of allocated rats were scored through specific behavioural tests that capture different manifestations of affective-like responses (i.e., forced-swim, novelty-suppressed feeding, sucrose preference and/or open field). Moreover, the long-term effects of temozolomide on cognition were evaluated with the Barnes maze (short- and long-term memory). In parallel, groups of rats (n=12 females, n=11 males) were also treated with the same administration paradigm (temozolomide: 2 cycles, 25 mg/kg, i.p., 5 days/cycle, 1 dose/day, 10 days total with 2 resting days in between cycles, n=5-6 per group/sex; or vehicle: DMSO, 1ml/kg, n=5-6 per group/sex). Brains were collected 24 h post-treatment and hippocampal samples were prepared for western blot (BDNF) or immunohistochemistry (NeuroD) experiments. Data were analysed through two-way ANOVAs (independent variables: Sex and Treatment). The main results showed that temozolomide did not induce any significant differences in the behavioural tests evaluated, proving no changes in affective- and/or cognitive-like responses when administered in adult rats of both sexes, at least through the battery of tests utilized. Moreover, and in terms of the neurochemical markers analysed, while temozolomide did not alter hippocampal BDNF levels, it decreased the number of NeuroD+ cells in the dentate gyrus for both sexes (****$p<0.0001$ vs. control-treated rats). In conclusion, even though a decrease on hippocampal neurogenesis has been associated with depressive-like symptomatology, in the present study temozolomide decreased neuronal differentiation by NeuroD without inducing a negative impact on affective-like behaviour and/or long-term cognition in adult rats of both sexes. Future studies will focus on evaluating the pharmacological interactions of this chemotherapeutic drug with potential antidepressants in order to select appropriate treatments in the context of cancer-associated depression.

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P2X7R and Pannexin overexpression correlates with retinal degeneration severity

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Inherited retinal dystrophies (IRD) are a group of progressive neurodegenerative disorders that lead to gradual vision loss and eventual blindness. The progression of these diseases is consistently accompanied by tissue inflammation and oxidative stress. Among the key mediators of the inflammatory response in neurodegenerative conditions are purinergic receptors, particularly the P2X7 receptor (P2X7R). This receptor plays a crucial role in both septic and sterile inflammation, and it is involved in diseases of the central nervous system (CNS). P2X7R is overexpressed in retinal degenerative conditions as glaucoma, age-related macular degeneration or diabetic retinopathy. P2X7R are often coexpressed with P2X4 receptors (P2X4R), and both are predominantly found in glial cells, retinal ganglion cells, and at synaptic sites. Activation and modulation of P2X7R can occur through ATP release mediated by Pannexin (PanX) channels. We are focused on investigating the role of P2X receptors and Pannexin channels in IRD, and their contribution to the persistent inflammatory environment characteristic of these disorders. Our main objective is to understand the role of P2X receptors in the neurodegenerative processes that occur in IRD, to test their potential use as therapeutic targets. We have studied the expression levels of P2X7R and P2X4R, along with pannexins 1 and 2, during the progression of retinal degeneration in two murine models of IRD: the rd10 mouse, a model of retinitis pigmentosa, and a model of central areolar choroidal dystrophy. These diseases are caused by mutations in the Pde6 and the peripherin genes, being autosomal recessive and dominant, respectively. We assessed retinal function through electroretinography, examined retinal morphology by immunohistochemistry, and analyzed P2X7R, P2XR4 and Pannexin expression using transcriptomic analysis, western blotting and flow cytometry. Our results show a progressive upregulation of P2X7R, P2X4R, and Pannexin 1 and 2 in both IRD models. The increased expression correlated with the disease severity, visual function loss, and morphological alterations. The expression patterns of these receptors and channels, as well as the disease progression, differ between the two mouse models. Hence, our findings suggest that the upregulation of P2X7R, P2X4R, and Pannexins 1 and 2 can contribute to retinal degeneration and constitute a promising therapeutic target for retinal diseases, as it has already been proposed for other pathologies characterized by chronic inflammation. Furthermore, as the retina is part of the CNS, new treatments for retinal degenerative diseases could potentially be translated to other disorders with similar inflammatory components.

P15

Impact of the E193K LRRK2 variant on gap junction organization in Parkinson´s Disease

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Parkinson's disease (PD) is a neurodegenerative condition clinically characterized by movement-associated symptoms such as tremor, muscle stiffness and impaired balance. Aetiology of PD is complex due to the interaction between ageing, genetics 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. At the pathophysiological level, the E193K variant, which is located at the ARM domain, modifies mitochondrial dynamics and proper vesicle trafficking[1] but its role in Gap Junctions (GJs) regulation is unknown. GJs are key elements for neuronal communication, and they are made up of proteins names connexins (CXs)[2].

By combining different methods, in this study, we assessed the impact of the E193K variant on GJs conformation. We found that GJs structure is modified in fibroblasts obtained from a E193K LRRK2 carrier compared to a healthy subject. We also found that the E193K mutation increased cellular toxicity upon 1-methyl-4-phenylpyridinium (MPP^+) exposure and LRRK2 binding to GJs. In conclusion, our data demonstrate a crucial effect of the E193K LRRK2 variant controlling GJs organization.

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P16

Inhibition of purinergic receptor P2X7 changes the expression of calcium entry-channels

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Neurons are highly sensitive to fluctuations in intracellular Ca^{2+} levels. Disruption of calcium homeostasis can lead to cell death, potentially triggering or exacerbating neurodegenerative processes. Excessive Ca^{2+} influx has been linked to several retinal degenerative diseases, including retinitis pigmentosa (RP) and glaucoma, primarily through the activation of calpain-dependent apoptotic pathways and calcineurin-mediated signaling (reviewed in [1]). We previously demonstrated that retinal degeneration is associated with increased expression of calcium-permeable channels, such as transient receptor potential melastatin 7 (TRPM7) in a mouse model of ischemia-reperfusion [2], and purinergic receptors P2X7R and P2X4R in rd10 mice, a model of retinitis pigmentosa [3]. We hypothesize that altering the calcium influx through calcium-permeable channels may regulate the expression of other calcium entry pathways. The objective of this study was to analyze the effect of *in vivo* administration of FF807, a P2X7R inhibitor on the expression of calcium-permeable channels in the rd10 mouse model. We administered the drug intraperitoneally, from postnatal day (P)18 to P25. We assessed retinal function through electroretinography and we evaluated the expression of P2X7R, TRPM7, TRPV1, and TRPV4 using immunohistochemical analysis. Our preliminary results show a significant decrease in the expression of P2X7R and there is also a trend showing a decrease in the expression of TRPM7. No changes were observed in the expression of TRPV1 or TRPV4 channels. Under these conditions, P2X7R inhibition did not alter the progression of retinal degeneration, as no differences in retinal morphology or function were detected in treated mice. Our findings suggest that P2X7R blockade may modulate the expression of other calcium entry pathways. Further investigation is needed to determine the long-term implications of these expression changes.

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P17

Multielectrode arrays: A method for studying exocytosis in human platelets

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Amperometry is the most widely used technique for studying the exocytosis of biological amines. However, the scarcity of human tissues, particularly in the context of neurological diseases, poses a challenge for exocytosis research. Human platelets, which accumulate 90% of blood serotonin, release it through exocytosis. Nevertheless, single cell amperometry with encapsulated carbon fibers is impractical due to the small size of platelets and the limited number of secretory granules on each platelet. The recent technological improvements of amperometric multi-electrode array (MEA) devices allow simultaneous recordings from several electrodes with high performance. In this meeting, we present a comparison of three MEA boron-doped diamond (BDD) devices for studying serotonin exocytosis in human platelets: i) BDD-on-glass MEA, ii) BDD-on-silicon MEA, and iii) BDD on amorphous quartz MEA (BDD-on-quartz MEA). Transparent electrodes offer several advantages for observing living cells, and in the case of platelets, they control activation/aggregation. BDD-on-quartz offers the advantage over previous materials of combining excellent electrochemical properties with transparency for microscopic observation. These devices are opening exciting perspectives for clinical applications.

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Pain and Inflammation

P18

Extracellular vesicles from the plasma of patients with morbid obesity show altered content of immune cell markers and bariatric surgery modified them

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Obesity is a major public health challenge of the 21st century and a growing global epidemic, projected to affect 51% of the world's population within the next decade. Morbid obesity, defined as a body mass index (BMI) of 40 kg/m² or higher, is the most prevalent chronic metabolic disease in developed countries and is associated with numerous comorbidities. Its management involves lifestyle modifications, including dietary changes, increased physical activity, and behavioral adjustments. When these measures prove insufficient, bariatric surgery becomes the most effective treatment for significant and sustained weight loss.

The rising prevalence of obesity is driven by complex genetic, environmental, and behavioral factors. In addition to its systemic effects, obesity triggers chronic low-grade inflammation, leading to cellular and molecular alterations that impact overall health. In this context, extracellular vesicles (EVs) have gained significant attention due to their role in intercellular and interorgan communication, as well as in the regulation of metabolic processes. These nanostructures, released by all cell types, carry bioactive cargo such as proteins, which can modulate the function of recipient cells. EVs also act as mediators of inflammation, and their composition is influenced by the originating cell type and its physiological state.

This study aimed to characterize EVs isolated from plasma and assess the presence of immune cell markers in plasma-derived EVs from patients with morbid obesity before and after bariatric surgery, as well as in healthy controls.

Patients with morbid obesity (BMI > 40, aged 30–60) from the surgical obesity treatment program at Hospital Clínico de Valencia, along with healthy controls, were recruited. EVs were isolated from frozen plasma using size-exclusion chromatography (qEV2 70 nm, IZON) and characterized by electron microscopy, nanosight, and western blot. Immune cell markers were also analyzed by western blot.

Our findings showed that plasma EVs from morbidly obese patients expressed both general EV markers and specific markers related to their cellular origin. Notably, the number of plasma EVs decreased after bariatric surgery. Additionally, the cellular sources of immune system- derived EVs underwent significant changes in obesity and following surgery. These findings suggest that plasma EVs could serve as biomarkers for monitoring metabolic changes and comorbidities in obesity and after bariatric surgery. Further studies are needed to explore their role.

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Cyanocobalamin-loaded dissolving microneedles relapses cutaneous inflammation symptoms in a delayed type of hypersensitivity murine model

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Inflammatory skin conditions affect a broad population worldwide. The primary treatment for these diseases involves the use of topical corticosteroids. However, their application is restricted, particularly in chronic and acute cases, as it can result in various side effects. Cyanocobalamin (vitamin B12) shows promising effects in alleviating symptoms like inflammatory dermatitis, due to its anti-inflammatory and antioxidant properties. Unfortunately, due to its high molecular weight (>500 Da), cyanocobalamin cannot be effectively administered through the skin, as it hinders the drug's diffusion through the upper skin layers. In this context, we have developed dissolving microneedles loaded with cyanocobalamin to facilitate the painless and efficient delivery of the drug through the skin.

Cyanocobalamin-loaded dissolving microneedles (B12@DMAPs) were fabricated by the solvent casting method using a mixture of solvable and biocompatible polymers (poly-(vinyl pyrrolidine and poly-(vinyl alcohol). The B12@DMAPs were characterized in vitro terms of deformation, insertion, and release. The morphology of the B12@DMAPs was studied using Scanning electronic microscopy. Insertion parameters were also studied ex vivo in murine skin and the skin delivery of B12 was studied using Franz Diffusion Cells in porcine skin.

Finally, the effectiveness of the formulation was studied in a delayed-type hypersensitivity murine model. Briefly, mice were sensitized with oxazolone (OXA) 3% at day, then animals were challenged with OXA 1% in the shaved back, and then formulations were administered. At day 7, DMAPs were removed (when corresponding) and then animals were injected with Luminol (200 mg/kg, *i.p.*) and bioluminescence imaging was performed. Afterwards, animals were killed, and skin biopsies were taken for histopathological studies and cytokines detection.

B12-DMAPs in vitro characterization showed that the formulation has optimal properties for its application onto the skin. *In vitro* and *Ex vivo* studies showed that microneedles patches successfully achieved a controlled release of the drug during 24 h and delivered the drug across the skin layers.

Moreover, the B12@DMAPs performance in the *in vivo* model showed an alleviation of the inflammatory symptoms. Bioluminescence imaging, histopathological study and cytokine detection showed a similar trend: No differences were observed between healthy mice or mice treated with topical corticosteroids and the animals treated with B12@DMAPs ($p < 0.05$), whereas untreated mice and mice treated with no-drug-loaded DMAPs showed a significant difference in pro-inflammatory signs ($p > 0.05$).

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P20

Upregulation of the CXCL16/CXCR6 axis in individuals at risk for COPD and in early-stage COPD: Potential as a peripheral biomarker and therapeutic target

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Chronic obstructive pulmonary disease (COPD), mainly triggered by prolonged cigarette smoke exposure, involves systemic inflammation that contributes to disease progression. The immune characterization of early-stage COPD (GOLD 1) appears to be essential for the identification of early biomarkers and therapeutic approaches. CXCL16/CXCR6 axis, known for mediating leukocyte trafficking, has been previously associated with COPD, but its role in GOLD 1 remains unclear. This study aimed to assess the expression of this axis in early COPD and individuals at risk.

Blood samples from 27 GOLD 1 patients, 27 long-term smokers with normal lung function (pre-COPD), and 14 non-smokers were analyzed by flow cytometry to determine CXCR6 expression in platelets, leukocytes, and leukocyte-platelet aggregates. Additionally, CXCL16 plasma levels were measured by ELISA, while lung function was evaluated by spirometry.

GOLD 1 patients showed significantly higher CXCL16 plasma levels and increased CXCR6 expression on platelets, classical monocytes, B-cells, and leukocyte-platelet aggregates compared to both pre-COPD individuals and non-smokers. Plasma CXCL16 was also elevated in the pre-COPD group, despite no significant differences in CXCR6 expression. Notably, CXCL16/CXCR6 expression negatively correlated with the FEV1/FVC ratio.

These findings provide the first evidence of early upregulation of the CXCL16/CXCR6 axis in COPD, with CXCL16 emerging as a potential biomarker for early detection. Considering the role of the CXCL16/CXCR6 axis in leukocyte trafficking, it may offer a promising target for therapeutic intervention to reduce immune cell infiltration in the lungs, preventing COPD onset and progression.

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P21

Effect of fibroblast-released PGE_2 on keratinocytes and monocytes

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The interplay between human dermal fibroblasts (HDF) and other cell types, such as keratinocytes and monocytes, is essential for skin homeostasis and inflammatory responses. Previous studies suggested that defective c-Jun N-terminal kinase (JNK) signaling in psoriatic fibroblasts impairs their ability to resolve inflammation due to decreased cyclooxygenase-2 (COX-2) expression and thus contributing to the chronification of this disease (1). This study aims to explore the significance of JNK signaling in HDF for their role in modulating immune responses and their interplay with monocytes. In addition, we analyzed the potential anti-inflammatory role of prostaglandin E_2 (PGE_2) from HDF supernatants on keratinocyte activity.

Primary HDF and keratinocytes were isolated from foreskins of adult healthy donors by dispase II treatment and collagenase IA (dermis) and trypsin (epidermis) digestion. HDF were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). Keratinocytes were grown in KGM media in a serum-free low-Ca^{2+}. Keratinocytes were treated with PGE_2 (30 ng/mL), the concentration previously determined in supernatants of HDF stimulated with interleukin (IL)-1β (2.5 ng/mL) for 24h, in the presence or absence of tumor necrosis factor (TNF)-α (10 ng/mL). Human peripheral blood monocytes were incubated with conditioned media (CM) from HDF previously stimulated with IL-1β (2.5 ng/mL, 24h) in the presence or absence of the non-selective COX-inhibitor indomethacin (10 µM) or the JNK inhibitor SP600125 (SP) (50 µM). Release of IL-6, IL-8, and human beta-defensin 2 (hBD2) by keratinocytes, as well as release of TNF-α and IL-10 by monocytes was determined by ELISA.

As expected, TNF-α induced the release of IL-6, IL-8, and hBD2 by keratinocytes. Interestingly, exogenous addition of PGE_2 at concentrations present in fibroblast supernatants significantly reduced the release of these cytokines (45.1%, 47.8% and 43.5%, respectively) in keratinocytes stimulated by TNF-α, indicating an anti-inflammatory effect of PGE_2 in activated keratinocytes. Furthermore, monocytes incubated with CM from IL-1β-stimulated HDF showed increased IL-10 and decreased TNF-α release. In contrast, previous treatment of IL-1β-stimulated HDF with indomethacin or SP abolished this effect, confirming that PGE_2 released by HDF favors the polarization of stimulated monocytes towards an anti-inflammatory, pro-resolving phenotype and that JNK signaling is involved in the paracrine crosstalk between HDF and monocytes during immune responses.

These results are consistent with an important role for dermal fibroblasts-derived PGE_2 in the regulation of inflammation in skin through interaction with keratinocytes and monocytes. Our findings also suggest that JNK activity is crucial for the immunosuppressive function of HDF, and its downregulation may contribute to the persistence of inflammation in psoriasis.

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Involvement of protein A and IL-16 in the hyperalgesia and allodynia evoked by Staphylococcus aureus in mice

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Staphylococcus aureus (SA) is a Gram-positive microorganism that evokes painful infections[1,2]. It has been recently shown that its cell wall-anchored protein A (SpA) triggers the release of interleukin-16 (IL-16)[3]. IL-16 is an inflammatory mediator that is converted into its mature form following the cleavage of pro-IL-16 by caspase-3. Considering that IL-16 has recently been implicated in inflammatory painful responses in mice[4], we investigated the participation of SpA and IL-16 in SA-induced hypernociception. Swiss male mice received the intraplantar (i.pl.) injection of SpA or 10^3–10^6 colony forming units (CFU) of SA. In separate experiments, SA was also injected into the knee joint.

Thermal hyperalgesia and mechanical allodynia were measured and the effect of the administration of an anti-IL-16 antibody was tested. In the injected tissues, ELISA and qRT-PCR assays together with immunohistochemical analysis were performed.

The i.pl. injection of SpA (0.3–3 µg, 1 h before testing) induced thermal hyperalgesia and mechanical allodynia, which were effectively blocked by the subcutaneous (s.c.) administration of an anti-IL-16 antibody (0.3–1 µg/kg, 1 h before testing). Furthermore, the i.pl. administration of SA 6 hours before, also evoked hyperalgesia and allodynia, alongside elevated local IL-16 levels and increased expression of caspase-3 mRNA, without affecting IL-16 mRNA levels. Immunofluorescence analysis revealed that neutrophils were the primary IL-6-expressing cells following i.pl. SA injection. Reinforcing the functional role of IL-16 in SA-mediated nociception, treatment with an anti-IL-16 antibody (1–30 µg/kg) significantly reduced both modalities of hypernociception induced by SA but had no effect on pain responses triggered by Streptococcus pyogenes, a Gram-positive bacteria that does not express SpA. Since one of the main painful settings related to SA infection is septic arthritis[1], we next injected SA (2×10^5 CFU, 5 days before) into the knee joints of mice. This procedure also led to hyperalgesia, allodynia, and local upregulation of IL-16 measured by ELISA; its expression being mainly associated with synovial macrophage-like cells. In accordance, the administration of the anti-IL-16 antibody also completely prevented the hypernociceptive responses obtained in this model. Altogether, our findings highlight IL-16 as a potential novel target for managing pain associated with SA infections.

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P23

Association Between Diet and Gut Microbiota in hypertensive women with fibromyalgia: the Genus NK4A214 as a Potential Microbial Biomarker

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The interaction between gut microbiota, chronic pain, and diet is gaining clinical relevance due to its role in inflammatory, metabolic, and neurological processes[1]. This cross-sectional observational study aimed to evaluate differences in gut microbial composition in women with fibromyalgia, considering their clinical status and dietary patterns, and to identify potential microbial biomarkers associated with these factors.

Eighty-three women over 50 years old were recruited from community pharmacies, meeting voluntary participation and inclusion criteria. Of these, 46 were healthy controls (HC) without chronic diseases or medication, and 37 had fibromyalgia. The fibromyalgia group was further divided into those with hypertension (FMHT, n=10) and those with fibromyalgia only (FM, n=27). Faecal microbiota samples were analysed using 16S rRNA amplicon sequencing on the Illumina MiSeq platform[2]. Bioinformatics and statistical analyses were performed in R v4.4.2. Alpha and beta diversity indices were calculated. Clinical covariates were explored using principal component analysis (PCA). Differential abundance was assessed using Kruskal-Wallis tests, and linear models were used to explore associations between microbial taxa and dietary intake (categorized by food groups and individual nutrients).

Alpha diversity was significantly lower in the fibromyalgia group compared to healthy controls (p-value: 0.013 for Shannon; p-value: 0.011 for Simpson). Beta diversity showed significant differences between the three clinical groups (p-value: 0.001, Bray-Curtis). No significant differences were found in macronutrient intake or overall dietary frequency across the three groups.

Among all identified taxa, the genus NK4A214 showed significant differences across clinical groups (p-value: 0.001) and was linearly associated with intake of leafy greens (p-value: 0.046), sugary products and pastries (p-value: 0.035), and dark chocolate (p-value: 0.005), suggesting a potential diet-microbiota-disease relationship.

In conclusion, NK4A214 emerges as a potential microbial biomarker in women with fibromyalgia and hypertension. Its abundance appears to be influenced by specific dietary patterns. These findings support the relevance of diet in modulating gut microbiota in clinical contexts and point toward future personalized nutritional interventions.

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Parallel effects of PACAP-38 on electrical excitability and voltage-gated Ca^{2+} currents in adrenomedullary chromaffin cells and primary sensory neurons from neuropathic rats

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In the chronic constriction injury (CCI) model of sciatic nerve neuropathic pain, we have previously demonstrated parallel plastic changes in both rat adrenomedullary chromaffin cells and primary sensory neurons. These changes involve the functional expression of nicotinic acetylcholine receptors, low voltage-activated (T-type) Cav channels, and P2X receptors (1). This plasticity leads to enhanced electrical excitability in both cell types and increased catecholamine release from chromaffin cells. Notably, adrenal catecholamines contribute to the mechanical and thermal allodynia observed in CCI rats (2).

The neuropeptide PACAP (pituitary adenylate cyclase-activating polypeptide) functions as a co-transmitter of acetylcholine at the splanchnic nerve-chromaffin cell synapse and plays a predominant role under conditions of heightened secretory demand (3). PACAP is also overexpressed in primary sensory neurons following sciatic or spinal nerve transection and is known to modulate Nav1.7 channel activity (4).

In this study, we used patch-clamp electrophysiology and electrochemical techniques to examine the effects of PACAP-38 on electrical activity, voltage-gated ion channel function, and catecholamine secretion in cultured chromaffin cells and isolated adrenal glands from control and CCI-induced neuropathic pain rats. In parallel, we investigated the electrophysiological properties of lumbar dorsal root ganglion (DRG) neurons from the same animals. Chromaffin cells and DRG neurons from CCI rats exhibited increased spontaneous and evoked action potential firing. In chromaffin cells, this hyperexcitability was associated with a depolarized resting membrane potential and enhanced Ni^{2+}- and mibefradil-sensitive low voltage-activated (T-type) Ca^{2+} currents, alongside increased catecholamine release, as measured by membrane capacitance and carbon-fiber amperometry.

PACAP-38 (1 nM) further stimulated both spontaneous and evoked electrical activity, as well as Ca^{2+} currents through high (L-type) and low voltage-activated (L- and T-type) Cav channels in chromaffin cells and DRG neurons from both control and CCI animals. These channels are key regulators of action potential firing. Consistently, PACAP-38-induced excitability was reduced by T-type (1 µM Ni^{2+}, and 1 µM mibefradil) and L-type (1 µM nifedipine) Cav channel blockers. Moreover, PACAP38-evoked catecholamine secretion was significantly greater in adrenal glands from CCI animals, and this effect was sensitive to Ni^{2+}, indicating a role for T-type channels.

These results implicate PACAP-38 in peripheral sensitization in this experimental model of neuropathic pain, acting directly on DRG neurons and indirectly via the adrenal chromaffin cells. In both cell types, T-type Cav channels appear to mediate the effects of PACAP-38.

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P25

Dual antidepressants in neuropathic pain: a systematic review and meta-analysis of preclinical studies

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Chronic pain affects 20% to 30% of adults and is often debilitating and disabling[1]. Many patients fail to respond adequately to conventional treatments, highlighting the urgent need for advancements in drug development and therapeutic innovation. Such advancements require a deeper understanding of the mechanisms underlying the efficacy of existing treatments, mainly dual antidepresasant[2]. These drugs are widely used to manage chronic pain, including neuropathic pain, due to their dual-action mechanism and demonstrated analgesic efficacy[3,4]. Preclinical research is crucial for understanding the action mechanisms of these compounds. We conducted a meta-analysis to evaluate the effects of dual antidepressants on hypersensitivity in neuropathic pain models, focusing on how preclinical experimental parameters influence their analgesic efficacy. Following stablished guidelines and PROSPERO pre-registration (CRD42024567186)[5,6], we conducted searches in the Scopus, EMBASE, and Web of Science databases to identify studies involving rodents with neuropathic pain treated with dual antidepressants, compared to a vehicle group, and subsequently evaluated for at least one of the following pain modalities: mechanical allodynia, mechanical hyperalgesia, cold allodynia, and heat hyperalgesia. We conducted a peer-blinded screening of titles and abstracts, followed by a full-text review, identifying 90 studies for inclusion in the meta-analysis. We used a random-effects multilevel model, followed by moderator analysis to assess the influence categorical and continuous variables. Duloxetine, Venlafaxine and Milnacripran significantly reduced pain in rodents subjected to neuropathic pain. Subgroup analyses indicated no significant influence of species, sex, drug or the type of neuropathic pain model on the analgesic effect. However, chronic administration appeared to be more effective than single-dose treatments in hyperalgesia-like pain modalities. Most studies were conducted on male rats using duloxetine. Meta-regression analyses revealed that the number of doses in chronic treatments and the dosage in single administrations significantly influenced analgesic effects. This meta-analysis revealed that dual antidepressants significantly alleviate preclinical neuropathic pain across four modalities of nociceptive stimuli, with variations observed based on administration types and dosages. These findings underscore the importance of optimizing experimental variables and enhancing reporting practices to improve transparency and reproducibility in future pharmacological studies. Also, we emphasize the importance of studying sex differences in behavioral outcomes, given the underrepresentation of female rodents.

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Dual G9a inhibition as a novel epigenetic strategy for treating autism spectrum disorder in BTBR mice

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Autism spectrum disorder (ASD) is a serious neurodevelopmental condition currently affecting more than 1% of children worldwide. ASD causes are complex and multifactorial, with several associated genes and environmental risk factors. Clinically, ASD involves difficulties in communication and social interaction, along with repetitive and stereotyped behaviors, and restricted interests. On a molecular level, several pathways are disrupted, including those related to chromatin remodelling, RNA transcription and splicing, and gene expression. Notably, histone-modifying enzymes, such as G9a, are elevated in individuals with ASD, suggesting that changes in chromatin structure may play a key role in the disorder's development. G9a is responsible for methylating Histone 3 at lysine 9 (H3K9me2), being capable of repressing the expression of genes related to learning and memory formation as well as social interaction. Overactivation of G9a has been linked to cognitive impairment, synaptic plasticity reduction, autophagy dysfunction, increased oxidative stress and neuroinflammation. This study aimed to determine whether increasing doses of a dual pharmacological approach targeting G9a could have a beneficial effect in BTBR T+ Itpr3tf/J (BTBR) mice. These mice are an inbred strain presenting a severely reduced hippocampal commissure and complete agenesis of the corpus callosum. They are commonly used as an idiopathic autism mouse model, and they present repetitive and compulsive-like behaviors. In this experiment, the test compound was administered intraperitoneal injection at two doses (5 mg/Kg and 10 mg/Kg). Marble burying test (MBT) and nestlet shredding test (NST) were performed to study the behavioral phenotype. The C57BL/6J mouse strain served as the control. An improvement in both cognition and behavior was observed after dual G9a inhibition. These findings support the hypothesis that epigenetic modulation may be a potential therapeutic avenue. Indeed, fragment-based design strategies play an important role in multitarget drug discovery. We used this approach to design dual inhibitors by identifying common pharmacophoric features in previously reported G9a inhibitors. Our dual approach is an innovative and encouraging multifaceted therapeutic strategy for future ASD treatment, particularly for individuals in whom traditional interventions have proven insufficient.

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Physical and functional interaction between the mu-opioid receptor and the adenosine 1 receptor

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μ-opioid receptor (MOR) agonists are considered the most effective drugs to treat chronic pain[1], which is a common pathological condition affecting almost 26% of the Spanish population[2]. MORs belong to the superfamily of G protein-coupled receptors (GPCRs) and are involved in the modulation of pain relief (analgesia), but also in the development of addiction and other side effects associated with opioid use[1]. Moreover, it is known that GPCRs can form oligomeric entities that modify their pharmacological and functional properties[3,4], making them promising therapeutic targets for the development of new effective and safer drugs. More specifically, previous studies have evidenced that a co-administration of MOR and A1R agonists triggers a synergic antiallodynic effect[5]. Therefore, the main aim of this project was to determine the existence of a complex between MOR and adenosine 1 receptor (MOR-A1R heteromer) and describe its fingerprint. By proximity ligation assays performed ex vivo on mouse tissue slices, we have observed that the MOR-A1R heteromer is present in the striatum. Moreover, using bimolecular luminescence complementation assays, we determined the heteromerization of MOR-A1R in mammalian transfected cells, as well as its interface of interaction, which is suggested to be between the 4th transmembrane domain of MOR and the 5th of A1R. Furthermore, to characterize the MOR-A1R heteromer, we conducted G-protein activation and cAMP production assays in transfected cells with MOR or both MOR and A1R to study possible allosteric modulations resulting from the formation of the heteromer. The results showed a reduced response of both receptors when being co-activated by their respective agonists, suggesting a negative cross-talk between MOR and A1R. Moreover, cross-antagonism in the heteromer was also detected by studying the negative effect of an antagonist on the activation by an agonist of the partner receptor. In the near future, further investigation is required to deepen our knowledge on the allosteric interactions between MOR and A1R and assess the role of this heteromer in the development of new effective opioid drugs with minimized side effects for the treatment of chronic pain.

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Impact of galanin 1 receptor presence on opioid-induced intracellular signaling

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μ-opioid receptor (MOR) agonists are the most effective drugs for treating chronic pain. MORs belong to the G protein-coupled receptor (GPCR) family and mediate both the analgesic and addictive effects of opioids[1]. It has been shown that MOR and galanin 1 receptor (Gal1R) form oligomeric complexes in the ventral tegmental area and nucleus accumbens. Within these areas, where MOR is mainly present as part of the MOR–Gal1R heteromer, activation of MOR leads to stimulation of the brain reward system[2-4]. The role of receptor oligomerization, reported to be tissue-specific, results in MOR exhibiting distinct pharmacological and functional properties when it oligomerizes with Gal1R. To explore how the heteromerization of MOR with Gal1R modulates opioid pharmacology, we performed various in vitro pharmacological assays in cells expressing either MOR alone or co-expressing MOR and Gal1R. A key aspect in performing these assays is the concept of receptor reserve – the maximal biological response that can be achieved even when only a fraction of the receptors is activated. Since ligand efficacy differences are more detectable under low receptor reserve[5], we investigated whether varying MOR expression levels impact ligand efficacy using cAMP accumulation assays. Additionally, we screened a library of opioid ligands using a G-protein BRET activation assay with Gαo1 and Gαi1subunits to evaluate how ligand potency and efficacy vary depending on the G protein subtype and on whether MOR is expressed alone or with Gal1R. We also examined β-arrestin recruitment and receptor internalization to assess how Gal1R heteromerization affects MOR trafficking and signaling bias. Under our experimental conditions, all opioid agonists at high MOR expression remained full agonists at lower levels (8–9 times lower, similar to native tissues).

Furthermore, we observed that all opioid ligands tested, except PZM21 in Gαi1, exhibited a trend toward reduced potency in both Gαo1 and Gαi1 when expressed in MOR-Gal1R cells. Specifically, (S)-methadone, buprenorphine, SR17018, tramadol, and morphine consistently showed decreased potency and/or efficacy in these cells, suggesting potential functional modulation by Gal1R. To further investigate these effects, we performed molecular dynamics simulations to identify differences in ligand-receptor interaction in the presence or absence of Gal1R, and to identify the key amino acids involved in selectivity. Overall, Gal1R modulates intracellular MOR signaling, favoring certain pathways. These allosteric interactions within the receptor complex, as well as the different structural features of opioids involved in the interaction, could potentially be exploited for the design of new analgesic drugs with fewer adverse effects.

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Natural Products Pharmacology

P29

Targeting NLRP3 Inflammasome with Dehydroisohispanolone: A Promising Strategy Against Gouty Inflammation

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Natural products remain a valuable source of pharmacologically active compounds, with hispanolone derivatives recognized for their wide biological activities [1].
Dehydroisohispanolone (DIH), a labdane diterpene, exhibited notable anti-inflammatory activity acting on multiple inflammatory signaling pathways [2]. This study aimed to investigate DIH as a potential pharmacological inhibitor of the Nod-like receptor protein 3 (NLRP3) inflammasome and evaluated its therapeutic potential in a murine model of gouty arthritis. DIH significantly inhibited NLRP3 inflammasome activation, with a significant reduction of IL-1β release and pyroptotic cell death in response to a variety of NLRP3 activators (Nigericin, ATP,
Imiquimod and Silica) in Lipopolysaccharide (LPS)-primed macrophages. Mechanistically, DIH disrupted the oligomerization of the adaptor apoptosis-associated speck-like protein (ASC), thereby preventing the assembly of the inflammasome complex. DIH selectively inhibited NLRP3 inflammasome activation without affecting the absent in melanoma-2 (AIM2) or NOD-like receptor family CARD domain containing 4 (NLRC4) inflammasomes. In vivo, the diterpene significantly attenuated joint inflammation induced by monosodium urate (MSU)
crystals, reducing pro-inflammatory cytokine production and myeloperoxidase (MPO) activity in joint tissues. These findings provide novel insights into the anti-inflammatory properties of the diterpene DIH by targeting NLRP3 inflammasome and supported its potential as a therapeutic agent for gout and other NLRP3-driven inflammatory conditions.

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Oleacein regulated IL-1β-induced inflammation and oxidative stress in the human synovial fibroblast cell line SW982.

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Inflammatory arthritis is a term used to describe a diverse group of rheumatic diseases including rheumatoid arthritis, psoriatic arthritis, and osteoarthritis, characterised by inflammation of the synovial joints and excessive growth of synovial fibroblasts (SFs). SFs are primary stromal cells residing in the sublining and lining layers of the joint synovium. In healthy joints, they maintenance the homeostasis between synovial tissue, the extracellular matrix, and the synovial fluid [1]. However, in arthritis, SFs transition from a protective role to adopting a destructive phenotype, actively participating in the chronic inflammation [2] and in joint destruction [3]. Dietary intervention has demonstrated the capacity to ameliorate disease activity by mitigating inflammation and oxidation, as well as through its salutary effects on the gut microbiota[4]. Along with drug treatment, nutritional therapy has been described as an interesting alternative to help reduce the progression of these types of diseases and improve patients' quality of life. In this context, oleacein (OLA), one of the most abundant secoiridoids in extra virgin olive oil, has previously been shown to have anti-inflammatory and immunomodulatory effects[5,6]. This study aimed to explore the antioxidant and anti-inflammatory effects induced by IL-1β in the human synovial cell line (SW982). This cell line is an important *in vitro* tool for studying inflammation in arthritis. Its usefulness depends on its capacity to replicate the behaviour of human fibroblast-like synoviocytes, which are pivotal in the development of this condition[7]. Cell viability was determined using the Sulforhodamine B assay. Levels of inflammatory markers (TNF-α, IL-6, IL-1β, PGE2, MMP-1 and MMP-3) were assessed using ELISA. Protein expression of pro-inflammatory enzymes (COX-2 and mPGES-1) and signalling pathways were evaluated by Western blotting. OLA was found to exhibit anti-inflammatory and antioxidant effects by regulating key inflammatory signalling pathways, such as MAPKs, NF-κB and the Keap1/Nrf2/HO-1 axis. Additionally, OLA reduced the production and expression of pro-inflammatory markers (COX-2, mPGES-1, MMP-1, MMP-3, IL-8, IL-6, TNF-α and PGE2). These results suggest that OLA is a promising regulator of the inflammatory response in rheumatic diseases.

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Reversal of Aspirin Non-Responsiveness in Patients with Type 2 Diabetes Mellitus: A Comparative Study Between Extra Virgin Olive Oil Supplementation and Aspirin Dose Increase

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Background:
Acetylsalicylic acid (ASA) is the antiplatelet treatment of choice for patients at high cardiovascular risk or with established cardiovascular disease, provided no contraindications exist. However, our research group has reported a prevalence of inadequate response to ASA therapy of approximately 50% (Ortega Hombrados L, et al. BCPT 2024; 135 (suppl.1): 22).
Various mechanisms have been investigated to explain this non-responsiveness, including increased oxidative stress—particularly in patients with diabetes mellitus.
Objective:
To evaluate the effect of extra virgin olive oil (EVOO), as an antioxidant agent, on ASAmediated platelet inhibition compared to increasing ASA dosage, in patients with type 2 diabetes mellitus exhibiting ASA non-responsiveness.
Methods:
We conducted a single-blind, controlled clinical trial with parallel groups. Patients with confirmed ASA non-responsiveness were randomized into two treatment arms: 100 mg/day of ASA plus 40 mL/day of EVOO (preferably at breakfast) versus 200 mg/day of ASA. Antiplatelet response was assessed using the PFA-100 system. ASA IC50 was determined in vitro before and after treatment using collagen-induced electrical impedance aggregometry. Residual platelet activation was evaluated by flow cytometry (anti-CD62 antibody), and oxidative stress and vascular inflammation biomarkers were quantified using commercial ELISA kits.
Results**:**
A total of 36 patients with type 2 diabetes and ASA non-responsiveness were included (18 per group; 39% female, 61% male; median age 69 years). After 1 month of follow-up, both treatment arms demonstrated restored antiplatelet response. ASA IC50 decreased by 29% in the ASA (100 mg) + EVOO group and by 32% in the ASA (200 mg) group compared to baseline, with no **statistically** significant difference between groups. Residual platelet activation was reduced in both groups. Biomarkers of oxidative stress and vascular inflammation showed
statistically significant reductions compared to pre-treatment values in both groups, again without significant differences between them.

Acknowledgements: PI-0120-2020. Efectividad de la asociación aceite de oliva virgen extra- ácido acetilsalicílico vs. ácido acetilsalicílico cada 12 h sobre la resistencia al tratamiento antiagregante en pacientes con diabetes mellitus tipo 2. CONSEJERÍA DE SALUD Y FAMILIAS.

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Influence of the interaction between hydroxytyrosol and triterpenes on the neuroprotective effect of extra virgin olive oil

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The neuroprotective effect of extra virgin olive oil (EVOO) is well known, mainly due to the antioxidant action of its phenolic components, among which hydroxytyrosol (HTy) stands out. Recently, importance has been given to the presence of triterpenic derivatives (oleanolic, maslinic and ursolic acid) (TTp).
The aim of the study was to assess the neuroprotective effect of an EVOO rich in TTp+HTy, compared to an EVOO rich in Hty, in an experimental model of diabetes mellitus, using a hypoxia-reoxygenation model in brain slices. On the other hand, to assess the possible interaction between HTy and TTp in this neuroprotective effect.
Ex vivo experiments. Streptozotocin-diabetic rats (10 rats/group), followed up for 2 months, were used. Groups: non-diabetic controls (NDR), control diabetic rats (DR) and DR treated with 0.5 ml/kg/day of HTy-rich EVOO (destoned olives -AOVE-HTy-) or HTy+TTp-rich EVOO (destoned and dehydrated olives -AOVE-HTy+TTp-). In vitro experiments. Brain slices from healthy rats, incubating TTp and HTy in the same proportion contained in both types of EVOO were analysed. Brain slices were subjected to a hypoxia-reoxygenation model and cell death (LDH) and oxidative and nitrosative stress variables were determined.
The DR group produced 128% more LDH than NDR, 136% more lipid peroxides, 141% more peroxynitrites and 64% less glutathione. The administration of EVOO-HTy reduced lipid peroxides by 75% and EVOO-HTy+TTp by 82%, peroxynitrite production by 36% and 57%, respectively, and glutathione production by 20% and 47%, respectively. In all variables, the effect of EVOO-HTy+TTp was significantly higher, the only difference between the two EVOOs being the TTp content.
In the in vitro experiments, the compounds used inhibited LDH production in a concentrationdependent manner, showing an IC50 of 17.7 ± 0.5 µM for HTy and 17.3□0.8 µM for TTp; when HTy was incubated in the presence of a concentration of TTp that only inhibited LDH production by 16%, the IC50 of HTy was reduced to 5.2 ± 0.07 µM. A similar behaviour was observed when analysing the production of lipid peroxides and glutathione.
In conclusion, there is a positive interaction between HTy and TTp, in the same proportion in an EVOO rich in HTy and one rich in HTy+TTp, which may explain the greater ex vivo neuroprotective effect found for the latter in the experiments in diabetic rats.

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2- and 3-Prenylated quinolines and tetrahydroquinolines with PPAR activity as potential candidates for metabolic syndrome

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Background: Peroxisome proliferator-activated receptors (PPARs) represent highly valuable therapeutic targets for the treatment of type 2 diabetes and hypertriglyceridemia. Selective PPAR agonists display limited effects on glucose or lipid metabolism and are associated to serious adverse effects, however, the pan-PPAR modulators are promising agents for the treatment of metabolic disorders.
Material and methods: Prenylated quinolines and tetrahydroquinolines (THQs) have been synthesised using the Friedländer cyclodehydration, Grignard reaction and Johnson-Claise rearrangement sequence. Transactivation assays were used to determine the PPAR activity at each human PPAR (α, β/δ, γ). Computational studies by molecular docking and molecular dynamic simulations were used to assess the structural features of each ligand-PPAR complex. Cytotoxicity was evaluated by flow cytometry using an annexin V-FITC staining on the human macrophage cell line THP-1. Six-week-old ob/ob (C57BL/6.Cg-Lepob/J) mice received THQ 5a (10 mg/kg/day) via gavage for 2 weeks. Plasma samples were obtained for biochemical assessment, and liver and epididymal white adipose tissue were obtained for qPCR studies.
Results: Two series of prenylated quinolines either at the 2- or 3-position, and their THQs have been prepared. The compounds with a seven-carbon prenylated side chain at the 2-position (series a: THQ 5a and 6a) displayed a pan-PPAR agonism, while compounds with a six-carbon prenylated side chain at the 3-position (series b: THQ 5b and 6b) had stronger selectivity for PPARα activation. The administration in ob/ob mice of THQ 5a improves total cholesterol, non-HDL-c, HOMA-IR index and lipid metabolism without increasing liver enzymes.
Conclusions: THQ 5a is a promising lead compound in the development of agents for treating metabolic disorders (T2D and dyslipidaemias), which may prevent further cardiovascular comorbidities associated with MetS.

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Anti-inflammatory activity of 3-*O*-methyl rosmarinic acid isolated from Greek endemic *Salvia teddii*

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The genus *Salvia* L., the most species-rich genus within the Lamiaceae family[1], comprises 1.036 currently accepted species[2]. *Salvia teddii* Turrill is a Greek endemic species, restricted to the northeastern part of the country, yet its non-volatile constituents remain unexplored[3,4]. Thus, the main components of the methanol:water (5:1) extract were isolated and identified by chromatographic and spectroscopic techniques. Overall, the structural elucidation of five compounds was performed, including two caffeic acid derivatives, namely rosmarinic acid (**1**) and 3-*O*-methyl rosmarinic acid (**2**); two flavonoids, luteolin-7-*O*-rutinoside (**3**) and chrysoeriol-7-*O*-glucoside (**4**); and one megastigmane, salvionoside A (**5**), using 1D and 2D NMR spectroscopy. The present study aimed to evaluate the in vitro anti-inflammatory activity of the crude extract and the isolated compounds (**1**–**5**) in the murine RAW 264.7 macrophage cell line. Two assays were employed: measurement of nitric oxide (NO) inhibition in LPS-stimulated cells and suppression of reactive oxygen species (ROS) in TPA-stimulated cells.
Among the samples tested, 3-*O*-methyl rosmarinic acid (**2**) exhibited the most potent dual activity, significantly reducing NO levels (inhibition percentage of 23% at 50 µM, *p<0.05, and 48% at 100 µM, ****p<0.0001 Dunnett's *t* test) and completely inhibiting ROS production at both doses, surpassing the activity of rosmarinic acid at 100 µM (22% and 37%, respectively). In the ROS assay, compound **2** showed a low IC_{50} value of 2.96 µM, highlighting its strong antioxidant potential. This is the first report on the phytochemical composition of the endemic *S. teddii* and its promising anti-inflammatory and antioxidant properties.

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Valorization of Citrus Waste via Green Technologies: A Fatty Acid-Centered Approach to Functional Extracts

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The extraction of pharmacologically relevant lipids from agro-industrial by-products through sustainable methods represents a valuable strategy for therapeutic innovation. In this study, Citrus sinensis peel—a waste product rich in bioactive compounds—was processed using two green extraction techniques: supercritical fluid extraction (SFE) with CO_2 and accelerated solvent extraction (ASE) with ethanol. The objective was to evaluate their efficacy in recovering antioxidant compounds, carotenoids, and particularly unsaturated fatty acids with therapeutic interest.

SFE, although yielding lower total extract (5.9–9.3%), demonstrated high selectivity for lipophilic fractions, notably enriching oils in linoleic (34–38%), linolenic (17–20%), and palmitic acids (20–25%). These fatty acids are known for their roles in modulating inflammatory responses, improving endothelial function, and contributing to the maintenance of membrane integrity in neural and cardiovascular tissues. ASE, operated at 150 °C and 1500 psi, favored the recovery of phenolic compounds (≈9 mg GAE/g dry weight) and exhibited strong antioxidant capacity. However, it showed reduced efficiency in extracting long-chain unsaturated lipids compared to SFE.

The pharmacological relevance of the fatty acid profile lies in its potential to support the development of lipid-based formulations aimed at managing chronic inflammatory conditions, dyslipidemia, and neurodegenerative disorders. The high proportion of linoleic and α-linolenic acids provides precursors for eicosanoid synthesis and omega-3/6 balance, critical in immune modulation and metabolic regulation.

Both techniques offer solvent-free or GRAS-compatible protocols that align with pharmaceutical quality and environmental standards. This study supports the integration of SFE and ASE in drug discovery pipelines for generating bioactive lipid-rich extracts from botanical waste, with direct applicability in nutraceutical and therapeutic product development.

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Green Extraction of Bioactive Lipids and Phenolics from Wasted Tiger-Nut Derived Flour via Supercritical CO_2

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Supercritical fluid extraction (SFE) with carbon dioxide ($scCO_2$) represents a green, residue-free technology with growing interest in the recovery of pharmacologically relevant compounds from plant-derived matrices. This study investigates the potential of wasted tiger-nut-derived flour (WTNDF), a by-product of beverage manufacturing, as a source of bioactive lipids with therapeutic relevance. Extractions were conducted under both pure $scCO_2$ and 2% ethanol-doped conditions, at laboratory and pilot scale, and the resulting oils were characterized for antioxidant properties, phenolic content, carotenoids, and lipid profile.

SFE enabled the recovery of lipid fractions enriched in oleic (>70%) and linoleic acids (~10%), key unsaturated fatty acids with documented anti-inflammatory, cardioprotective, and metabolic regulatory activities. NMR and GC-FID analyses confirmed the presence of acylglycerols and a lipid profile consistent with functional dietary oils. The absence of ethanol enhanced the extraction of phenolic compounds, yielding a maximum total phenolic content (TPC) of 37.9 mg gallic acid equivalents/g oil, nearly doubling the yield compared to 2% ethanol-doped extractions. Total antioxidant capacity (TAC) remained consistent across conditions (0.75–0.90 µmol Trolox equivalents/g oil), suggesting that the extraction preserves redox-active compounds with potential cytoprotective effects. Carotenoid content was low in all cases (<2.1 µg/g oil), indicating limited relevance of these pigments in WTNDF extracts.

The pharmacological interest of these findings lies in the combination of antioxidant phenolics and unsaturated lipids within a single, cleanly extracted matrix. Given their roles in oxidative stress modulation, inflammation, and lipid metabolism, these compounds may support the development of nutraceuticals or adjuvants in metabolic and cardiovascular disorders. Moreover, the scalability and solvent-free nature of this process enhance its suitability for pharmaceutical applications requiring high-purity bioactives. Overall, this study highlights SFE as a sustainable platform for generating pharmacologically promising extracts from agri-food residues.

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Pharmacogenetics and Precision Pharmacology

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Is it necessary Edoxaban plasma levels´ monitoring in patients with renal dysfunction?

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Objective: To determine the possible relationship between renal dysfunction and altered plasmatic levels of direct oral anticoagulants (DOACs) with the incidence of bleeding in patients with atrial fibrillation (AF) and other cardiac pathologies.

Methods: Observational and retrospective study in patients with atrial fibrillation (AF) enrolled between 2021–2023. Inclusion criteria: Age >18 years; Treatment with dabigatran, rivaroxaban, apixaban, or edoxaban; Informed consent by telephone. Epidemiological, clinical, and laboratory data were analyzed (reason for DOAC use, thrombotic risk, CHA2DS2VASC, intervention, Has-Bled bleeding risk, creatinemia, glomerular filtration rate (GFR), INR, APTT, Tp, plasmatic levels (PL) of DOACs, bleeding).

Results and discussion: In the 70 patients included (51.4% men, mean age 75±1.41 years, AF diagnosis 97.1%, mean GFR 59.3±2.65 ml/min/1.73m^2 and high prevalence of chronic pathologies), only 18.6% presented PL in the therapeutic range: 58.6% on apixaban presented subtherapeutic PL (41.4% were >75 years old). 58.3% on dabigatran and 55% on edoxaban presented supratherapeutic PL. Edoxaban PL were inversely correlated with GFR. 58.6% of patients (N=41, P=0.021) experienced bleeding (cranioencephalic 28.6%, gastrointestinal 21.4%). Bleeding occurred in 51.21% (P=0.034) of patients receiving DOACs´ supratherapeutic PL. Edoxaban showed the highest incidence of bleeding (N=10, 76.9%, P=0.05) followed by dabigatran (N=4, 57.1%). Most bleeding events (N=21, 51.2%, P=0.05) were observed in patients with renal dysfunction, supratherapeutic PL (N=14, 34.1%, P=0.05), and with a non-significant inverse correlation between decreased renal function and DOACs supratherapeutic PL (-0.255; P=0.108). Edoxaban showed the highest incidence of bleeding in conditions of altered renal function (61.5%, N=8). Apixaban showed a lower incidence of bleeding in patients with therapeutic and supratherapeutic range and with both normal and abnormal GFR. Regardless of the DOACs PL, patients >75 years showed a higher incidence of bleeding with subtherapeutic (N=8, 66.7%), therapeutic (N=6, 75%), and supratherapeutic (N=14, 66.7%) PL. Dabigatran had the best adverse event profile, and edoxaban the worst, in this population.

Conclusion: Routine determination of DOACs plasmatic levels is necessary to ensure they are within the therapeutic range, especially in elderly patients with renal impairment, to prevent adverse events.

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Perioperative use of antibiotics in companion animal reproductive surgeries: A retrospective five-year study

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Antibiotics are essential for treating human and animal infectious diseases. Surgical site infections have been reported as a complication in 3.0-8.7% of small animal surgeries, depending on the procedure (1). Consequently, surgical antimicrobial prophylaxis (SAP) has been developed to reduce their impact. However, in veterinary medicine SAP is an underexplored field, and varies with the clinical setting, nature or surgical workload, and geographical área (2). Although general guidelines on antibiotic use in small animals have been established (2), there is a lack of information on perioperative antibiotic use in pets, particularly in Spain. This study aimed to describe the perioperative use of antibiotics in companion animals' reproductive surgeries performed at the Veterinary Teaching Hospital of the University of León (HVULE), Spain.

An observational, retrospective and descriptive study was carried out among dogs and cats who were treated surgically at the HVULE between 2018 and 2022. Data collection, processing and storage was carried out in accordance with Spanish regulations. Information was processed and analysed with Microsoft Excel (2019) and SPSS Statistics 26.

A total of 1785 reproductive surgeries were performed (63.2% cats and 36.8% dogs). Many of them were females (67.2%), purebred (85.5%), and adults (90.9%), being 65.9% of the surgery's ovariohysterectomies. Antibiotics were prescribed in 62.4% of the interventions. Three antibiotics were employed: marbofloxacin (49.3%), followed by amoxicillin/clavulanic acid (46.8%), and cefazolin (3.9%). A significant year-on-year increase in perioperative antibiotic use was observed (χ^2 =1077.8, $p < 0.001$). Cats were 47 and 9 times more likely to use marbofloxacin (OR = 46.7; 95% CI: 25.2–86.6; $p < 0.001$) and amoxicillin/clavulanic acid (OR = 8.7; 95% CI: 6.9–11.0; $p < 0.001$), respectively. Cefazolin was more likely employed in dogs (OR = 1.9; 95% CI: 1.0–3.8; p=0.047) and geriatrics animals (OR = 2.7; 95% CI: 1.2-6.0; p=0.013). According to the EMA Categorization of antibiotics in animals (3), 49.3% of the active ingredients administered were classified as Restrict (marbofloxacin), and 50.7% as Caution (amoxicillin/clavulanic acid and cefazolin). Considering the list of essential medicines for cats and dogs, marbofloxacin should not be used as a preventive treatment, amoxicillin/clavulanic acid is widely used for management of soft tissue infections associated with staphylococci, and cephazolin is recommended as the first choice when SAP is indicated (4).

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Pharmacokinetics of albendazole metabolites after oral administration in cattle

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Albendazole is one of the most employed benzimidazoles in veterinary medicine. It is a broad-spectrum anthelmintic, highly effective against gastrointestinal roundworms and lungworms, as well as tapeworms and adult liver flukes in cattle. In this animal species, pharmacokinetic behavior has been described after intraruminal administration, but little is known when the drug is orally administered, under field conditions. Thus, the goal of this study was to characterize the plasma disposition of albendazole in cattle when the anthelmintic was administered by the oral route. Six parasite-free 5-month Holstein calves received the drug orally at a dose of 7.5 mg/kg. Samples of jugular blood were collected over 60 hours, and analysed by high-performance liquid chromatography with a photodiode array detector1. Concentration-time data were evaluated by non-compartmental methods (Phoenix WinNonLin 8.4). The parent drug was quickly and extensively metabolized to albendazole sulfoxide (active metabolite) and albendazole sulfone (inactive metabolite). Albendazole was not detected at any sampling time. Both compounds (sulfoxide and sulfone) were present for 48 h and 30 h, respectively. Albendazole sulfoxide achieved a peak plasma concentration (Cmax) of 2.20 ± 0.68 µg/mL at 9.0 ± 3.5 h post-treatment (tmax), with an area under the curve (AUCtotal) of 33.73 ± 10.96 µg·h/mL, whereas Cmax for albendazole sulfone was nearly the half (1.04 ± 0.14 µg/mL) and observed later (tmax = 11.7 ± 0.8 h). AUClast was also smaller for the latter metabolite (15.05 ± 2.22 µg·h/mL). Compared to pharmacokinetic parameters calculated at the same dose after intraruminal administration2, albendazole sulfoxide Cmax and AUC were slightly lower, and tmax achieved before. As for albendazole sulfone, Cmax and AUC were clearly lower and tmax earlier. Regarding other studies with higher intraruminal doses, results were more variable. The study has made it possible to define the absorption kinetics of albendazole in this ruminant species under the conditions commonly used.

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Retrospective assessment of perioperative antibiotics treatment in dogs undergoing elective orthopedic surgery in a veterinary teaching hospital

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The occurrence of surgical site infections (SSI) is a frequent complication of surgery and is associated with increased cost to the owner and suffering for the patient (1). One of the objectives of human and veterinary medicine is to reduce the rate of SSI, and the prophylactic administration of antibiotics is one way to minimize it (2). However, the use of antimicrobial prophylaxis (AMP) in clean and clean-contaminated procedures is increasingly controversial (3). In addition, the type of procedure, potential risk factors, the duration of the surgery or the use of implants, are indicators for the administration of AMP (4). Therefore, the aim of this retrospective study was to evaluate the perioperative antibiotics use in dogs undergoing elective orthopedic surgery at the Veterinary Teaching Hospital of the University of León (Spain). Patient data were collected from 1st January 2018 to 31st December 2022. Processing was carried out in accordance with Spanish regulations and analyzed using Microsoft Excel and SPSS Statistics 26.

A total of 589 dogs were eligible for inclusion. Dogs age ranged from 2 months to 18 years, and they were mainly males (54.8%) and pure breed (78.4%). A higher frequency of traumatological surgery was observed in adult animals (67.9% *vs*. 29.4% in geriatric ones). The most common procedures were laminectomies (26.8%), dislocations (19.9%), fractures (14.9%) and prosthesis placement (14.6%). The number of surgeries remained constant over the five years ($\chi^2 = 5.97$; $p = 0.202$). However, antibiotic use increased over the years ($\chi^2 = 56.53$; $p < 0.001$). 71.6% of the patients received perioperative antibiotics. The three drugs employed were cefazolin (92.1%), which is in accordance with other studies (2), amoxicillin/clavulanic acid (7.5%) and marbofloxacin (0.4%). Cefazolin was more likely employed in laminectomies (OR = 1.91; 95% CI: 1.11–3.29; $p = 0.020$). A high use of perioperative AMP has been observed, which highlights the importance of addressing rational antibiotic use through evidence-based guidelines.

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P41

Prescription Profile of Oral Pre-Exposure Prophylaxis (PrEP) and Its Impact on HIV Infection in Colombia: A Cross-Sectional Study (2019–2024)

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Oral pre-exposure prophylaxis (PrEP) has become a key strategy in HIV prevention, with its efficacy supported by multiple clinical studies and endorsed by international public health authorities (1,2). Despite its inclusion in prevention guidelines, real-world implementation particularly in low- and middle-income countries (LMICs)—remains poorly characterized (3,4). In Colombia, limited information exists regarding prescribing patterns, geographic distribution, and inequities in access to PrEP within the national health insurance system (2). From an expanded pharmacovigilance perspective, monitoring the use and prescribing practices of PrEP offers valuable insights to identify gaps in safe and effective integration and to guide rational and equitable use of preventive antiretrovirals across diverse populations (4,5).

A cross-sectional observational study is currently being conducted using anonymized national-level dispensing data obtained from the largest pharmaceutical benefit manager in Colombia. The dataset spans the period from 2019 to 2024 and includes variables such as patient age, sex, geographic region, health insurance scheme (contributory or subsidized), prescribed PrEP regimens, and ICD-10 codes related to HIV infection. Descriptive analyses are underway to identify temporal trends and regional variations in PrEP prescription. Stratified analyses are also being performed to assess differences across demographic groups and explore potential associations between PrEP coverage and subsequent HIV diagnoses. Data processing and statistical procedures are being conducted in accordance with good pharmacovigilance practices and institutional ethical standards.

The analysis of national dispensing data is currently underway. It is expected to identify temporal trends in PrEP prescribing patterns between 2019 and 2024, as well as differences in access based on demographic and geographic factors. The study aims to explore whether there are disparities in prescription frequency related to age, sex, region, and insurance scheme. Additionally, potential associations between PrEP coverage and HIV diagnosis rates will be evaluated using stratified analyses. These upcoming results will contribute to a better understanding of real-world PrEP use and its role in public health and pharmacovigilance in Colombia.

This study will generate real-world evidence on PrEP prescribing practices in Colombia, contributing to public health strategies and pharmacovigilance frameworks. By identifying trends and potential gaps in access, it aims to support the safe, equitable, and rational use of preventive antiretrovirals in national HIV prevention efforts.

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P42

Pharmacogenetics association with long-term clinical evolution of kidney transplant patients

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Background: Kidney transplantation is currently the only curative therapeutic option for end-stage renal diseases that severely impair normal kidney function. These patients need lifelong administration of immunomodulatory pharmacological agents, such as tacrolimus, aimed at modulating the patient's immune response to promote graft tolerance. Despite its effectiveness, tacrolimus has a narrow therapeutic range and is associated with several adverse effects, including nephrotoxicity, increased susceptibility to infections, post-transplant diabetes mellitus, neurotoxicity, hypertension, and malignancies, which may limit its use Pharmacogenetic variability has been reported to influence the efficacy and safety of immunosuppressive therapies in early stages of kidney transplantation. This study investigates long-term associations between pharmacogene variants and clinical outcomes in a cohort of kidney transplant recipients over a 12-year follow-up.

Materials and Methods: We analyzed the genotype of both donors and recipients in 37 SNPs from 14 genes involved in drug metabolism and transport in a cohort of 79 kidney transplant patients. Clinical parameters, including survival, renal function, tumor occurrence, and pharmacokinetics of tacrolimus, during follow-up and its association with specific pharmacogene variants were evaluated. Logistic regression and Kaplan-Meier analyses assessed associations between gene variants and clinical outcomes. Association of genetic variants with plasma concentration of tacrolimus and renal clearance rate along time was also studied.

Results: Variants in metabolizer (*CYP3A5*, *CYP2B6*) and transporter (*ABCB1*, *ABCC2*) genes were associated with 12-year survival rate. Increased tumor risk correlated with *ABCC2* rs2273697 AA and AG variants in donors and decreased risk with *CYP2B6* rs3745274 GT variant in recipients. Renal function was influenced by variants in *ABCB1, ABCC2, CYP3A5, CYP3A4,* and *CYP2B6*. Tacrolimus dose-dependent (C_0/D) concentration was affected by variants in *CYP3A4, CYP3A5, CYP2C19, ABCB1*, and *SLCO1B1*. Increased nephrotoxicity risk was associated with donor's AA variant in *CYP2C19* rs4244285 and reduced by *SLCO1B1* rs2306283 AA and AG variants. Gene variant interactions between metabolizer and transporter genes were also associated with altered risk of events incidence, such as *CYP2B6-ABCB1* with the risk of acute rejection and nephrotoxicity or *CYP3A5-SLCO1B1* with exitus incidence.

Conclusions: Our findings support that pharmacogene variants influence transplant outcomes. Remarkable associations include survival rate related to *ABCB1* and *ABCC2* variants, tumor occurrence associated to *CYP2B6* rs3745274, and renal function affected by multiple pharmacogenes. Variants in *CYP2C19* and *SLCO1B1* significantly impacted tacrolimus pharmacokinetics and nephrotoxicity risk. These results underline the importance of pharmacogenetic testing for personalized management in kidney transplantation and the need of employing multi-variant panels for genotyping although further validation in larger cohorts is necessary.

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META-PRIME: Repurposing drug metabolites in precision medicine

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Precision oncology currently benefits only a minority of patients while approved drugs target <4% of the human proteome. In this context, drug metabolites, seldom comprehensively characterized in terms of their broad pharmacological activity, represent an often-overlooked source of pharmacological diversity. Importantly, certain drug metabolites can reach significant concentrations in the bloodstream and exhibit distinct *in vivo* activities compared to their parent drugs – potentially opening new repurposing opportunities. The META-PRIME project, funded by the Spanish Ministry of Science and Innovation as part of the *Proyectos de Generación de Conocimiento* program, leverages machine learning (ML), chemical synthesis, and biological testing to expose and exploit this diversity. The project builds on our proof-of-concept discovery of unique targets for the metabolite of the cancer drug rucaparib, which translated into unique anti-Parkinson activity on iPSC neurons [1]. To assess whether drug metabolites could represent new sources of repurposing opportunities comprehensively, consistently, and at scale, we analyzed the 224 FDA-approved antineoplastic drugs, from which we obtained a curated chemical library of 18 drug-metabolite pairs. These metabolites are present in human plasma at concentrations >10% and showed a differential off-target profile from their parent drug as predicted through different ML methods. Selected drug metabolites were synthesized and screened using *in vitro* binding/functional assays on isolated protein targets that we predicted computationally. Moreover, the compounds were also sent to EU-OPENSCREEN to be part of the European Academic Compound Library and benefit from additional screening and cell painting characterization. In addition, we are also quantifying their effects on proliferation over a broad panel of cancer cell lines designed considering the parent drugs indications. Our experimental validation confirms our hypothesis that a significant number of drug metabolites (>25 %) bind to targets that are different to their parent drugs, thus extending our drug repurposing arsenal. Using selected examples, we demonstrate how the broad characterization of drug metabolites can (i) rationalise unexplained clinical efficacy and toxicity, (ii) reveal new repositioning avenues in disease of high unmet medical need such as oncology, neurodegenerative diseases and antimicrobials, and (iii) expand the actionable target landscape of precision pharmacology. Leveraging drug metabolites' pharmacology promises more effective, patient-tailored therapies and better exploitation of our existing pharmacopeia.

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Teaching innovation in Pharmacology

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Face to face debriefing impact on medicines administration and tracheal intubation skills assessing through analysis of student´s-developed self-video recording in medicine degree

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Debriefing is an Anglo-Saxon military term that refers to a series of meetings held at the end of a mission, in which soldiers and commanders exchanged views on the strategy that had been implemented. Over time, medical personnel adopted this concept, and although a hospital is not a war zone, the idea is the same: to review a practice and exchange opinions on what happened in a simulation.

Objective: To evaluate the face to face debriefing impact on medicines administration and tracheal intubation skills assessing through analysis of Medicine Degree´s students-developed self-video recording.

Methods. Four-year prospective study in which students of the Medicine Degree (Anaesthesia subjects) were encouraged to voluntarily make self-video recording during endotracheal intubation simulation practice. Were analyzed: the performance of pre-intubation reference anatomical measurements, use of sedation-relaxation medications, ventilation performance, intubation/extubation performance, and use of recovery medications. The self-video recording were uploaded to the virtual campus and analyzed. And a face-to-face debriefing was conducted with each student to assess the acquired competencies and demonstrated skill development. The impact of the face to face debriefing on student grades and satisfaction were analyzed.

Results. 672 students were enrolled, 63.2% women, 20±2.8 years old. The average time spent by students completing the self-movies was 11.45±8.4 min and attending the face to face debriefing was 9.15±5.7 min. This activity increased their success rate in the final evaluation question related to tracheal intubation (+35.7%) compared with the control group without any debriefing. The percentage of students who were satisfied with this activity was 98.9%.

Conclusion. The face to face debriefing about self-video recording on medicines administration and tracheal intubation skills performed with Medicine undergraduate students improved their results in the final evaluation and their communication skills.

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Improving Student Engagement throughout Collaborative Oral Presentations in General Pharmacology

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Background: Oral presentations are a key evaluation component of the General Pharmacology course (23025) in the Medicine Degree at the University of the Balearic Islands. Traditionally, groups of 4 students presented pharmacological topics in groups, although this format offered limited active engagement and non-equitable participation. To address this challenge, a new activity was implemented in which group members assumed distinct roles; while 2 students delivered the presentation, the remaining 2 responded to questions posed by peers and instructors. This division of tasks aimed to foster critical thinking, collaborative learning, and dynamic interaction [1]. Additionally, a digital forum extended the discussion beyond the classroom, allowing for further reflection and content clarification [2].

Methods: The instructional strategy was structured into 5 key stages to ensure comprehensive student engagement and learning outcomes: (1) Topic Selection: groups chose pharmacologically relevant topics, including drug mechanisms, adverse effects, interactions, or clinical case studies, aligning content with course objectives. (2) Content Preparation: each group prepared a 10-12 minutes PowerPoint presentation, focusing on clear structure, evidence-based content, and the use of visual aids to facilitate comprehension and maintain audience engagement. (3) Oral Presentation: 2 members of each group presented the content to the class, adhering to time constraints and employing effective communication techniques to enhance audience attention. (4) Q&A Session: following the presentation, the remaining 2 members responded to questions posed by classmates and faculty, allowing assessment of content mastery and the ability to articulate complex concepts under pressure. (5) Digital Forum Participation: a dedicated digital forum remained active for one-week post-presentation to promote further discussion, question resolution, and reflective learning. This asynchronous component allowed all students to revisit key concepts and engage in evidence-based discussions beyond the classroom.

Results and Conclusions: The restructured format encouraged active involvement from all group members, enhancing skills in communication, critical analysis, and rapid response. The digital forum deepened engagement, allowing traditionally less participative students to contribute meaningfully to discussions. Feedback from students indicated increased in equitability participation, while faculty observed improved content comprehension and group dynamics. This approach not only aligned with course objectives, but also prepared students for their future clinical practice, where clear communication and teamwork will be essential.

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Introduction of Problem-Based Learning methodology in Ocular Pharmacology: towards social dissemination

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Background: Problem-based learning (PBL) is an active teaching methodology that places students at the centre of the learning process by engaging them in solving real-world problems.
This approach promotes critical thinking, collaboration, and the practical application of knowledge. In this teaching innovation project, we have introduced for the first time this methodology in the subject of "Ocular Pathology and Pharmacology" in parallel with an objective of social diffusion.
Material and Methods: A practical case or question was presented to students, which they had to address by producing a video, audio presentation or any format with the objective to communicate the proposed solution to a non-scientific audience. Specifically, the question raised was: "Bacterial conjunctivitis vs viral conjunctivitis". The population of this study comprises the students enrolled in the subject Ocular Pathology and Pharmacology in the academic course 2024-25 (32 students). Students were randomly divided in groups of 3-4 members and this task was performed during four tutorial sessions of one hour. During the fifth session, students had to present their task to the rest of the students. After the activity, a satisfactory survey of 15 items with a five-point Likert scale was provided in order to assess the students´ satisfaction.
Results: All students enrolled in this subject participated in the innovative activity and 8 different groups were composed. Each group independently decided the presentation format and the sector of society. Specifically, the chosen formats were very diverse including oral presentations, posters, videos, triptychs, survey, blog, tiktok, scientific experiment, board gameand even a song. Regarding the sectors of society, they were also very varied including children's and teenagers' classes, pool lifeguards, pet owners and parents with children. The activity was rated satisfactorily by all the students (4.59 out of 5 and 93.75% of students were very or quite satisfied), showing their willingness to participate in similar initiatives. In fact, most of the students would repeat or recommend this activity in future editions of the subject (4.74 out of 5 and 100% of students scored it ≥ 4). Regarding the methodology, all students indicated that the contents worked on during the activity were relevant to their learning (4.56 out of 5) and the methodology used has favoured the understanding of the concepts (4.70 out of 5). Finally, concerning the organization of the activity, students stated that the task was well organized and structured (4.85 out of 5) and the instructions and objectives were clear from the beginning (4.81 out of 5).
Conclusions: The introduction of PBL in the Ocular Pathology and Pharmacology has proven to be a highly engaging and effective educational strategy. The activity not only enhanced students' understanding of the subject matter but also encouraged creativity, teamwork, and the ability to communicate scientific knowledge to non-expert audiences. The high levels of student satisfaction and their willingness to repeat the experience underscore the value of integrating socially oriented, active learning methodologies into pharmacology education.

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Pharmaceutical Advice in the Digital Era: Innovative Educational Strategies Using Instagram

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Social media platforms, notably Instagram, have emerged as influential tools for public health communication. Leveraging this trend, our project aimed to enhance pharmacy students' skills in scientific dissemination and effective communication within digital contexts. Conducted during the first semester of the academic year 2024-2025, this initiative was integrated into seminars of the course "Clinical Pharmacy and Pharmaceutical Care", targeting fifth-year students of the Pharmacy and dual Pharmacy-Nutrition degrees at Universitat de València.

Students, grouped in teams of 4-5, developed educational content on assigned topics related to minor ailments and rational drug use (such as women's health, sun protection, anxiety, acne, venous insufficiency, rational use of antibiotics, among others). Content was structured as Instagram posts (two image posts and one video reel per group), complemented by interactive stories featuring quizzes and engaging information. Teachers acted as account moderators, guiding students in producing scientifically rigorous content that was also accessible, engaging, and directly relevant to patients and the general public. All materials were published on the Instagram Business account @farmaceuticxalrescate, specifically created for this educational project.

The project achieved substantial engagement, reaching 6,476 Instagram accounts, generating 4,457 profile visits, and significantly impacting health communication through diverse formats, primarily reels, followed by image posts and stories. A satisfaction survey among participants (n=115) indicated high approval: 84% of students found the project highly valuable for learning communication skills, and 95% considered the content useful for general public health awareness. Additionally, students highlighted the project's role in reinforcing and expanding their existing knowledge and in developing the ability to communicate rigorously yet in an approachable manner.

These findings demonstrate the effectiveness of using Instagram as a pedagogical tool, significantly enhancing students' professional communication skills and actively contributing to health promotion through scientifically sound and patient-friendly advice. This innovative educational approach aligns with UNESCO Sustainable Development Goals, specifically targeting health and quality education (SDG3 and SDG4), and can serve as a transferable model for similar initiatives in other educational contexts.

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Enhancing Pharmacology Education: A Comparison of Case-Based and Computer-Based Learning

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The Pharmacy curriculum at the University of Barcelona includes Pharmacology and Therapeutics as a core subject, delivered through two compulsory courses—Pharmacology and Therapeutics I and II—offered in the 7th and 8th semesters, each being a 6-ECTS-credit course. Traditionally, practical training for these courses has relied on computer simulation programs and demonstration videos. However, in the 2024-2025 academic year, we introduced, exclusively in the Pharmacology and Therapeutics II course, case-based learning with group tutoring to replace the computer-based practical sessions. The same year, we assessed and compared student perceptions of competency acquisition across both courses, utilizing a standardized survey. Following the completion of their respective practical sessions, 178 out of 310 students enrolled in Pharmacology and Therapeutics I (computer-practice group, CP) and 132 out of 362 students participating in Pharmacology and Therapeutics II (case-based learning group, CBL) provided survey responses. Across all skill-related survey questions, students engaged in CBL expressed significantly greater satisfaction. For instance, 64.9% of CBL students *vs.* 20.7% of CP students—strongly agreed that the sessions motivated them to further explore the course content. Similarly, 66.2% of CBL students strongly agreed that the practices had helped them develop specific competencies in pharmacology, compared to 25.1% of CP students. Regarding the acquisition of general skills, 67.7% of students in the CBL group strongly agreed that the sessions improved their ability to learn autonomously, whereas only 25.1% of CP students shared this view. Additionally, 70.8% of CBL students felt the sessions enhanced their critical analysis, problem-solving, and decision-making abilities. In contrast, only 24.6% of CP students fall into this category. Other aspects rated more positively among students in the CBL-based Pharmacology and Therapeutics II course included the relevance of the practical sessions in preparing them for their future roles as pharmacists (63.4% *vs.* 19.7%) and the level of instructor support received (85.5% *vs*. 53.1%). Lastly, the survey addressed to Pharmacology and Therapeutics II students featured a direct question comparing the effectiveness of computer-based *vs.* case-based sessions in facilitating learning and competency acquisition. Subjectively, a substantial majority—77.1%—strongly agreed that clinical case-based sessions improved their learning and skill development.

In each subject, practical activities accounted for 15% of the total grade (15 points). Students in the CBL group achieved higher scores (13.7±0.9) compared to those in CB sessions (6.5±3.7). However, these results are not directly comparable. The CP assessments were conducted at the end of the semester and focused on practical pharmacology competencies, whereas the CBL evaluations were conducted immediately after completing the activity and assessed skills related to patient care, drug interaction observation, and medication management planning.

Overall, students' feedback indicated a marked preference for CBL over CP practical sessions, particularly in terms of engagement, competency development, and satisfaction with their training experience.

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Use of AI in Learning through the Case Method in Pharmacology

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Background: generative Artificial Intelligence (AI) is a tool already integrated into students' daily lives. According to recent reports by the European Commission and CRUE, it is imperative to incorporate AI into higher education, training students to use it ethically and safely due to potential risks related to information quality and reliability. The case method, an active learning approach, allows evaluation of AI by challenging it with complex clinical reasoning tasks. Pharmacology education demands strong scientific knowledge and applied clinical skills, which can be fully developed with active realistic learning methods. The growing complexity of pharmacological treatments, polypharmacy, and personalized therapy makes this challenge even more pressing. This project aims to enhance learning in Pharmacology II for Pharmacy and Pharmacy & Nutrition degrees students with a dual purpose: a) to train students in clinical case resolution, simulating real-life professional contexts and b) to develop critical capacity for therapeutic decision-making using both traditional scientific sources and generative AI tools, encouraging evaluation and correction of AI-generated outputs.

Methods: during practical sessions (16 students/group, 4 hours), students work in subgroups to resolve complex clinical pharmacology cases using two approaches: i) information retrieval from validated primary and secondary sources (e.g., official drug databases like Bot PLUS); and ii) AI-assisted resolution (primarily using ChatGPT). Cases are based on real anonymized patient scenarios prepared in advance by students and refined by professors. Sessions include: students answer of case-related questions prior to information search, information retrieval and group analysis using both data sources, group presentations and peer comparison of the information obtained and the accuracy of different sources employed, final collective discussion with instructors and a rubric-based evaluation to assess both learning outcomes and students' perceptions of AI utility and limitations. Pedagogical Strategies: Problem-Based Learning to encourage contextualized knowledge construction and realistic clinical application, collaborative learning that enhances peer interaction and shared reasoning and peer assessment, which promotes critical thinking through mutual evaluation. These align with Sustainable Development Goals 3 (Good Health & Well-being) and 4 (Quality Education).

Results: based on surveys from 75 students, over 80% expressed high satisfaction with the method, noting improvement in theoretical understanding and real-world application. Around 78% of students reported enhanced collaboration. Around 60% found AI useful for quick, autonomous information access. Students identified weaknesses in AI performance during complex clinical reasoning, especially due to outdated or incomplete information.

Conclusions: the case method is highly valued by students for fostering meaningful learning and practical skills. Integrating AI promotes critical professional judgment. Students appreciated the opportunity to contrast AI outputs with validated sources, gaining greater autonomy and awareness of AI's capabilities and limitations, this reinforcing the students' self-confidence in their abilities to solve potential real situations. Future courses will incorporate improvements suggested such as shorter, more dynamic clinical cases and simulated real-world pharmacy scenarios.

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Psoriasis therapeutics: the triangle patient - pharmacist - research staff as a didactic tool in Pharmacology

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The present educational initiative aimed to facilitate student learning of Pharmacology through an innovative and dynamic approach aligned with the United Nations Sustainable Development Goal 3: Health and Well-being. Recognizing the need for improved coordination among healthcare professionals, the purpose was to familiarize students with the important role of pharmacists in the therapeutic management of chronic diseases, incorporating the views of patients, healthcare providers, and researchers. Psoriasis, a chronic immune-mediated inflammatory skin disorder, was selected as a case study to explore this multidisciplinary perspective.

The activity was conducted as a seminar of the subject *Pharmacology I* (compulsory, 6 ECTS, second semester of the third year of the Degree in Pharmacy and the Double Degree in Pharmacy and Human Nutrition and Dietetics, at the University of Valencia, with approximately 230 students distributed in 4 groups). The three key learning objectives were: (1) understanding the pathogenesis of psoriasis; (2) exploring current pharmacological treatments, including mechanisms of action, side effects, and contraindications; and (3) reviewing novel pharmacological targets and investigational drugs in preclinical and clinical studies. A blended learning strategy was adopted, highlighting the Round Table (RT). Students worked in teams (4–5 members) to prepare a portfolio prior to the RT (1h of distance learning), which was evaluated by the faculty. Each team also formulated several open-ended questions to be posed during the RT, which featured a diverse panel: a patient, a physician, a pharmacist, a biomedical researcher, and faculty members. Following the RT, students completed a 10-minute self-assessment questionnaire in the virtual classroom. All students passed the test, with an average score of 8.6 out of 10. Finally, student feedback was collected via an anonymous survey consisting of 7 questions, 5 of which used a 6-point Likert scale (strongly disagree, disagree, neither agree nor disagree, somewhat agree, agree and strongly agree).

Across the four groups, responses showed a consistent trend: 76% agreed or strongly agreed that the topic was interesting, and 79% found the activity more useful and formative than other academic experiences. Notably, 59% of students reported using AI tools to complete the pre-RT task. In conclusion, this seminar introduced an engaging, interdisciplinary learning format, fostering student interest and perceived educational value, while highlighting the pharmacist's essential role in managing chronic diseases and supporting SDG 3.

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"Molecular Pharmacology" in the Degree in Biochemistry and Biomedical Sciences at the University of Valencia from the students´ perspective

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Introduction: The *Degree of Biochemistry and Biomedical Sciences* at the University of Valencia offers 6 optional subjects in the 4th year and *Molecular Pharmacology* is one of them (4,5 ECTS credits). Typically, there are 15-18 students, in one single group, and the language employed is Spanish. Face-to-face activities include theoretical classes (20h), practicum (12h), seminars (6h) and tutorials (individual and group ones, 3h). The objective of this work was to analyze the students´ opinion about the different programmed learning activities and how they studied the subject.

Methods: An anonymous voluntary survey of 17 questions was conducted. The first question had 4 options to choose from, 15 others contained a Likert scale of: "Strongly disagree", "Disagree", "Neutral", "Agree" and "Strongly agree" and we also included one question regarding the use of artificial intelligence (AI) whose answer options were "Never", "Very rarely", "Sometimes", "Often" and "Very often".

Results: A total of 15 students (100% of those enrolled in the subject in the academic year 2024/2025) took the survey. When it came to study materials, half of the students (53.3%) used only the slides provided through the Virtual Classroom, whereas the other half supplemented the slides with their own Internet searches. Surprisingly, no students reported the use of textbooks, whether physical copies from the library or e-books available online, in addition to the slides. Half of the students (53.3%) expressed preference for having access to all slides at the beginning of the course, and 66.7% strongly favored slides that contained substantial text. All the students either "agreed" or "strongly agreed" that attending theory classes significantly helped them understand the subject better. Additionally, 60% admitted using slides from previous academic years, while 66.7% stated they have used AI "often" or "very often". Notably, only 46.7% reported keeping up with the subject consistently throughout the course. Regarding the knowledge of previous subjects in the curriculum, 86.7% of the participants stated that they found it necessary for studying *Molecular Pharmacology* effectively, while 80% stated that in their case, their knowledge from previous subjects was sufficient. Regarding assessment, the vast majority (93,3% "agreed" or "strongly" agreed that it is appropriate for a significant portion (50%) of the final grade to come from continuous evaluation, including practical sessions, seminars, tutorials (individual and group) and questionnaires in the Virtual Classroom. Perceptions of these learning activities varied significantly: only 20% of the students found seminars useful, while 53.3% and 86.7% reported the same for the tutorials and the practicum, respectively. Finally, 73.3% of students stated that *Molecular Pharmacology* had sparked significantly more interest than other subjects studied previously.

Conclusion: This survey has allowed us to acknowledge the students' opinion about this subject and the way they approach studying it. The results we obtained will help us to improve the organization of the activities and the materials provided in following years.

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Developing Communication Skills in Biomedicine: PechaKucha as a Visual Storytelling Approach to Promote Active Learning in Master's Students

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Effective communication is a fundamental skill in scientific education,yet often underemphasized in postgraduate biomedical training. In the context of the "Advances in Cell Culture course", part of our Master's program in Biomedicine, we implemented a PechaKucha-style presentation activity. PechaKucha is a storytelling presentation format that originated in Japan, designed to keep presentations concise and fast-paced. Each presenter shows exactly 20 slides for a specific period (20 seconds each), resulting in a total presentation time of 6 minutes and 40 seconds. The automatic slide progression forces speakers to be precise and focused, encouraging clarity, creativity, and visual communication. We carried out the activity with 10 master's students, working in pairs to encourage collaboration and shared learning. Each group selected one cell line from a predefined set of eight (HULEC, Hematopoietic Stem Cells, Jurkat, HEK-293, COS-7, HeLa B, HUVEC, HL60). The tasks were to research the origin of the cell line, identify the suitable culture medium and its composition, find a scientific article that uses the cell line, and prepare a concise, visually driven 6-minute and 40-second presentation (20x20) aimed at synthesizing and clearly communicating the biological relevance of their assigned cell model. A brief how-to introduction to the PechaKucha style was given to the students, including a video using this format. Then, the students were then given 80 minutes to prepare their presentations. Following the activity, students completed an anonymous survey assessing their learning experience. The survey had both open-ended and multiple-choice questions with the options *strongly agreed*, *agreed, neither agree nor disagree*, *disagree*, *strongly disagree*. Our results indicated that most students agreed or strongly agreed that the activity improved their understanding of cell line concepts (80%), enhanced their ability to synthesize and orally present information (90%), and promoted effective teamwork (90%). The PechaKucha format was perceived as facilitating clear presentation of complex information and encouraging active engagement (90%). All participants agreed or strongly agreed that the activity encouraged them to reflect on how to communicate science in a more accessible and effective way (100%). Overall, the activity was positively received, with a majority recommending its continued use in future sessions (90%) or as a preferred model for presentations (70%). However, feedback also highlighted the need for slightly more preparation time, as a most of them reported that the given time was not sufficient (70%). In open-ended questions about which aspects of the activity could be enhanced, time was the only mentioned element. When asked about the appropriate amount of time needed, the average response was 125 ± 32 minutes (Mean ± SD). These findings support the inclusion of structured communication exercises such as PechaKucha to foster synthesis, collaboration, and science communication competencies in postgraduate biomedical students.

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P53

Implementation of a Practical-Theoretical Activity Based on Protocol Design and Scientific Project Simulation in a Master's Degree

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In postgraduate biomedical education, it is essential to incorporate active methodologies that promote applied competencies, critical thinking, and realistic problem-solving. To achieve this objective, a practical-theoretical activity was implemented within the "Advances in Cell Culture" subject in the *Biomedicine Master's degree at the University of Alicante. This exercise aimed* to engage students in designing experimental protocols using cell culture and simulating the initial stages of a research funding process application. This methodology aligns with widely supported active learning strategies in higher education, which have been shown to improve student engagement and comprehension (1,2). The activity was carried out during a 5-hour session. Students were divided into pairs or groups of three. Subsequently, a draw was held to assign the different research projects and the initial budget available to each group for conducting the preliminary experiments. The assigned projects and their budget were: (1) Evaluation of a new chemotherapeutic drug for cervical cancer (€20,000); (2) Identification of early biomarkers and/or predictors for atherosclerosis (€10,000); (3) Discovery of a novel subtype of pro-inflammatory myeloid-lineage cells (€3,000); and (4) Preclinical testing of miRNA-based therapy to inhibit angiogenesis in diabetic retinopathy (€7,000). The activity was introduced as a simulation of a research funding pitch: students had to design and defend a project proposal to convince their peers and an evaluation panel to fund their initiative with a hypothetical €500,000 grant. For the first 90 minutes, students were not allowed to use computers or mobile devices, working solely with printed theoretical materials provided by the instructors. This constraint encouraged active discussion, teamwork, and critical resource management. After this initial phase, they were permitted to use computers to refine their experimental designs and budget adjustments. The session concluded with a final hour dedicated to project presentations. Each group delivered a short pitch in free format to advocate for their proposal. At the end of the presentations, a peer voting process was conducted to select the winning project. Finally, students were asked to complete an anonymous survey, to assess the activity's impact and perception. The results were highly positive: over 95% of the students highlighted the usefulness of facing a complete experimental design and especially valued the opportunity to make autonomous decisions. They also noted that this methodology contributed to consolidating prior knowledge and improving their understanding of standard procedures in cell culture. This experience demonstrates the educational value of active, simulation-based methodologies in enhancing both laboratory techniques and transferable soft skills essential for future biomedical professionals, offering a replicable model for other postgraduate programs.

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Scientific divulgation on antimicrobial resistance: An experience through podcasting

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In recent years podcasts have gained popularity as their affordability and ease of accessibility have made them a good e-learning tool. More specifically, they help spread knowledge among population, act against misinformation on social networks, and engage people in the search for solutions to the problem disseminated. We describe the design and creation of podcasts as a way to reach large audiences. They were generated by the students enrolled in an annual course funded by the European Commission (project RESIST-UE), focused on the fight against antimicrobial resistance (AMR). Once the activity was ended, they answered a satisfaction survey. The number of students who successfully completed the course was 21. Of those, 17 defined the topics, planned the content of the podcasts, and created the episodes (81.0%). These students were mostly females (76.5%), undergraduates (76.5%), and studied the Degree in Veterinary Medicine (47.1%), Nursing (17.6%) or Podiatry (17.6%). The topics presented in the AMR-related podcasts and the way they were dealt with were very different, and usually related to the Degree studied or the professional activity of the author. All of them are available in the most popular and widely used podcast hosting platforms. A total of 311 downloads have been recorded and actually heard. These hearings were mainly made with a browser (32 %), Spotify (23 %), and Pocket Cast (5 %). Moreover, most of them were downloaded to a PC (41 %) or a mobile (28 %). The satisfaction survey was answered by 11 students (64.7% of those who took part in the activity). In their opinion, the activity had been satisfying (45.5%) or highly satisfying (36.4%), and they would do it again. Thus, podcasts represent a promising educational tool to increase and expand awareness about topics of great interest to the public, such as AMR.

P55

Promoting attendance and active participation among undergraduates: an innovative experience

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Nowadays, non-attendance of lectures appears to be a growing trend among undergraduates. Thus, it is an issue that has attracted the attention of teachers and educators, as attendance rates are often considered an indicator of student engagement, and may have a significant impact on academic performance [1]. The study describes the development of an innovative strategy carried out in a compulsory subject of the Degree in Veterinary Medicine (Pharmacy and Pharmacotherapy, with 15 theoretical lectures), in which a loyalty card was used to improve attendance and active participation in the theoretical lectures. The loyalty card was stamped daily if the students posed a question related to the topic reviewed, answered questions presented by the lecturer, or participated in the discussions raised. Attendance rate and the number of stamps affixed to the loyalty card were used as indicators of the strategy, as well as a satisfaction survey. 20 students (28.2% of those enrolled in the subject) voluntarily took part in the activity, and all of them were in their first enrolment (20% males). The number of stamps/student ranged between 0 and 15 (mean of stamps/lecture 9,8). Male students achieved a mean of 9.0 ± 3.9 stamps, whereas female ones 6.9 ± 5.5. As for attendance, students attended class a mean of 10.5 ± 4.4 times, showing males greater engagement (11.3 ± 3.9 vs. 10.6 ± 4.6 in females). Regarding final marks, only 1 student failed the subject in this group (0.05%). Those who passed the course, achieved a mean of 7.0 ± 1.2 points (out of 10). For those who chose not to take part in the strategy (the remaining 71.8%); 27.5% were males; they had enrolled in the subject 1.1 ± 0.3 times; the mean attendance rate was 3.3 ± 3.7; and the mean final mark was 6.1 ± 1.0 (4 students did not the exam). A total of 12 students fulfilled the satisfaction survey (60% participants in the strategy; 16.7% males). They pointed out that the loyalty card had notably increased their interest to participate during the lectures (66.7%), and that they were more comfortable with those discussions performed during the lectures (58.3%). Moreover, the loyalty card had increased their interest for the subject (58.3%), and they were satisfied (25%) or very satisfied (50%) with the innovative strategy. In summary, the activity carried out seems to be a good initiative to increase the interest of students attending class.

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P56

Development of a peer-assessment rubric for Pharmacology undergraduate students

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Peer assessment allows students to evaluate their peers, foster collaborative learning, develop students' evaluation skills, and enhance their engagement with certain subject. On the other hand, rubrics are nowadays widely used as instructional and learning instruments. The study describes the design and use of a rubric specifically designed for peer-assessment among students in the subject Veterinary Pharmacology. A rubric was specifically designed to evaluate their abilities related to synthesize and communicate information in a friendly way. It contained 9 criteria with a rating scale to define student performance and activity requirements. The rubric was shared with students at the beginning of the semester, and applied to the collaborative activity carried out by students in the subject throughout the semester, in which they had to elaborate a poster and a video on a topic of Pharmacology. A total of 98 students (74.5% females) took part in the activity the academic course 2024-25, which was performed in groups of 3-5 people. Each group created two activities to be shared and disseminated to their peers. Almost all of them (99%) assessed the activities of their peers (not participating in peer assessment would be negatively considered in the final marks of the subject). Mean scores for each activity evaluated ranged from 8.1 to 9.2. Among female students mean marks varied between 7.7 and 9.2, whereas among male ones did between 7.6 and 9.0. Significant differences were found between both sexes in scores given (Mann-Whitney U test, $p < 0.05$). A satisfaction survey was also used to know students' opinion about the strategy carried out, showing that they were highly satisfied with it. In conclusion, we have seen that the rubric has had a positive effect and enabled peer assessment through clear assessment criteria and a structured format.

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Learning pharmacology by teaching it: empowering future pharmacists as health educators

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Pharmacists are increasingly recognized as health educators [1], yet this role is often underdeveloped during undergraduate training. To strengthen this aspect, an educational innovation project was implemented in the Pharmacy Degree at the University of Salamanca, aiming to promote active learning through direct patient education. The aims were to reinforce knowledge of pharmacological treatment for fibromyalgia [2], to develop students' skills in health communication and patient education, and to promote the pharmacist's role in rational medication use. Over four months, ten volunteer students were grouped to prepare some educational sessions for patients suffering from fibromyalgia, in collaboration with AFIBROSAL (*Association of people with fibromyalgia and chronic fatigue syndrome of Salamanca*). Each student group addressed one therapeutic group (e.g., antidepressants, antiepileptics, analgesics, or phytotherapy) and created both oral presentations and information in leaflets to patients. The sessions were held in AFIBROSAL's community centre, and the pharmacology professors supervised all the content development, besides the students received feedback through individual tutoring sessions. The activity also included student participation in public events for International Fibromyalgia Day. All students reported enhanced understanding of pharmacology and improved skills in scientific communication, empathy, and adapting language for patient audiences, expressing 100% willingness to participate in similar future initiatives related to pharmacology. Patient feedback was overwhelmingly positive: 74% rated presentations as excellent and 100% would recommend the sessions to other patients. Despite the relatively low participation rate (around 5% of the course cohort), due largely to extracurricular timing and academic workload, the activity had a high impact on those involved and was recognized as meaningful both educationally and personally. In conclusion, involving pharmacy students in real patient education effectively enhances pharmacological knowledge and professional skills. This teaching project promoted critical skills aligned with the pharmacist's evolving role and has been well received by both students and patients. The success of this initiative supports its future implementation in other pharmacology courses.

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Enhancing regulatory competencies through simulation: an active learning experience in Pharmaceutical Legislation

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The ability to correctly classify pharmaceutical products and detect falsified or mislabelled items is essential for future healthcare professionals, yet often underrepresented in pharmacology curricula. To address this gap, we developed an active learning activity within the course "Pharmaceutical Legislation and Deontology" at the University of Valencia, aiming to strengthen students' understanding of regulatory categories, labelling requirements, and public health implications of counterfeit medicines.

The intervention targeted fourth-year Pharmacy students and fifth-year double-degree students, and was structured in four phases: (1) a pretest consisting of multiple-choice questions to assess baseline knowledge; (2) an individual viewing of an educational video explaining legal classifications such as drugs, medical devices, cosmetics, food supplements, and biocides; (3) a collaborative simulation in which students, acting as health inspectors, examined real product packaging and wrote a justification report; and (4) a posttest and a satisfaction survey.

Results showed a 68.6% improvement in posttest scores and a 31.2% reduction in average response time, suggesting greater confidence and content mastery. Students also reported a high level of engagement, appreciation for the real-world relevance, and interest in repeating similar activities in other courses. These findings support the integration of simulation-based and experiential learning strategies into pharmacology-related education [1], not only to improve knowledge acquisition but also to enhance motivation, teamwork, and professional readiness.

By simulating real inspection scenarios, students develop a practical understanding of pharmaceutical regulation, reinforcing key competencies in critical thinking and ethical-legal analysis. This approach aligns with global educational goals in pharmacy to prepare graduates for increasingly complex regulatory environments.

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Phytotherapy in Childhood: Educational Strategies for Safe Use of Medicinal Herbs

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The use of herbal medicinal products has significantly increased in recent decades, likely due to the search for more natural alternatives in disease treatment. In pediatric settings, families are typically the intermediaries in their use, often underestimating the risks and interactions associated with medicinal herbs, due to the widespread perception that natural equals safe. However, the lack of reliable information in this context can lead to inappropriate or unsafe use, particularly when based on traditional knowledge not supported by official sources. The aim of this study is to propose an educational intervention to enhance knowledge of phytotherapy among children and prevent its improper use. Medicinal herbs with proven efficacy for children over six years old, according to official sources such as the EMA and ESCOP, were selected. These plants were categorized according to four systems: respiratory, digestive, nervous, and skin. Educational materials were developed for children, including games, short stories, and practical activities. In addition, informative materials for parents were designed, providing useful, practical, and safe information. This intervention may be valuable in future applications within school settings or health workshops

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An innovative action by pharmacy students through collaborative learning in high schools

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The third-year Pharmacognosy students from the Pharmacy Degree at the University of Valencia (UV) have been participating in an educational innovation initiative for high school students in last years. University students apply the knowledge acquired in the Pharmacognosy course and strengthen their communication skills to provide reliable information, based on the biological properties of natural substances and their synthetic analogues with recognized addictive potential, to high school students. The aim is to convey this message through closeness and generational affinity, to prevent the consumption of addictive substances.

Third-year Pharmacognosy students participate in service-learning (SLE) methodology. The project began with a questionnaire. The teaching methodologies used were flipped classroom and gamification using Google Forms®, the Pharmacognosy Virtual Classroom teaching platform and Wooclap create interactive presentations. This was followed by a practical demonstration on tobacco leaves.

Pharmacognosy students conducted an innovative educational activity during the 2024-25 with students from a secondary education school (SES) of Burjassot (Valencia) to educate them about problems associated with substance abuse. Pharmacognosy students gathered information (Learning) on the consequences of addictive substance use, given the perceived risk and visibility of the drug problem to prevent drug use and undertake preventive actions in this population group (Service). Of the 23 participants of the SES aged from 16 to 18 years (32% women), the 13% reported having smoked tobacco sporadically and 74% drank alcohol at some point (30% monthly and 4% weekly). 22% SES students drank energetic drinks daily, 17% have consumed amphetamines, and 9% had used anxiolytics. Regarding illegal drugs, nobody reported having used cannabis at some point. At the SES, the university students presented the main herbal drugs used by adolescents as first-time users. This was followed by a practical demonstration on tobacco leaves to capture their interest and facilitate understanding. Along the presentation by Wooclap the SES students participate using a participation link or a QR code. They concluded with a game, receiving immediate feedback. At the end, all participants completed a survey and provided positive feedback on the activity. It was enriching for the university students and highly appreciated by the SES faculty.

The university students developed skills in the teaching-learning process aimed at promoting the health of IES students by motivating them to improve their habits, reconsidering their risk perception, and discouraging them from drug use.

Cardiovascular pharmacology

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Telomere length is associated with ventricular fibrillation during acute myocardial infarction

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Introduction: Telomeres are terminal sequences, TTAGGG, of linear eukaryotic chromosomes that are involved in maintaining genomic stability and regulating cellular proliferation. Telomeres shorten with every cell division, as DNA polymerase is not able to fully replicate the 3′ end of the DNA strand. Telomere length has been shown to be associated with biological ageing and pathogenesis of various cardiovascular diseases. However, the length of the telomere has not been studied in patients that present ventricular fibrillation (VF) during acute myocardial infarction (AMI).

Purpose: The aim of this study is to analyze telomere length an its potential association to VF in AMI.

Methods: Blood samples of 95 AMI and 62 VF in AMI patients were included in the study. DNA isolation was performed, and telomere length was measured in quadruplicate by quantitative PCR method, and 36B4 (S) was used as reference gene. The T/S ratio was calculated using these efficiency values: T/S ratio = efficiency−CpTel/efficiency−Cp 36B4. Data was analyzed using SPSS software version 20 (Chicago, Ilinois, USA). Mann-Whitney U-test was performed for homogeneity testing. Multivariate analysis using logistic regression analysis was performed to assess T/S ratio and age and gender as associated risk factors of FV in AMI patients. Significance was defined as $p < 0.05$.

Results: Telomeres were significantly longer in patients with VF during AMI (AMI= 194,08±41,08 (n=95) *vs* VF in AMI=211,98±45,25 (n=63) P<0.008). Multivariate analysis by binary logistic regression did not reveal significant association with age (p=0.857) nor gender (p=0.196) with FV in AMI patients. A longer telomere length is significantly associated with FV in AMI patients and may be a risk factor associated with VF (OR:1.010; p=0.024).

Conclusions: Patients with VF during AMI present higher length of telomeres than patients with AMI, independently of sex and age.

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Absence of CCR3^{+} hematopoietic cells worsened atherosclerosis lesion formation

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Atherosclerosis is one of the leading causes of morbidity and mortality in Western countries and the balance of proinflammatory mechanisms and resolution of inflammation dictates the ultimate clinical outcome [1]. We evaluated the impact of an atherogenic diet in the lesion formation in apoE$^{-/-}$ CCR3$^{+/+}$ and apoE$^{-/-}$CCR3$^{-/-}$ mice fining increased lesion formation in the latter. Therefore, here we have evaluated if CCR3^{+} cells are involved in the increased atherosclerotic lesion found in apoE$^{-/-}$CCR3$^{-/-}$ mice.

Two months old mixed chimeras were generated by transferring whole bone marrow (BM) of apoE$^{-/-}$CD45.1 mice to irradiated apoE$^{-/-}$CCR3$^{-/-}$ CD45.2 recipient animals and viceversa. Animals were fed with an atherogenic diet during two additional months. Lesion formation, macrophage, CD8^{+} lymphocyte, eosinophil, eotaxin-1/CCL11, IL-4, pSTAT-6, and M2-macrophage content were determined within the lesion though histological and immunohistochemical techniques. Statistical significance was determined using a Two-way ANOVA followed by Bonferroni's post hoc test on raw data.

The atheroma in apoE$^{-/-}$ CD45.1 mice transplanted with BM from apoE$^{-/-}$CCR3$^{-/-}$ animals was significantly greater than that developed in apoE$^{-/-}$CCR3$^{-/-}$ mice transplanted with BM from apoE$^{-/-}$CD45.1 animals. Examination of the atherosclerotic lesions revealed that the number of infiltrated macrophages and CD8 lymphocytes were significantly higher in apoE$^{-/-}$ CD45.1 mice transplanted with apoE$^{-/-}$CCR3$^{-/-}$ bone marrow fed with an atherogenic diet. No significant or reduced expression of CCL11/eotaxin-1, eosinophils, IL-4, STAT6 phosphorylation or anti-inflammatory M2-like macrophages were found in apoE$^{-/-}$ CD45.1 transplanted with BM of apoE$^{-/-}$CCR3$^{-/-}$ animals.

Our fundings confirm that hematopoietic CCR3^{+} cells may exert a protective effect in the development of the atherosclerotic process.

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Blocking the CCL25–CCR9 chemokine axis reduces leukocyte-endothelium interactions in patients with morbid obesity

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Obesity is a chronic and multifactorial condition that represents a major challenge to global public health. Chronic low-grade inflammation contributes to insulin resistance and the development of diabetes in obese patients. This study investigates the expression of the proinflammatory chemokine CCL25 and its receptor CCR9 in visceral and subcutaneous adipose tissue from patients with severe morbid obesity, as well as their association with diabetes development. In addition, the consequences of CCR9 blockade on leukocyte-vascular endothelium interaction, an early step in atherogenesis, was analyzed.

Samples of subcutaneous (SCAT) and visceral adipose tissue (VCAT) from morbidly obese patients undergoing bariatric surgery were analyzed to measure the expression of CCR9 and CCL25 by RT-PCR, western blot, and immunohistochemistry. The effects of ex vivo CCR9 neutralization on leukocyte adhesion to endothelial cells were also evaluated using a laminar flow chamber assay.

RT-PCR analyses showed that CCL25 and CCR9 levels were significantly higher in visceral fat compared to subcutaneous fat ($p < 0.05$) in morbidly obese patients. Additionally, both CCL25 and CCR9 were significantly elevated in the visceral fat of diabetic patients compared to non-diabetic patients ($p < 0.05$). Immunohistochemical studies revealed that CCL25 is predominantly localized in endothelial cells and macrophages within visceral fat. Using the laminar flow chamber model, it was demonstrated that blocking endothelial CCR9 function with a specific antagonist significantly reduced leukocyte adhesion to dysfunctional endothelium, a key event in atherogenesis, in patients with morbid obesity.

This preliminary research suggests that the CCL25/CCR9 axis could represent a potential pharmacological target in morbid obesity and diabetes.

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Canagliflozin as a Potential Senostatic Agent in Human Vascular Smooth Muscle Cells

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Gliflozins are antidiabetic drugs known for their role in managing Diabetes Mellitus (DM), a highly prevalent condition and a major cardiovascular risk factor. These drugs exert their glucose-lowering effect by inhibiting the sodium-glucose cotransporter 2 (SGLT2) in the renal tubules. Among them, canagliflozin has been reported to provide cardiovascular benefits through mechanisms that remain to be fully elucidated. This study aims to investigate whether canagliflozin acts as a senostatic agent by modulating interleukin-1β (IL1β)-induced and glucose-induced senescence in human aortic vascular smooth muscle cells (VSMCs).

In vitro, human VSMCs stimulated with IL1β (10 ng/mL) and cultured under basal glucose (5.5 mM), or high glucose (22 mM) conditions exhibited a significant increase in senescence-associated secretory phenotype (SASP) markers and β-galactosidase activity, indicative of cellular senescence. Treatment with canagliflozin (1 μM) ameliorated these effects. Furthermore, bioenergetic profiling revealed that IL1β and high glucose conditions promoted a metabolic shift toward glycolysis at the expense of oxidative phosphorylation. To investigate the mechanism underlying this shift, the expression of glucose transporters in vascular smooth muscle cells (VSMCs) was assessed. The cells were found to express GLUT1, SGLT1, and SGLT2, which location and expression were altered in response to IL1β (10 ng/mL) and high glucose conditions (22 mM). Under these conditions, glucose consumption, measured using a glucose oxidase (GO) enzymatic assay, was significantly increased. This increase was prevented by treatment with canagliflozin (1 μM), indicating a potential role of SGLT-and GLUT-mediated glucose uptake in the observed metabolic response.

These findings suggest that canagliflozin mitigates the deleterious effects of IL1β and high glucose on human VSMCs, potentially by modulating senescence and cellular metabolism. Canagliflozin appears to act as a senostatic drug, targeting cellular senescence, with glucose regulation as a central mechanism. This may contribute to the cardiovascular protective effects observed in diabetic patients treated with canagliflozin.

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miR-149-5p Negatively Regulates Angiogenesis and Migration in Human Endothelial Cells Exposed to Inflammatory and Ischemic Stimuli

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MicroRNAs are crucial regulators of endothelial function and cardiovascular disease. Among them, miR-149-5p has emerged as a potential modulator of vascular biology, yet its role under inflammatory and ischemic conditions remains poorly defined. Preliminary data from our murine model show a decrease in miR-149-5p expression following acute myocardial infarction. This study aims to explore its expression and function in human endothelial cells under conditions partially mimicking myocardial infarction and atherosclerosis.

Cultured Human Umbilical Vein Endothelial Cells (HUVECs) were exposed to TNF-α (10 ng/ml 4 or 24 h), oxidized LDL (oxLDL 100 ug/ml 24 h) and hypoxia (3 % O2 1, 4 or 24 h). Bioinformatic prediction and Gene Ontology enrichment analysis of miR-149-5p targets were performed using TargetScan, miRDB, micro-T-CDS and DAVID tools. For the functional assays, which included tube formation, to assess angiogenesis, and wound healing, to assess migration, HUVECs were transfected with either 50 nM of miR-149-5p mimic or 100 nM inhibitor. Expression levels of the miRNA and its potential target genes were measured by RT-qPCR (using TaqMan and SYBR Green, respectively). Statistical significance between groups was assessed by *t* test and 1-way ANOVA when appropriate. Values are expressed as mean ± SEM in percentage.

The expression of miR-149-5p was significantly downregulated in HUVEC following the ischemic and proinflammatory conditions (oxLDL 13.65 ± 5.30 %, $n = 9$; $p < 0.05$; TNF-α 64.71 ± 4.52 %, $p < 0.00$, $n = 12$; hypoxia 50.1 ± 19.15 %, $p < 0.05$, $n = 6$). In silico analysis revealed enrichment of predicted target genes of the Wnt/β-catenin pathway, which is involved in angiogenesis, cell migration, and vascular remodelling. In vitro experiments were performed to assess the involvement of miR-149-5p and its target genes in these functions. miR-149-5p mimic reduced angiogenesis in terms of number of nodes (74.91 ± 13.0 %, $p < 0.001$, $n = 4$) and migration rate (22.97 ± 8.58 %, $p < 0.05$, $n = 3$), while inhibition of miR-149-5p induced the opposite effects, enhancing both angiogenesis (3 ± 0.10 %, $p < 0.05$, $n = 3$) and migration (17.29 ± 7.17 %, $p < 0.05$, $n = 4$). The expression of both predicted target genes FZD5 and PPAP2B increased following TNF-α treatment (165.34 ± 34.06 %, $p < 0.001$ and 200.5 ± 19.38 %, $p < 0.0001$, $n = 6$ respectively), while only the PPAP2B expression was increased under hypoxia (75.17 ± 13.28 %, $p < 0.0001$, $n = 6$). Furthermore, miR-149-5p mimic restored TNF-α-induced-PPAP2B expression to control levels (87.43 ± 15.74 %, $p < 0.05$, $n = 3$).

Our findings indicate that miR-149-5p regulates key genes in angiogenesis and migration under inflammatory and ischemic stress —central processes to cardiac remodelling— highlighting its potential as a therapeutic target in ischemic heart disease.

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Effects of metformin on the NLRP3 inflammasome and metabolic alterations in human vascular smooth muscle cells

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The NLRP3 inflammasome is a key mediator of the innate immune response and has been implicated in different cardiovascular pathologies, such as atherosclerosis and diabetes mellitus. Metformin, a drug widely used in the treatment of type 2 diabetes mellitus, has demonstrated beneficial cardiovascular effects beyond glycemic control, although the mechanisms responsible for these effects are not yet fully understood.

In this study, we examined IL1β (10 ng/ml)-induced NLRP3 inflammasome activation in primary human vascular smooth muscle cell cultures, in the absence or presence of metformin (10 μM) or anakinra (1 μg/ml), a specific IL1 receptor blocker. Both the priming and activation phases of the NLRP3 inflammasome were examined by quantifying proteins such as NLRP3, p-P65, and IL1β using Western blot, as well as detecting ASC (Apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain) specks by indirect immunofluorescence. Additionally, to explore the interaction between inflammatory and metabolic pathways, bioenergetic parameters were measured using the Seahorse XF metabolic flux analyzer. Moreover, senescence associated β-galactosidase assay was performed to study the potential senomorphic effect of metformin in our cells.

Our preliminary results indicate that metformin reduces the expression of NLRP3, p-P65, and IL-1β, as well as the formation of ASC specks, suggesting that it inhibits both the priming and activation phases of the NLRP3 inflammasome although to a lesser extent than anakinra. The metabolic alterations produced by IL1β stimulation were reduced also by metformin, as well as the senescence associated β-galactosidase staining.

In conclusion, metformin attenuates NLRP3 inflammasome activation and IL1β-induced metabolic alterations and senescence, suggesting a potential therapeutic effect in inflammatory vascular diseases, beyond its conventional use in diabetes.

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The gut microbiota regulates hypertensive vascular damage in patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease associated with increased risk of renal and cardiovascular complications. In murine models of lupus, gut dysbiosis has been implicated in the development of hypertension. The main objective of this study was to characterize the gut microbiota of hypertensive SLE patients and evaluate its contribution to vascular dysfunction using germ-free (GF) mice.

Fecal and plasma samples were collected from healthy controls (Control), normotensive SLE patients (N-SLE), and hypertensive SLE patients (H-SLE). Microbiota composition was analyzed via shotgun metagenomics, and plasma short-chain fatty acids (SCFAs) were quantified using gas chromatography. GF mice were colonized with fecal microbiota from each group over two days and maintained for 10 weeks. Systolic blood pressure (SBP) was measured by tail-cuff plethysmography. Aortic endothelial function was assessed by wire myography, and immune cell populations were analyzed by flow cytometry.

SLE patients exhibited reduced microbial richness, with significantly lower Shannon diversity in the H-SLE group. Butyrate-producing bacteria were reduced in N-SLE, while H-SLE showed marked depletion of propionate-producing bacteria, notably *Akkermansia*, correlating with reduced plasma propionate. Both SLE groups showed diminished plasma acetate levels. GF mice colonized with lupus microbiota developed elevated SBP, more pronounced in H-SLE recipients, accompanied by left ventricular hypertrophy, proteinuria, hepatomegaly, and increased anti-dsDNA antibodies and plasma cells. Th17 cells were elevated in lymphoid tissues and plasma, with a decrease in Tregs observed in the H-SLE group. Only H-SLE-colonized mice showed impaired aortic endothelium-dependent relaxation, reversible by NADPH oxidase inhibition, associated with Th17 infiltration, Treg depletion, and increased vascular collagen deposition.

The gut microbiota of SLE patients, particularly those with hypertension, contributes to the transfer of a hypertensive and vasculopathic phenotype in GF mice. Alterations in microbial composition and SCFA production may influence vascular immune regulation and endothelial function.

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Impact of Chronic Antiretroviral Exposure on Profibrotic Gene Expression and Extracellular Matrix Remodeling in a Murine Model

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Cardiac fibrosis, characterized by excessive extracellular matrix (ECM) deposition, leads to myocardial stiffness and impairs ventricular function. Although antiretroviral therapy (ART) is essential for HIV treatment, it has been associated with cardiovascular (CV) complications, including cardiac fibrosis. Integrase strand transfer inhibitors (INSTIs), widely used in ART, may increase CV risk —such as myocardial infarction (MI) and heart failure (HF)—specifically in individuals with preexisting CV comorbidities. However, the specific effects of individual ART drugs on cardiac remodeling processes remain unclear. This study investigated the chronic effects of exposure to commonly prescribed INSTIs [dolutegravir (DTG), bictegravir (BIC), raltegravir (RAL), cabotegravir (CAB)] and doravirine (DOR) on profibrotic gene expression and ECM remodeling in a murine model in order to identify potential drug-specific cardiotoxic profiles.

Male C57BL/6J mice were orally and daily administered with one of the following antiretroviral drugs at clinically relevant doses for four weeks: DTG (10.79 mg/kg), BIC (10.75 mg/kg), RAL (179.31 mg/kg), CAB (6.48 mg/kg) or DOR (20.50 mg/kg). A vehicle-treated group served as control. At the end of the treatment period, cardiac tissues were harvested for analysis. Profibrotic gene expression (*Col1a1, Col3a1, Fn1, Acta2, Tgf-β1, Cdh2,* and *Lox*) was assessed using RT-qPCR. ECM stiffness was estimated by calculating the *Col1a1/Col3a1* ratio. Additionally, nuclear cardiac cell size was quantified in histological heart sections using the image analysis software QuPath. Statistical comparisons were performed to assess differences among the groups using GraphPad.

RAL, but not BIC, significantly reduced the expression of *Lox* (0.75±0.09 RAL *vs.* 1 Veh) -a key enzyme involved in collagen cross-linking-, and ECM stiffening (0.80±0.08 RAL *vs.* 1 Veh); suggesting that RAL may attenuated ECM rigidity by inhibiting collagen cross-linking formation. Additionally, BIC significantly decreased ECM stiffness (0.85±0.06 BIC *vs.* 1 Veh), indicating modulation of ECM remodeling through non-collagen-dependent mechanisms. Despite its effect on stiffness, BIC was associated with an increase in nuclear cardiac cell size, suggesting a possible pro-hypertrophic response to treatment.

These findings highlight distinct, drug-specific effects of ART agents on cardiac ECM remodeling and cellular responses with RAL exerting protective effects by reducing ECM rigidity and BIC inducing compensatory hypertrophic responses. A more comprehensive understanding of these differential effects is essential for optimizing ART regimens, particularly in patients with underlying CV risk.

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A new SGK1 inhibitor protects pulmonary endothelium and reduces inflammation in acute and chronic lung injury models

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Pulmonary inflammation plays a central role in the onset and progression of both acute and chronic lung diseases such as acute respiratory distress syndrome (ARDS) or pulmonary hypertension (PH). The inflammatory response is a complex process involving the release of pro-inflammatory cytokines, chemokines, and adhesion molecules in which both immune and endothelial cells play a relevant role. However, failure to resolve the inflammatory response or the presence of an excessive immune reaction can lead to pulmonary dysfunction, compromising the integrity of the alveolar-capillary barrier and contributing to vascular damage. In this context, serum and glucocorticoid regulated kinase 1 (SGK1) has emerged as a key regulator in multiple signalling pathways, playing a significant role in the immune and vascular response. Also, several studies have linked SGK1 dysregulation to hyperinflammatory responses and vascular remodelling. Consequently, we propose the use of novel SGK1 inhibitors, recently discovered by our research group, as a potential experimental therapy for the treatment of both acute and chronic pulmonary vascular and inflammatory diseases. To validate the efficacy and safety of SGK1 inhibitors, we established three complementary models: an *in vitro* model using human pulmonary artery endothelial cells (HPAEC), an acute *in vivo* mouse model simulating ARDS, and a chronic *in vivo* model of PH. Multiple molecular assays were performed to assess the levels of inflammatory mediators such as TGF-β, IL-6, and VCAM-1 among others. Our findings demonstrate that inhibition of SGK1 reduces TGF-β release from HPAEC after stimulation with TNF-α, and decrease VCAM-1 expression. In addition, in the acute mouse model, we observe a reduction in the number of neutrophils both in bronchoalveolar lavage fluid and tissue samples, along with an anti-inflammatory phenotype within this model. The endothelial damage marker, VCAM-1 is downregulated in treated mice lung tissues, indicating a reduced endothelial injury. These results correlate well to hemodynamic data found in our chronic PH model in which treated mice showed decreased right ventricular systolic pressure, thus demonstrating amelioration of the vascular function. In conclusion, the use of our novel SGK1 inhibitors appears to be a promising therapeutic strategy in the context of pulmonary inflammation, endothelial dysfunction and vascular disease.

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Evaluation of the Effects of Nintedanib in an *In Vivo* Model of Post-Myocardial Infarction Cardiac Fibrosis

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Cardiac fibrosis is characterized by the excessive deposition of extracellular matrix (ECM), mainly type I collagen and fibronectin. This accumulation increases myocardial stiffness and contributes to progressive cardiac dysfunction. Following myocardial infarction (MI), the activation of cardiac fibroblasts (CF) and local inflammation contribute to the development of adverse left ventricular remodelling and heart failure (HF). Despite its clinical relevance, current therapeutic options specifically targeting cardiac fibrosis remain limited. Nintedanib (NTB), a tyrosine kinase inhibitor that targets PDGF, FGF, and VEGF receptors, is currently approved for the treatment of idiopathic pulmonary fibrosis and other fibrotic conditions. However, its antifibrotic effects on cardiac tissue remain unclear.

The aim of this study was to evaluate the antifibrotic effects of NTB in a murine model of cardiac fibrosis by assessing its impact on fibrosis-related gene expression, ECM stiffness, and plasma biomarkers of cardiac injury, in order to determine whether NTB exerts protective or deleterious effects on injured cardiac tissue.

The antifibrotic potential of NTB was evaluated in a murine model of MI-induced cardiac fibrosis. MI was induced in male C57BL/6J mice by temporary ligation of the left anterior descending (*LAD*) coronary artery. NTB (52.7 mg/kg) or vehicle (drinking water) was administered orally and daily for 14 days post-infarction. At the study endpoint, cardiac tissue was harvested to assess mRNA expression of fibrosis-related genes (*Col1a1*, *Col3a1*, *Fn1*, *Acta2*, *Mmp9*, *Timp1*, *Tgf-β1*, *Cdh2*, and *Lox*) using RT-qPCR. ECM stiffness was estimated by calculating the *Col1a1/Col3a1* ratio. Plasma levels of cardiac injury biomarkers (Troponin I, BNP, and Galectin-3) were also quantified. Statistical analyses were conducted to identify significant changes between the treatment groups.

NTB treatment did not attenuate cardiac fibrosis; instead, it significantly upregulated ($p \leq 0.05$) the relative mRNA expression of *Col1a1* (36.9±13.2 NTB *vs.* 11.9±4.1 vehicle), *Fn1* (49.8±16.0 NTB *vs.* 9.4±2.8 vehicle), *Timp1* (136.8±59.4 NTB *vs.* 17.5±4.5 vehicle) and *Lox* (118.1±35.4 NTB *vs.* 23.9±8.3 vehicle). Additionally, ECM stiffness (1.5±0.1 NTB *vs.* 0.9±0.1 vehicle) and plasma Troponin I levels (9.2±2.0 NTB *vs.* 4.0±1.3 vehicle) were elevated with NTB treatment, suggesting that the drug exacerbated myocardial injury. These findings indicate that, in the context of ischemia-induced cardiac damage, NTB may exert deleterious effects rather than protective effects. Instead of mitigating fibrosis, NTB appeared to promote fibrotic remodeling, increase matrix rigidity, and potentially contributing to the progression of HF.

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New approaches to drug design, development and administration

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Functional Impact of CaV1.2 and CaV1.3 on Neurite Outgrowth and Oxidative Stress in Differentiating F11 Cells

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Voltage-gated calcium channels (VGCCs), particularly the L-type subtypes CaV1.2 and CaV1.3, are critical regulators of neuronal differentiation in immortalized peripheral F11 neurons (1). Calcium influx through these channels activates intracellular signalling cascades involved in neurite outgrowth and excitability. We previously showed that, under basal conditions, CaV1.3 overexpression in F11 cells promoted a 20% increase in neurite outgrowth and significantly enhanced calcium influx, supporting its role in the acquisition of early neuronal traits (2).

The aim of the present study was to further investigate the roles of CaV1.2 and CaV1.3 during differentiation of F11 cells, focusing on their functional impact under differentiating conditions.

F11 cells were transfected with expression vectors encoding CaV1.2 or CaV1.3 and cultured under neuronal differentiation protocols. Neurite outgrowth was quantified by image analysis; calcium influx and reactive oxygen species (ROS) levels were assessed using fluorescence-based assays.

CaV1.3 overexpression under differentiation conditions significantly reduced neurite outgrowth by more than 50% ($p < 0.001$, ANOVA followed by Dunnett's post-hoc test) and calcium response by 30% ($p<0.001$, ANOVA followed by Dunnett's post-hoc test) but increased oxidative stress by 14% ($p < 0.001$, ANOVA followed by Dunnett's post-hoc test). In contrast, CaV1.2 overexpression resulted in only a mild reduction in neurite outgrowth, without significant changes in calcium influx or ROS production.

These findings reveal a context-dependent role for CaV1.3 in neuronal differentiation: while it promotes neurite extension in undifferentiated cells, its overactivation during differentiation impairs neurite growth and elevates oxidative stress. Altogether, the data underscores the importance of tightly regulated calcium signalling via L-type VGCCs for proper neuronal development.

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Molecular modeling and preclinical evaluation of novel pyrazole-core based Estrogen Receptor alpha modulators

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Estrogen receptors are druggable nuclear receptors to develop drugs with clinical relevance in endocrine cancers, osteoporosis, neurodegenerative or altered metabolism-associated diseases. *In silico* protein-ligand docking methodology has been successfully used to predict the affinity and bound pattern of ERα selective and full agonist 1,3,5-triaryl-4-alkyl-pyrazole. However, the 1,3,5-triaryl-pyrazole-core remains to be explored as a pharmacophore model to design and develop novel ERα modulators. In our work, *in silico* protein-ligand docking methodology was successfully used to predict the affinity and optimal binding pattern of unique full or partial agonists. The *in silico* and biological evaluation support the hypothesis that the location of hydroxyl groups in specific rings plays a decisive role to reach an optimal orientation of compound inside the ligand-binding-domain of human ERα, a molecular event which is critical for agonist activity. The best Glide scores were established for di-hydroxylated compounds that incorporated the two hydroxyl groups on the C-ring, or one hydroxyl group on the B-ring and another one on the C-ring. In addition, compounds incorporating a bromine or cyan group in the *para* position of the aromatic ring A, and hydroxyl groups on the B and C rings revealed optimal stabilization of the ligand-ERα complex. Then, unique pyrazole-core based compounds were synthesized for evaluation of biological effects on ERα positive cells. The relative efficacy of these compounds to induce ERα-dependent transcription and cell proliferation in human adenocarcinoma cell line varied from being a full to a partial agonist/antagonist. Notably, these compounds did not modify neither Androgen Receptor, Glucocorticoid Receptor-, STAT3-, nor STAT5-dependent transcription. The estrogenic activities of these compounds were inhibited by methyl-piperidino-pyrazole, an ERα-specific antagonist. Clinically relevant, ADME analyses predicted all criteria for these compounds to be considered drug candidates with optimal oral and brain bioavailability.

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Modulation of Cigarette Smoke-Induced Oral Mucosa Senescence via Pharmacological Nrf2 Activation: A Potential Strategy to Prevent Early Carcinogenic Progression

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Findings previously reported by our group suggest that chronic exposure to cigarette smoke promotes cellular senescence in the oral mucosa, potentially contributing to premalignant lesions development. This process involves upregulation of senescence markers (p16, p21) and pro-inflammatory cytokines (IL-6, IL-8) linked to the senescence-associated secretory phenotype (SASP) [1]. Nuclear factor erythroid 2–related factor 2 (Nrf2) is a key transcription factor regulating cellular redox homeostasis and signal transduction. It mitigates intracellular oxidative stress, delays cellular senescence onset, and prevents age-related diseases progression [2], making it a promising target to counteract senescence-related tissue damage [3]. This study evaluates the effect of Nrf2 activation on cellular senescence induced by cigarette smoke in human oral mucosa models.

Oral mucosa biopsies from 15 smokers and 14 non-smokers were analysed by RT-qPCR and immunohistochemistry for p16 and p21 gene and protein expression. Human oral keratinocytes (KOH), fibroblasts (FOH), and 3D oral mucosa models were exposed to 2% cigarette smoke extract (CSE) to induce senescence. Senescence markers p16 and p21 were assessed by RTqPCR

and Western blotting, β-galactosidase (β-gal) activity by flow cytometry, γH2AX by immunofluorescence, and IL-6 and IL-8 release by ELISA. The Nrf2 activator NK-252 was tested at 10^{-7}, 10^{-8}, and 10^{-9} M.

Smoker biopsies showed elevated p16 and p21 expression. CSE exposure significantly increased expression of p16, p21, IL-6, IL-8, γH2AX, and β-gal activity. NK-252 treatment significantly ($p<0.05$) reduced gene and protein expression of p16 and p21, attenuated DNA damage, suppressed IL-6 and IL-8 secretion, and lowered β-gal activity, indicating a broad anti-senescent effect.

Nrf2 activation by NK-252 counteracts cigarette smoke-induced senescence in human oral mucosa models, supporting its potential as a preventive strategy against the progression of premalignant oral lesions and early carcinogenic processes associated with tobacco exposure.

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The pharmacological characterization of the major metabolite of infigratinib clarifies clinically observed side-effects and opens repurposing opportunities

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Infigratinib is a pan-FGFR inhibitor that was acceleratedly approved by the FDA for FGFR2-positive cholangiocarcinoma; it was voluntarily withdrawn in 2024 due to difficulties in recruiting sufficient patients for a Phase 3 trial given the relative rarity of this cancer. Interestingly, the recent landmark Phase-2 PROPEL-2 clinical trial demonstrated clinically meaningful height-velocity gains in children with achondroplasia – opening an exciting repurposing opportunity for this drug [1]. Infigratinib is primarily metabolized by CYP3A4 into a major human metabolite, BHS697, that has similar *in vitro* binding affinities for FGFR1-3 compared to the parent drug; it is therefore considered pharmacologically equivalent. However, our machine learning (ML) predictions and follow up *in vitro* validation on isolated proteins revealed that the metabolite displays a different polypharmacology compared to the parent drug. Specifically, the parent drug promiscuously binds to several GPCRs with more potent affinities than the reported Cmax of the drug in human plasma; therefore, this activity could be modulating or exacerbating some of the side effects observed on patients taking infigratinib, such as decreased appetite. Moreover, our experimental validation using the Incucyte® real-time imaging platform –which provides continuous, high-content read-outs ideally suited to small-molecule screening– in two cholangiocarcinoma cell lines show that BHS697 achieved roughly two- to three-fold greater growth inhibition than the parent drug. Collectively, these results suggest that the metabolite could be a safer and more effective candidate to treat children with achondroplasia and be considered in future drug combinations in oncology. Overall, our results underline the importance of broadly characterizing the pharmacology of drug metabolites to maximize the uses of our pharmacopeia.

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Therapeutic activation of Nrf2 attenuates senescence markers in COPD

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Cellular senescence is increasingly recognized as a key driver in the pathogenesis and rogression of chronic obstructive pulmonary disease (COPD), contributing to impaired tissue repair, inflammation, and structural lung damage. The transcription factor Nrf2 regulates the expression of antioxidant and cytoprotective genes and has emerged as a potential therapeutic target to counteract senescence-associated cellular dysfunctions.
This study aimed to evaluate the role of Nrf2 activators in attenuating cigarette smoke extract (CSE)-induced senescence in epithelial cells derived from healthy donors and IPF patients.
Lung parenchyma samples were obtained from control subjects (n=33) and IPF patients (n=18) to assess the expression of key senescence markers. Furthermore, primary epithelial cells isolated from bronchial tissue were treated with Nrf2 activators following CSE-induced senescence.
Our findings demonstrate that senescence markers such as p16^INK4a, p21^CIP1, and telomere shortening are significantly upregulated in COPD lung tissue. Treatment with Nrf2 activators led to a reduction in the expression of p16, p21, β-galactosidase, γ-H2AX, and an increase in intracellular glutathione (GSH) levels. Moreover, Nrf2 activation attenuated the senescenceassociated secretory phenotype (SASP) and mitigated multiple detrimental effects of CSE exposure, including cell cycle arrest, reduced cell proliferation, and DNA damage.
In conclusion, Nrf2 activators effectively counteract multiple hallmarks of cellular senescence induced by oxidative stress, supporting their potential as therapeutic agents in mitigating senescence-related pathologies in COPD.

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Comparative Evaluation of Antitussive Efficacy of Traditional and Novel Drugs in a Guinea Pig Model of Cough

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Cough is a common protective reflex aimed at clearing the airways. However, when prolonged, it may indicate underlying pathology and significantly impact patients' quality of life. Current antitussives such as codeine and dextromethorphan have limited utility due to adverse effects. Novel agents like gefapixant, a selective P2X3 antagonist, may offer improved therapeutic alternatives. Preclinical models such as guinea pigs provide valuable insight into antitussive efficacy.To compare the antitussive effects of traditional (codeine, dextromethorphan) and novel (cloperastine, levodropropizine, gefapixant) drugs in a citric acid-induced guinea pig cough model, using both behavioral and acoustic analysis.

Dunkin Hartley guinea pigs (200–250 g) were divided into 13 groups: a saline control group, a citric acid (0.4 M) challenge group, and multiple treatment groups pre-treated with test drugs at various doses (oral gavage) prior to citric acid exposure. Thirty minutes post-treatment, animals were exposed to aerosolized citric acid for 7 minutes. Coughs were recorded and analyzed acoustically (onset, count, power spectral density). Histological analysis of tracheal tissue was performed post-experiment. Statistical analysis used ANOVA with Bonferroni post hoc test ($p < 0.05$).

Citric acid significantly increased cough frequency (mean 24.5 ± 3). Codeine (at 6-24 mg/kg), cloperastine (at 6-24 mg/kg) and gefapixant (at 12–24 mg/kg) reduced cough counts and delayed onset significantly ($p < 0.05$). Levodropropizine (32 mg/kg) and dextromethorphan (72mg/kg) did not produce significant effects. Acoustic analysis revealed attenuation in signal intensity and spectral power with effective treatments. No histological damage was observed post-exposure.

This study demonstrates that codeine, cloperastine, and gefapixant exhibit significant antitussive effects in a guinea pig model. Gefapixant, in particular, shows promise as a non-opioid alternative. Our findings support the use of acoustic and behavioral parameters for preclinical antitussive evaluation and highlight the potential of novel agents for chronic cough management.

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Setting up a FOLFOX-treated rat model to evaluate the short- and medium-term adverse effects of chemotherapy: preliminary results.

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Cancer survival has increased in the last few decades thanks to the improvement in diagnostic and therapeutic approaches. Among treatments, cancer chemotherapy is key, but it may produce severe adverse effects not only during treatment, but also after it has finished, causing sequels that may affect different organs and systems, such as the peripheral nervous system or the brain-gut axis. Thus far, most animal models have used antitumoral drugs given singly to study chemotherapy-induced adverse effects, whereas most often cancer patients receive combinations of two or more drugs as a standard regimen. For example, colorectal cancer (CRC) patients may receive FOLFOX, a combination of 5-fluorouracil (5-FU) + oxaliplatin, drugs that cause mucositis/diarrhoea and neuropathic pain, respectively as characteristic adverse effects. Our aim is to study the short-/medium-term effects of FOLFOX on general health and behavioural parameters, including nociceptive thresholds, in a preclinical animal model. We used young male Wistar rats (250-300 g), which received FOLFOX (previously shown to reduce tumour growth in a CRC liver metastatic rat model[1]): oxaliplatin (6.1 mg/kg, i.p.) on day 1 and 5-FU (71.4 mg/kg, i.p.) with leucovorin (14.3 mg/kg, i.p.) on day 1 and 2. Control rats received the corresponding vehicles. Body weight, food and water intakes and general health (including the occurrence of diarrhoea) were recorded regularly along 6 weeks. In cohort 1, exploratory behaviour and anhedonia were evaluated using the hole-board test and the splash test, respectively. In cohort 2, nociceptive thresholds were recorded using the von Frey test (to detect mechanical tactile allodynia), the plantar test (to detect heat tactile hyperalgesia) and the PAM test (to detect mechanical muscle hyperalgesia), and locomotor activity was evaluated with an actimeter. Compared with controls, in which no mortality was detected, in FOLFOX-treated rats, 12.5% rats died during the second week after treatment. In the remaining FOLFOX-treated rats, compared with control rats, body weight gain and food intake (but not water intake) decreased during the first week after treatment, but both parameters recovered afterwards. Signs of diarrhoea were observed early after FOLFOX, but not in control rats. Compared with the control group, in FOLFOX-treated rats, exploration of the central area of the hole-board test, was reduced at 1, 2 and 6 weeks after treatment, suggesting some level of anxiety, whereas signs of painful neuropathy (tactile allodynia) were apparent at week 2 (without a reduction in spontaneous locomotor activity in the actimeter), but not at weeks 1 or 6 after treatment. No signs of tactile mechanical hyperalgesia or anhedonia were apparent at weeks 1, 2 or 6. Our preliminary results in male young rats suggest that FOLFOX causes several of the typical short-/medium-term adverse effects (body weight loss, anorexia, diarrhoea, painful neuropathy, anxiety) that affect cancer patients. Our model is ready to be used for the study of FOLFOX impact on specific organs and systems, like the brain-gut axis.

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The antiretroviral drug Rilpivirine interferes with the expression of mitoDEGs during TGF-β-induced hepatic stellate cell activation

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Background: The transdifferentiation of hepatic stellate cells (HSCs) from a quiescent state to a myofibroblast-like phenotype (activated HSCs, or aHSCs) is a key event in the liver's response to injury through liver fibrosis. This transformation is energetically demanding, making mitochondrial reprogramming a critical component. Our group previously demonstrated that the anti-HIV drug Rilpivirine (RPV) exerts hepatoprotective effects in animal models of chronic liver injury. It the context of its antifibrotic action in vitro, RPV inhibits the process of TGF-β-induced activation of HSC impeding the alterations of mitochondrial function and morphology. This work aimed at a large-scale assessment of the effect of RPV on the expression of genes that encode mitochondrial proteins in cells treated with TGF-β [1].

Material and Methods: We used primary human HSCs obtained from hepatic resections (donors form Hospital Clínico de Valencia) activated with TGF-β (2.5 ng/mL) and co-treated with clinically relevant concentrations of RPV (4 or 8µM) for 48h. RNA sequencing was performed at the core research facility at Universitat de València. cDNA libraries were quantified by fluorimetry, their size verified using the Agilent Bioanalyzer and an equimolar pool of all samples was sequenced on the NGS NextSeq 550 platform (Illumina) using single-read sequencing for 75 cycles (1x75 bp). The bioinformatic analysis was carried out by CIBERehd and DESeq2 package (R software) was used on raw counts to identify differentially expressed genes (DEGs).

Results: mRNA sequencing analysis resulted in the generation of expression data for 13156 protein-coding sequences. Mitochondria-related genes were identified among the DEGs using the MitoCarta 3.0 database. Altered expression of mitoDEGs was detected in cells exposed to TGF-β, with 139 up- and 199 down-regulated genes. Co-treatment with RPV vs TGF-□ alone led to significant changes in 90 mitoDEG (4µM RPV) and 186 mitoDEG (8µM RPV). Notably, a significant portion (31 in 4µM and 80 in 8µM, and 25 shared between both concentrations) of these genes displayed reversed expression compared to TGF-□. The mitoDEGs that stand out are FK506-binding protein 10 (FKBP10) and mitochondria-associated myosin 19 (MYO19) whose expression is upregulated by TGF-β and reversed upon co-treatment with RPV4 or RPV8. Investigation into the involvement of mitoDEG common for both RPV concentrations in mitochondrial pathways revealed essential processes such as the "Mitochondrial central dogma", "Metabolism", and "OXPHOS" (upregulated), and "Protein import, sorting and homeostasis", "Signaling", and "Mitochondrial dynamics and surveillance" (down-regulated).

Conclusions: These findings shed light on the mechanisms of RPV's action as an antifibrotic agent, offering insights into novel therapeutic targets for liver fibrosis in HSCs which is of interest in repurposing of existing drugs like RPV.

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Metabolic and immune effects of the LXR agonist T0901317 in a mouse model of diet-induced NAFLD

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Non-alcoholic fatty liver disease (NAFLD) represents a growing global health burden, characterized by increasing prevalence, lack of approved pharmacological treatments, and high mortality linked to cardiovascular complications. Liver X receptors (LXRs) are nuclear receptors that regulate lipid metabolism, inflammation, and vascular function[1]. In this study, we investigated the impact of the synthetic LXR agonist T0901317 on hepatic histology and immune cell profiles in a diet-induced mouse model of NAFLD.

Male C57BL/6 mice were fed a control or NAFLD-inducing diet[2]. After 8 weeks, NAFLD mice received T0901317 (25 mg/kg/day) or vehicle via subcutaneous osmotic minipumps for 28 days. Liver steatosis was evaluated by H&E staining and measurement of hepatic triglyceride levels. Leukocyte subsets in blood and liver were analysed by flow cytometry. Data are presented as mean±SEM (n=6–9), and statistical significance was evaluated using unpaired two-tailed Student's t-test.

Decreased hepatic fibrosis was found in T0901317-treated NAFLD mice vs vehicle. Moreover, it significantly increased liver triglyceride content (235.8±8.1 vs 162.8±8.8 mg/g, $p<0.001$) and liver weight (14.5±0.7% vs 8.6±0.8% of body weight, $p<0.001$). Epididymal white adipose tissue (eWAT) weight decreased (0.7±0.08% vs 1.2±0.06% of body weight, $p<0.001$). Neutrophil counts rose in both blood ($2.21±0.49×10^9$ vs $1.65±0.37×10^9$ cells/L) and liver (5.33±0.78% vs 2.49±0.68% of viable hepatic cells) following T0901317 administration.

The LXR agonist T0901317 seems to exert hepatic anti-fibrotic effects while increasing hepatic steatosis in a NAFLD mouse model, possibly via lipid redistribution from eWAT. Increased hepatic neutrophils suggest an immunomodulatory effect. This study provides insight into how LXR pathway modulation influences NAFLD progression, advancing our understanding of the metabolic–immune interplay as a basis for future biomarkers or combinatory therapies.

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Differential activation of the JAK/STAT pathway in ulcerative colitis: analysis of the inflammatory response and sex-based variability

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Ulcerative colitis (UC) is an inflammatory bowel disease characterized by an aberrant immune response. One of the pathways involved in this response is the JAK/STAT pathway. However, its exact role is not fully understood, which may explain the variable response to drugs that target JAK proteins.

This research aimed to analyze the activation of the JAK/STAT pathway in the colonic tissue of patients with UC. It also sought to identify potential differences between male and female patients. Sixty-one adult patients diagnosed with active ulcerative colitis (Mayo Endoscopic Score > 0) and undergoing routine colonoscopy were enrolled in a prospective, observational study conducted at a single hospital. During these procedures, biopsies were taken from both inflamed and non-inflamed areas of the colon. These samples were processed by chemical and mechanical digestion and subsequently analyzed by Western blot to measure the phosphorylation of the following proteins: JAK1, JAK2, JAK3, TYK2, STAT1, STAT3, and STAT4.

The results showed that JAK2, JAK3, TYK2, STAT1, STAT3, and STAT4 had higher phosphorylation levels in inflamed tissue. However, JAK1 did not show a significant difference. Additionally, some proteins, such as JAK2, JAK3, TYK2, and STAT3, appeared to be activated together, suggesting they may work in a coordinated manner. Male patients also showed higher activation of JAK2 and STAT3 compared to females.

In conclusion, the study highlights the diverse involvement of the JAK/STAT pathway in UC. It also emphasizes the importance of individualized assessment, including sex-based differences, to improve treatment strategies.

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Involvement of Peptidylprolyl Isomerase C in Liver Fibrosis: New Findings in Patients with Chronic Liver Disease

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Several members of the cyclophilin family are related with metabolic dysfunction-associated steatotic liver disease (MASLD). One of them is the peptidylprolyl isomerase C (PPIC), which is located in the endoplasmic reticulum (ER) and involved in the redox homeostasis and protein folding. It is overexpressed in the liver of animal models and in plasma of patients with chronic liver disease (CLD). Here we investigated the role of PPIC in CLD progression, its association with liver fibrosis, and its potential as a plasma biomarker.

PPIC expression was evaluated by RT-qPCR and Western blot *in vitro* (LX-2 cells and primary human hepatic stellate cells, both treated with TGF-β1) and in the liver of CCl_4-induced mouse model (4 weeks). In human plasma, PPIC levels (pg/mL) were determined by ELISA in three independent cohorts: 1) patients with CLD of different etiologies (n=44) and healthy controls (n=20); 2) MASLD patients (n=149); and 3) patients with type 2 diabetes (n=35) and healthy controls (n=19).

PPIC mRNA and protein levels were significantly increased in all the preclinical models of liver injury. In cohort (1), CLD patients displayed higher PPIC plasma levels compared to healthy individuals, showing similarities between the different etiologies (metabolic, alcoholic and HCV). Cirrhotic patients registered the highest PPIC levels, and there was a positive correlation between PPIC and AST or FIB-4, which suggests a relationship with the disease severity. In the MASLD cohort, patients with fibrosis grade F2-F4 presented an increase in their PPIC plasma levels independently of the NAS score, steatosis, or inflammation which evidences the relation of PPIC and fibrosis, and not liver injury in general. In the T2DM cohort, only diabetic patients with FIB-4≥1.3 had higher levels of PPIC in plasma, compared to healthy individuals, regardless of the glycemic control, reinforcing the specificity of PPIC as a biomarker of liver injury. Also, overall PPIC levels showed a positive correlation with age.

Across all cohorts, PPIC levels displayed a strong correlation with fibrosis stage (assessed as FIB-4) suggesting its potential as fibrosis-progression biomarker and therapeutic target in CLD.

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BP-2 as a leading pan-PPAR agonist for alleviating liver fibrosis in metabolic dysfunction-associated steatohepatitis

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Metabolic dysfunction-associated steatohepatitis (MASH) is characterized by hepatic lipid accumulation and lobular inflammation, often progressing to fibrosis. The associated proinflammatory milieu appears to play a central role in the pathogenesis of MASH and its complications. However, effective pharmacological therapies remain unavailable. BP-2, a pan-PPAR agonist synthesized by our group, has previously been shown to exert metabolic benefits and attenuate inflammation in a murine model of metabolic dysfunction. In this preliminary study, we assessed the therapeutic potential of BP-2 in ameliorating steatosis, fibrosis, and systemic inflammation in a murine model of MASH.

Six-week-old C57BL/6 mice were fed either a standard chow diet or a choline-deficient, high-fat diet for 12 weeks to induce hepatic features of MASH. From week 8 onwards, some MASH animals received subcutaneous administration of BP-2 (3 mg/kg/day) via osmotic pumps for 4 weeks. Then, blood samples were collected and liver sections were analyzed histologically using hematoxylin-eosin and picrosirius red staining to assess steatosis and fibrosis, respectively. Flow cytometry was used to assess circulating leukocyte activation and perform hepatic immune cell immunophenotyping following liver tissue digestion

While BP-2 had no observable effect on hepatic steatosis, it significantly reduced liver fibrosis by 24.3% in MASH mice. Peripheral blood analysis revealed no significant changes in leukocyte counts or activation, although BP-2 significantly reduced eosinophil and increased regulatory T-cell infiltration in the liver.

BP-2 may be a potential therapeutic candidate for reducing liver fibrosis in MASH, a condition without current pharmacological treatment. However, further transcriptomic, proteomic, and histological analyses are needed to explore the underlying mechanisms and signaling pathways.

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GPR84, which is expressed in intestinal macrophages, is upregulated in Ulcerative Colitis patients: Relevance on Macrophage Cytokine Profile

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Inflammatory Bowel Disease (IBD), which includes Ulcerative Colitis (UC) and Crohn's Disease (CD), is marked by a persistent and dysregulated immune response affecting the gastrointestinal tract. GPR84 has recently gained attention as a proinflammatory receptor involved in several inflammatory diseases. However, its contribution to IBD´s pathophysiology remains unclear. This study investigates GPR84 expression in intestinal tissues of IBD patients and evaluates its role in the cytokine secretion of macrophages.

Surgical intestinal resections were obtained from UC (n=5), CD patients (n=5) and healthy colon and ileum from colon cancer patients were used as non-IBD controls (n=10). Protein levels of GPR84 and the macrophage marker CD68 were analysed by Western Blot and immunohistochemistry. U937 monocytes were differentiated into macrophages using PMA (0.01µM, 48 h) and treated with decanoic acid (50–250µM) as a GPR84 agonist, in combination with a proinflammatory stimulus (LPS 0.1µM + IFNγ 20 ng/mL) for 24 hours. Some macrophages were co-treated with a selective GPR84 antagonist (10µM) or transfected with a specific siRNA against GPR84. Gene expression of inflammatory cytokines was measured by qPCR, while protein secretion was quantified in supernatants via Luminex assays. Statistical analysis was performed using t-tests or ANOVA, with significance set at $p<0.05$.

GPR84 protein expression was significantly higher in UC tissue compared to colonic control samples (150.3±11.75 vs 100.0±11.85), whereas no differences were found in CD patients versus ileal control samples. Immunohistochemical analysis showed slight GPR84 staining in epithelial cells, in parallel with a strong staining in cells of the lamina propria. Double immunohistochemistry confirmed that GPR84 is expressed in intestinal CD68+ macrophages in both UC and CD patients. In vitro, U937-derived macrophages treated with decanoic acid showed increased expression of IL1β (2.68±0.86) and IL8 (2.44±0.48) vs vehicle-treated macrophages (0.998±0.23 and 1.05±0.17, respectively), which was reinforced by elevated cytokine levels in the supernatants from these cells. Inhibition of GPR84 via antagonist treatment significantly reduced the expression of IL1β (0.99±0.29) vs vehicle-treated macrophages (1.94±0.26) and siRNA silencing significantly reduced the expression of IL8 (0.89±0.25) vs siCtrl macrophages (4.02±1.59).

GPR84 is overexpressed in the intestinal tissue of UC patients and is localized in intestinal macrophages. Its activation enhances proinflammatory cytokine production supporting its proinflammatory role in these cells, which point this receptor as a novel therapeutic target for UC patients.

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Different Intestinal Mycobiome Profiles in Surgical Samples from IBD Patients: Relevance on complicated-Crohn's Disease Phenotypes

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Inflammatory bowel disease (IBD), encompassing ulcerative colitis (UC) and Crohn's disease (CD), is a chronic condition marked by recurrent gastrointestinal inflammation and potential complications. While bacterial dysbiosis in IBD has been extensively studied, the contribution of the intestinal fungal community remains underexplored, particularly in surgically resected tissues. This study aims to investigate the intestinal mycobiome in surgical resections from IBD patients.

Fungal profiling was conducted on surgical specimens from 20 UC and 30 CD patients, using non-IBD ileal and colonic resections as controls. ITS regions were amplified from extracted fungal DNA and sequenced. Data were processed via DADA2, and statistical analyses were carried out using R. Multivariate analysis were assessed with Adonis tests, while richness and diversity metrics were analyzed using Wilcoxon tests. Visualization was performed through constrained correspondence analysis (CCA), and differential taxonomic abundance was evaluated using ANCOMBC2. FDR-adjusted p-values were used throughout. Correlations with bacterial communities were examined via Spearman analysis. Results were expressed as mean ± SEM, with significance defined at $p < 0.05$ using appropriate parametric and non-parametric tests.

UC resections displayed limited shifts in fungal richness and diversity compared to colonic controls, whereas CD samples showed significant changes at the genus level. Notably, Malassezia, especially *Malassezia globosa*, was enriched, while *Yarrowia lipolytica* was markedly reduced in CD versus ileal control tissues. UC samples displayed predominantly positive correlations among fungal taxa, with no statistically significant negative interactions, whereas CD resections exhibited fewer, weaker, and sometimes negative correlations. Interkingdom analysis revealed distinct bacterial-fungal interaction patterns. *Dothideomycetes unclassified* was a key taxon in UC correlations, while CD samples exhibited the loss of ileal control-associated negative interactions and emergence of new associations. In addition, comparative analysis across CD phenotypes revealed significantly higher levels of *Cryptococcus pseudolongus* and *Cladosporium unclassified* in B3 (penetrating) over B2 (stricturing) phenotypes. Conversely, *Dothideomycetes unclassified* was more abundant in B2. ROC analysis identified the fungal species *Dothideomycetes unclassified* (AUC = 0.823) and Cladosporium unclassified (AUC = 0.737) capable of distinguishing phenotypes. XLII Annual SEF Meeting. Valencia, September 10-12, 2025

This study offers the first comprehensive characterization of the intestinal mycobiome in surgical resections from both UC and CD patients. CD resections showed marked alterations in *Malassezia* and *Yarrowia* abundance. Notable fungal-bacterial associations were revealed, underscoring complex interkingdom dynamics. Importantly, the data suggest that distinct fungal taxa, particularly *Dothideomycetes* and *Cladosporium*, may serve as discriminative biomarkers in complicated-CD phenotypes.

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Activity of angiotensin-converting enzyme in the gut and feces of diabetic rats and the effect of endogenous angiotensin II and aldosterone

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Diabetes induces gastrointestinal complications that impact patients' quality of life. We recently described the activity of angiotensin-converting enzymes (ACE) in rat intestinal content, which was reportedly higher than the corresponding intestinal tissue (1). This study investigated intestinal ACE activity in streptozotocin (STZ)-induced diabetic rats and evaluated the effects of the angiotensin II type 1 receptor (AT1R) blocker, losartan, and the mineralocorticoid receptor antagonist, finerenone. Type 1 diabetes was induced in adult male Wistar rats via a single STZ injection (55 mg/Kg; i.p.). Diabetic rats were treated daily with losartan (20 mg/Kg; PO; STZ-LOS) or finerenone (10 mg/Kg; PO; STZ-FIN). Non-injected rats were used as controls (CTRL). After 14 days, jejunum, ileum and colon samples were collected, as well as the correspondent intestinal content, for assessment of systemic and local ACE activity through a fluorimetric assay using both Z-FHL and h-HL as substrates. The Mann-Whitney test was used to compared medians between groups. ACE-Z-FHL activity was higher in STZ rats than in controls in the colon (mmol/min/mg: STZ=15.13[7.50-19.28]; CTRL=5.07[4.32-7.53]; $p<0.05$), ileum (mmol/min/mg: STZ=21.51[11.78-39.31]; CTRL=6.62[4.26-9.51]; $p<0.05$) and jejunum (mmol/min/mg: STZ=44.56[19.90-83.82]; CTRL=24.91[7.44-34.41]; $p=0.0830$). ACE-h-HL activity was higher in STZ rats than in controls only in the jejunum (mmol/min/mg: STZ=13.05[7.81-18.59]; CTRL=3.63[0.47-6.65]; $p<0.01$). The ACE-Z-FHL/ACE-h-HL ratio was always higher than 1 and it was lower in STZ rats than in CTRL in the jejunum (STZ=3.52[2.28-5.91]; CTRL=8.02[5.26-11.85]; $p<0.01$). No differences were observed in the intestinal fecal contents. Losartan or finerenone did not affect ACE-Z-FHL or ACE-h-HL activities or ACE-Z-FHL/ACE-h-HL ratio in the intestinal tissue. However, paired analysis revealed that both ACE-Z-FHL and ACE-h-HL activities were higher in the intestinal fecal content than in the corresponding intestinal tissue across all segments of all experimental groups. The ACE-Z-FHL/ACE-h-HL ratio was similar between tissue and fecal content, except in the jejunum of CTRL (tissue=7.15[4.98-12.51]; fecal content=1.77[1.72-2.50]; $p<0.05$) and in the STZ+FIN group for the jejunum (tissue=8.10[3.22-12.80]; fecal content=2.25[1.75-2.44]; $p<0.05$), ileum (tissue=9.35[4.09-19.65]; fecal content=3.83[1.68-4.70]; $p<0.001$), and colon (tissue=9.02[4.84-18.87]; fecal content=3.92[1.82-5.56]; $p<0.05$). Taken together, these results suggest differential regulation of ACE activity between intestinal tissue and fecal content, warranting further investigation.

(1) Ferreira-Duarte M et al., 2023. doi: 10.1111/nmo.14598.