XLIII Annual Spanish Society of Pharmacology Meeting

Ilustre Colegio Oficial de Médicos de Madrid (ICOMEM)

Calle Santa Isabel, 51. 28012 Madrid

September 2-4, 2026

Book of Abstracts

SPONSORS:

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WELCOME

It is a pleasure to welcome you to the **XLIII Annual Meeting of the Spanish Society of Pharmacology**, which will take place at Ilustre Colegio Oficial de Médicos de Madrid (ICOMEM), in Madrid, Spain.

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 medicine, pharmacy, biomedical sciences and chemistry.

We have prepared a diverse and engaging scientific program that will cover cutting-edge topics in pharmacology and therapeutics, featuring renowned keynote speakers, insightful panel discussions, and innovative research presentations, including symposia, oral presentations, and poster sessions.

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. In order to encourage their participation through short research presentations, grants have been offered to help cover their expenses to attend the meeting.

The vibrant city of Madrid will provide an outstanding backdrop for this event. Its unique combination of historical heritage, cultural vitality, and modern infrastructure makes it an ideal place to host scientific exchange while also offering numerous opportunities for social interaction and networking.

Whether you are an established expert or a young researcher, we sincerely hope that this meeting will serve as a stimulating environment for sharing ideas, establishing new collaborations, and strengthening existing ones.

Luis Gandía / Concha Peiró / Carlos F. Sánchez Ferrer
Universidad Autónoma de Madrid
Organizing Committee of the XLIII Annual Meeting of the Spanish Society of Pharmacology

COMMITTEES

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Dolores Viña Castelao
Francisco Zaragozá García

CONGRESS TIME-TABLE

Day 1 02/09/2026	*Room 1*	*Room 2*
8:30-9:00	Delivery of documentation	
9:00-10:30	**Session 1:** Cardiovascular Pharmacology **Moderator:** Lucía Núñez (Universidade da Coruña) **Invited speaker:** Ricardo Caballero (Universidad Complutense de Madrid) "Reshaping the future of antiarrhythmic drugs: emerging therapeutic approaches for arrhythmias in heart failure ". **Selected oral communications:** Cristina González Correa (Univ. de Granada) Wilfrido Arrúa (Univ. Autónoma de Madrid). Oscar Lorenzo (Univ. Autónoma de Madrid) Iztzel Sofía Falla Moran (Fund. Universitaria de Ciencias de la Salud, Colombia)	**Session 2:** Young SEF Researchers Session "New Therapeutic Modalities". **Moderator**: Adrián Gironda (Universidad de Alcalá). **Invited speakers:** Lara García Varela (Instituto de Investigación Sanitaria de Santiago) Ferran José Duran (IRB Barcelona) Alfonso Alba Bernal (IdiPAZ/CNIO)
10:30-11:00	Coffee-break	
11:00-11:30	Official Opening	
11:30-12:30	**Invited Lecture 1: "**When blood vessels age: endothelial senescence and brain plasticity". **Moderator:** Mª Julia García Fuster (Universitat de les Illes Balears) **Invited speaker: Isabel Fariñas** (Universitat de València)	
12:30-13:30	**Round Table 1:** "Evaluation of technologies and access to innovation". **Moderator:** Francisco Zaragozá (Universidad de Alcalá de Henares) **Invited speakers:** Isabel Pieneros Andrés (Farmaindustria); Encarnación Cruz Marcos (Biosim); Mª Concepción Sánchez Monteo) (Consejo de Farmacéuticos de Castilla La Mancha)	
13:30-15:00	Lunch	
15:00-16:30	**Session 3:** Pain and Neural Pharmacology. **Moderator:** Raquel Abalo (Universidad Rey Juan Carlos) **Invited speaker:** Enrique Cobos (Universidad de Granada) "Digital Researchers: The Evolution of Preclinical Behavioral Pharmacology (With a Focus on Pain Research)"	**Session 4:** Natural products pharmacology **Moderator:** Carmen Montesinos (Universitat de València) **Invited speaker:** Marina Sánchez Hidalgo (Universidad de Sevilla) "Extra virgin olive oil and systemic lupus erythematosus prevention: Mechanistic insights and therapeutic potential"

	Selected oral communications: Amada Puerto-Moya (Universidad de Granada) Aitziber Mendiguren (Universidad del País Vasco) Neus Calza-Zamora (Universidad de Barcelona) Pedro Marín (Universidad de Murcia)	**Selected oral communications:** Javier Cano Lou (Universidad San Jorge) Jessica Bravo (Universidad Diego Portales; Chile) Ana María Sánchez-Tevar (Universidad de Málaga) Luis Rodríguez-Santos (Universidad de Santiago de Compostela)
16:30-17:30	**Session 5:** Pharmacogenetics and Personalized Medicine **Moderator:** Luis Gandía (Universidad Autónoma de Madrid) **Invited speaker:** Francisco Abad Santos (Universidad Autónoma de Madrid) "Clinical Implementation of Pharmacogenetic Biomarkers in Routine Practice: From Evidence to Personalized Prescribing" **Selected oral communications:** Luis Sendra (Universidad de València) Ángela Jiménez Ortas (Universidad de Granada)	**Session 6:** Teaching Innovation in Pharmacology **Moderator:** Josep Eladi Baños (Universitat de Vic) **Invited speaker:** Ricardo A. Peña Silva (Asociación Colombiana de Farmacología; ACF): "Innovación en educación en farmacología: metacognición e inteligencia artificial" **Selected oral communications:** Isabel García-Arnandis (Universidad de València) Inmaculada Bellido Estévez (Universidad de Málaga)
17:30-18:30	**Poster Session 1 (P1-P40)** Sala 3	
18:30-20:00	**Guided walking tour of Old Madrid** Starting point: ICOMEM (conference venue): at 18:30	
20:0-22:00	**Welcome Cocktail** La Corrala (C/ Carlos Arniches, 3, 5, Centro, Madrid)	

Day 2 03/09/2026	***Room 1***	***Room 2***
8:30-9:00	Delivery of documentation	
9:00-10:30	**Session 7:** Cardiovascular & Renal Pharmacology **Moderator:** Ana Briones Alonso (Universidad Autónoma de Madrid) **Invited speaker:** Mª Dolores Sánchez Niño (Hospital Universitario Fundación Jiménez Díaz; Universidad Autónoma de Madrid) "Pharmacological modulation of the kidney gerosuppressive function" **Selected oral communications:** Carla Maya López (Instituto de	**Session 8:** Pharmacology of Ion Channels **Moderator:** Ricardo Borges (Universidad de la Laguna)) **Invited speaker:** Antonio V. Ferrer Montiel (Universitas Miguel Hernandez) "TRP channels in chemotherapy induced peripheral neuropathy" **Selected oral communications:** Ricardo Caballero (Universidad Complutense de Madrid)

	Investigación Sanitaria Fundación Jiménez Díaz) Mª José Jiménez Aragón (Universidad de Granada) Isabel Sánchez Pérez (Universidad Autónoma de Madrid) Cristina Arce Recatalá (Universidad de Valencia)	Leandro Zúñiga (Universidad de Talca; Chile) Carmen Nanclares (Universidad Autónoma de Madrid) Pablo Aránguiz (Pontificia Universidad Católica de Valparaí so; Chile)
10:30-11:00	Coffee-break	
11:00-12:00	**Invited Lecture 2:** "From Bench to Patients: Redefining the Role of Academia in the Biomedical Innovation Pipeline". **Moderator:** Carlos F. Sánchez Ferrer (Universidad Autónoma de Madrid) **Invited speaker: César Romero** (Emory University; EEUU)	
12:00-13:30	**Round Table 2: "**Disruptive Therapeutic Innovation" **Moderator:** Antonio García García (Fundación Teófilo Hernando / Universidad Autónoma de Madrid) **Invited speakers:** Javier Milara (Universitad de Valencia; Hospital General Universitario Valencia) José Luis Díaz (Complejo Hospitalario Universitario A Coruña) Laia Bardolet (Almirall)	
13:30-15:00	Lunch	
15:00-16:30	**Session 9:** Digestive and Respiratory Pharmacology **Moderator:** Ana Mª Sahagún (Universidad de León) **Invited speaker:** Julio Gálvez (Universidad de Granada) "Bridging experimental research and clinical practice: Pharmacology of *Thymus serpyllum* extract (360GUT) in functional gastrointestinal disorders" **Selected oral communications:** Fernando de la Cuesta (Universidad Autónoma de Madrid) Patrice Marques (Universidad de Valencia) Carmen de la Fuente-Gómez (Universidad de Valencia) Juan José Enguix-Huete (Universidad de Granada)	**Session 10:** Pharmacology of Senescence & Aging **Moderator:** Concha Peiró (Universidad Autónoma de Madrid) **Invited speaker:** Guillermo Díaz-Araya (Universidad de Chile) "Cardiac fibroblast senescence and its impact on tissue remodeling and fibrosis: a pharmacological perspective" **Selected oral communications:** Jaime Rubio (Universidad de Castilla-La Mancha) Ana Cuadrado-Gómez (Universidad Autónoma de Madrid) Laura Rey (Universidad de Santiago de Compostela) Mabel Catalán (Universidad de Chile)
16:30-17:30	**SEF Assembly**	
17:30-18:30	**Poster Session 2 (P41-P79)** Sala 3	
21:00	**SEF Congress Dinner (prior reservation required)** Restaurante Zerain (Calle de Quevedo, 3, Centro, 28014 Madrid)	

Day 3 04/09/2026	***Room 1***	***Room 2***
8:30-9:00	Delivery of documentation	
9:00-10:30	**Session 11:** Neuropharmacolog **Moderator:** Dolores Viña Castelao (Universidade de Santiago de Compostela) **Invited speaker:** Manuela García López (Universidad Autónoma de Madrid) "NRF2 induction as a strategy for neuroinflammatory-related diseases" **Selected oral communications:** Victoria Maneu (Universidad de Alicante) María Álvarez-Rubal (Instituto Investigación Sanitaria Hospital Universitario la Princesa) Christian Griñán-Farre (Universidad de Barcelona) Abraham B Torregrosa (Universidad Miguel Hernández)	**Session 12:** Pharmacology of Inflammation **Moderator:** Mª Jesús Sanz (Universitat de València) **Invited speaker:** Mª Angeles Moro (Centro Nacional de Investigaciones Cardiovasculares, CNIC) "Immunothrombosis, a central mechanism in cerebrovascular disease" **Selected oral communications:** Elena Domingo (Instituto de Investigación Sanitaria-INCLIVA) Mª Carmen Carceller (Universidad de Valencia) Álvaro Gómez-Martín (Instituto de Investigación Sanitaria – INCLIVA) Alicia Villacampa (Instituto de Investigación Hospital Universitario La Paz – IDIPAZ)
10:30-11:00	Coffee-break	
11:00-12:00	**Invited Lecture 3:** "Advanced Therapies Based on the Genetic Modification of Human Hematopoietic Stem Cells" **Moderator**: Antonio Rodríguez Artalejo (Universidad Complutense de Madrid) **Invited speaker**: **Juan Antonio Bueren** (Centro de Investigaciones Energéticas Medioambientales y Tecnológicas; CIEMAT)	
12:00-12:45	**SEF Young Investigator Award and Lifetime Achievement Award in Pharmacology**	
12:45-13:00	**Closing ceremony and remarks**	


XLIII CONGRESO DE LA SEF
XLIII Annual Meeting of Spanish Society of Pharmacology

ABSTRACTS

INVITED LECTURES

When blood vessels age: endothelial senescence and brain plasticity

Isabel Fariñas

[1]*Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED),* [2]*Departamento de Biología Celular y Biología Funcional, 3Instituto de Biotecnología y Biomedicina (BioTecMed), Universidad de Valencia, 46100 Burjassot, Spain.*

E-mail: Isabel.farinas@uv.es

Aging is characterized by the progressive decline of tissue function, leading to loss of homeostasis, increased frailty, and a higher incidence of chronic diseases. Among the biological hallmarks of aging, the accumulation of senescent cells has emerged as a major driver of tissue dysfunction. Cellular senescence is triggered by diverse forms of stress, including DNA damage, oxidative stress, mitochondrial dysfunction, and telomere attrition, resulting in a stable cell-cycle arrest accompanied by profound metabolic and secretory changes. Although senescent cells no longer proliferate, they remain metabolically active and develop a senescence-associated secretory phenotype (SASP), which promotes chronic inflammation and disrupts tissue homeostasis by altering the function of neighboring cells. The central nervous system is particularly vulnerable to aging, with progressive impairment of brain plasticity contributing to cognitive decline and increased susceptibility to neurodegenerative disorders. While the accumulation of senescent cells in the aging brain is increasingly recognized, the mechanisms by which cellular senescence influences brain function remain poorly understood. In particular, little is known about how senescent cells within the brain vasculature alter the cellular and molecular interactions that support lifelong brain plasticity.

Our research aims to understand how cellular senescence impacts brain plasticity, with a particular focus on the neurovascular niche and adult neural stem cells. We investigate how senescent endothelial cells modify the local microenvironment of neurogenic niches through inflammatory and extracellular signals, and how these changes affect neural stem cell quiescence, activation, and regenerative capacity. By identifying the mechanisms through which senescence disrupts the communication between the vascular niche and neural stem cells, we seek to define how aging compromises adult neurogenesis and limits the brain's capacity for adaptation and repair. A better understanding of the interplay between cellular senescence and brain plasticity will not only provide fundamental insights into the biology of brain aging but may also uncover new therapeutic opportunities to preserve cognitive function during aging. In parallel, advances in the development of sensitive tools to detect senescent cells *in vivo* will facilitate the evaluation of senescence-targeting interventions and accelerate their translation into clinical applications.

From Bench to Patients:
Redefining the Role of Academia in the Biomedical Innovation Pipeline

Cesar A. Romero, MD, PhD,

Department of Medicine, Emory University-School of Medicine,
Atlanta, GA, USA.
Treasurer, International Society of Hypertension

E-mail: cesar.romero@emory.edu

Over the past decade, we have witnessed a true revolution in the development of innovative therapies for cardiovascular disease, cancer, and other major disorders.
Traditionally, biomedical innovation followed a well-defined model: academia generated the fundamental discoveries, while industry was responsible for their development and commercialization. However, this paradigm is changing. As academic research faces increasingly longer timelines and growing funding pressures, industry has expanded its role in the earliest stages of drug discovery, driven by emerging technologies, large-scale data, and artificial intelligence. Novel therapeutic strategies, biotechnology platforms, and AI have the potential to accelerate drug discovery at an unprecedented pace, fundamentally transforming how biomedical research is translated into patient care.

This transformation raises a fundamental question: What is the role of academia in today's biomedical innovation ecosystem?

In this lecture, we will review some of the most important recent therapeutic advances in cardiovascular medicine as examples of successful innovation and examine how these technologies evolve from an initial scientific idea to clinical implementation. We will discuss the key stages of the research and development (R&D) process, opportunities to strengthen academia's contribution, the impact of artificial intelligence, and new models of collaboration among universities, hospitals, and industry. The goal is to stimulate discussion on how to prepare the next generation of scientists to lead the future of biomedical innovation.

Advanced Therapies Based on the Genetic Modification of Human Hematopoietic Stem Cells

Juan A. Bueren

CIEMAT, CIBER de Enfermedades Raras e IIS Fundación Jiménez Díaz

E-mail: juan.bueren@ciemat.es

The development of viral vectors has allowed gene therapy to become a viable therapeutic option for numerous hereditary and acquired diseases. In recent years, we have witnessed the beginning of a new era in the history of medicine, in which several *ex vivo* and *in vivo* gene therapies are reaching maturity. Evidence of this is the progressive commercial approval of gene therapy medicines for monogenic diseases and cancer. For several decades, our laboratory developed new gene therapy tools based on the genetic correction of hematopoietic stem cells (HSCs) for the treatment of a variety of monogenic diseases affecting blood cells, mainly congenital anemias and primary immunodeficiencies.

In this presentation, I will focus on advances achieved in the gene therapy for patients with Fanconi anemia subtype A (FA-A) and congenital immunodeficiencies, particularly leukocyte adhesion deficiency type I (LAD-I). Approximately 80% of patients with FA develop bone marrow failure during the first decade of life, resulting in severe complications associated with bone marrow aplasia. In the case of severe LAD-I, patients suffer from infections which seriously compromises their survival in their early years. The therapeutic approach for both conditions is similar and based on the ex vivo genetic correction of the patients' hematopoietic stem cells (HSCs) using lentiviral vectors developed in our laboratory. Once the genetic defect is corrected, the HSCs are reinfused into the patients. While patients with LAD-I receive a pre-infusion conditioning with busulfan to facilitate the engraftment of the corrected cells, patients with AF do not undergo any pre-conditioning, given that our preclinical studies had previously demonstrated the proliferative advantage of the corrected HSCs.

The academic clinical trial in FA-A patients, initially sponsored by the Fundación Hospital Universitario Niño Jesús, demonstrated for the first time the engraftment and even hematopoietic regeneration of FA patients treated with gene therapy. Following these initial clinical studies, both the FA and LAD-I gene therapies were licensed to Rocket Pharmaceuticals, which then sponsored international clinical trials that confirmed the efficacy of both therapies. In March of this year 2026, the US FDA granted marketing authorization to Rocket Pharma for the medicinal product KRESLADI (genetically corrected HSCs from patients with LAD-I) for the treatment of patients with severe LAD-I, marking the first commercial approval of a gene therapy drug designed entirely in Spain. Currently, Rocket Pharma and our advanced therapies consortium continue to collaborate closely to facilitate access to new therapies for patients with monogenic diseases like LAD-I and FA.

References:

- *Haematopoietic gene therapy of non-conditioned patients with Fanconi anaemia-A: results from open-label phase 1/2 (FANCOLEN-1) and long-term clinical trials.* Río P …. Sevilla J, Bueren JA. Lancet. 2025 Dec 21;404(10471):2584-2592
- *Lentiviral Gene Therapy for Severe Leukocyte Adhesion Deficiency Type 1.* Booth C, Sevilla J, Almarza E, …. Bueren JA, Schwartz JD, Kohn DB. N Engl J Med. 2025 May 1;392(17):1698-1709

ROUND TABLES

Round Table 1: Evaluation of technologies and access to innovation

Moderator: Francisco Zaragozá
(Universidad de Alcalá de Henares)

Invited speakers:

Isabel Pieneros Andrés
(Farmaindustria)

Encarnación Cruz Marcos (Biosim)

Mª Concepción Sánchez Montero
(Consejo de Farmacéuticos de Castilla La Mancha)

Round Table 2: Disruptive Therapeutic Innovation”

Moderator: Antonio García García
(Fundación Teófilo Hernando; Universidad Autónoma de Madrid)

Invited speakers:

Javier Milara (Universidad de Valencia; Hospital General Universitario Valencia)

José Luis Díaz (Complejo Hospitalario Universitario A Coruña)

Laia Bardolet (Almirall)

ORAL SESSIONS

Session 1: Cardiovascular Pharmacology

Moderator:

Lucía Núñez (Universidade da Coruña)

Invited speaker:

9:00-9:30 Ricardo Caballero (Universidad Complutense de Madrid)
Reshaping the future of antiarrhythmic drugs: emerging therapeutic approaches for arrhythmias in heart failure

Selected oral communications:

9:30-9:45 Cristina González Correa (Univ. de Granada)
Adjunctive administration of Limosilactobacillus fermentum CECT5716 amplifies the blood pressure-lowering effects of amlodipine in spontaneously hypertensive rats

9:45-10:00 Wilfrido Arrúa (Univ. Autónoma de Madrid).
Protective effects of new SGK1 inhibitors on angiotensin II-induced cardiovascular damage

10:00:10:15 Oscar Lorenzo (Univ. Autónoma de Madrid)
Dapagliflozin reduces proinflammatory and profibrotic mediators in human cardiomyocytes exposed to lipotoxic stress

10:15-10:30 Iztzel Sofía Falla Moran (Fundación Universitaria de Ciencias de la Salud, Colombia)
Intravenous Haloperidol in Older Adults: QT Risk Description and ECG Documentation

Reshaping the future of antiarrhythmic drugs: emerging therapeutic approaches for arrhythmias in heart failure

Ricardo Caballero, Teresa Crespo-García, Josu Rapún, Sara Pérez-Martín, Alba De la Iglesia, Luis Loya, Eva Delpón

Department of Pharmacology and Toxicology. School of Medicine. Universidad Complutense de Madrid. Instituto de Investigación Gregorio Marañón. CIBERCV, Instituto de Salud Carlos III, Madrid, Spain

E-mail: rcaballero@med.ucm.es

Sudden cardiac death (SCD) due to ventricular arrhythmias such as ventricular tachycardia and fibrillation accounts for around 50% deaths among patients with heart failure with reduced ejection fraction (HFrEF) despite the use of guideline-recommended therapies, posing a major unmet medical need. HFrEF leads to anatomical, histological, and electrical remodelling which generates an optimal substrate for severe arrhythmia genesis and maintenance. Electrical remodeling is mainly characterized by the downregulation of Nav1.5 and Kir2.1 channel expression at the cardiomyocyte sarcolemma and of their currents, the sodium (I_{Na}) and inward rectifier K (I_{K1}), which decreases ventricular excitability and prolongs repolarization, predisposing to malignant ventricular arrhythmias. Indeed, a widening of the QRS and lengthening of the QT intervals is observed in the electrocardiogram of most of HFrEF patients. Classical antiarrhythmic drugs (AAD), which mainly act by blocking Na^+, Ca^{2+}, and/or K^+ channels, have limited efficacy (recurrences are frequently observed) and safety (they can produce severe proarrhythmic effects). Importantly, they are no longer the treatment of choice for ventricular arrhythmias associated to HFrEF as they increase mortality because they further decrease cardiac excitability and lengthen repolarization. Over almost 40 years, our group has explored new mechanisms of pharmacological modulation of cardiac ion channels in an effort to develop safer and more efficacious antiarrhythmic strategies. This trajectory eventually led to the creation and coordination of the ARCADIA Consortium, devoted to the design and development of advanced therapies potentially useful in the prevention and treatment of arrhythmias associated with HFrEF. The overarching hypothesis was that conventional AADs based on ion channel blockers is no longer useful and, thus, we pursue the development of next-generation AAD based on small molecules (e.g. sodium-glucose cotransporter 2-SGLT2 inhibitors) and peptide-based therapies to simultaneously increase I_{Na} and I_{K1} acting at different levels of the channel biology: i.e., protein transcription and trafficking, ion channel biophysical properties, etc. If successful, these novel therapies could lead to a paradigm shift in the field, resulting in the identification of innovative antiarrhythmic mechanisms that have long remained elusive.

Acknowledgements
This work was funded by Grants from the Comunidad de Madrid (ARCADIA S2022/BMD-7229), Spanish Ministry of Science, Innovation, and Universities (PID2023-150993OB-I00), and Instituto de Salud Carlos III [CB16/11/00303])

Adjunctive administration of *Limosilactobacillus fermentum* CECT5716 amplifies the blood pressure-lowering effects of amlodipine in spontaneously hypertensive rats

Cristina González-Correa[a,b,*], Javier Moleón[a,b,c,*], Sofía Miñano[a,b,*], Marta Toral[a,b,d], Iñaki Robles-Vera[e], Manuel Sánchez[a,b], Marta Olivares[f], Natividad Martín-Morales[g], Francisco O'Valle[b,g], María Jiménez-Aragón[a], Eduardo Guerra-Hernández[h], Manuel Gómez-Guzmán[a,b], Miguel Romero[a,b], Juan Duarte [a,b,d]

[a]Dept Pharmacology, School of Pharmacy and Center for Biomedical Research (CIBM), University of Granada, Spain; [b]Instituto de Investigación Biosanitaria de Granada, ibs.GRANADA, Granada, Spain. [c]Dept Translational Rheumatology and Immunology, Institute of Musculoskeletal Medicine, University of Münster, Germany; [d]Ciber de Enfermedades Cardiovasculares (CIBERCV), Spain.; [e]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; [f]Biosearch Life, Granada, Spain; [g]Instituto de Biopatología y Medicina Regenerativa (IBIMER), Dept of Pathology, School of Medicine, University of Granada, Granada, Spain; [h]Dept of Nutrition and Bromatology, University of Granada, Granada, Spain

E-mail: cristinagoncor@gmail.com

Background: *Limosilactobacillus fermentum* CECT5716 (LC40) has demonstrated antihypertensive effects and improved endothelial function in spontaneously hypertensive rats (SHR) (1). Amlodipine, a calcium channel blocker, lowers blood pressure and partially ameliorates gut dysbiosis in this model (2). Here, we investigated whether LC40 could potentiate the effects of amlodipine.

Material and methods: Twenty-weeks-old male SHR were randomly allocated into six groups (n = 8 per group): (1) untreated SHR receiving vehicle (1% methylcellulose), (2) SHR treated with LC40 (10^9 CFU day-1), (3) SHR treated with amlodipine at a dose of 1 mg kg-1 day-1 (AML1), (4) SHR treated with amlodipine at a dose of 5 mg kg-1 day-1 (AML5), (5) SHR co-treated with LC40 and AML1, and (6) SHR co-treated with LC40 and AML5. All treatments were administered by oral gavage once daily for five weeks.

Results: Co-administration of LC40 with low-dose amlodipine (1 mg kg^{-1} day^{-1}) enhanced blood pressure reduction and endothelial function compared with amlodipine alone. This combination improved key aspects of gut dysbiosis, including increased microbial diversity, reduced Firmicutes-to-Bacteroidetes ratio, and enrichment of acetate-producing bacteria. Functional microbiota analysis revealed a shift toward enhanced microbial electron-transfer pathways, which was associated with increased plasma concentrations of amlodipine and reduced fecal levels of its inactive metabolite, dehydro-amlodipine, suggesting improved intestinal drug bioavailability. Additionally, LC40 combined with amlodipine increased colonic mucin content and reduced circulating markers of gut permeability, including lipopolysaccharide and intestinal fatty acid-binding protein 2. In the central nervous system, adjunctive LC40 attenuated neuroinflammation, as evidenced by reduced microglial activation and lower mRNA expression of pro-inflammatory cytokines (IL-1β and TNFα) in the paraventricular nucleus of the hypothalamus, accompanied by decreased plasma noradrenaline levels. These changes were paralleled by immunomodulatory effects, including increased regulatory T cell infiltration and reduced vascular oxidative stress in the aorta. Overall, our findings identify gut microbiota as a therapeutic target to enhance the efficacy of low-dose calcium channel blockers.

Conclusions: LC40 co-administration improves amlodipine bioavailability and exerts complementary neuroimmune and vascular protective effects, supporting its potential as an adjunctive strategy for more effective hypertension management.

(1) Robles-Vera, Iñaki et al. "Probiotics Prevent Dysbiosis and the Rise in Blood Pressure in Genetic Hypertension: Role of Short-Chain Fatty Acids." *Molecular nutrition & food research* vol. 64,6 (2020): e1900616. doi:10.1002/mnfr.201900616

(2) González-Correa, Cristina et al. "Differing contributions of the gut microbiota to the blood pressure lowering effects induced by first-line antihypertensive drugs." *British journal of pharmacology* vol. 181,18 (2024): 3420-3444. doi:10.1111/bph.16410

Acknowledgements: This work was supported by Grants from Agencia Estatal de Investigación (AEI), (Ref. PID2024-159076OA-I00 funded by MICIU/AEI/10.13039/501100011033/FEDER, UE; PID2020-116347RB-I00 funded by MCIN/AEI/10.13039/501100011033), and Junta de Andalucía (CTS 164, P20_00193, A-CTS-318-UGR20) with funds from the European Union, and by the Ministerio de Ciencia, Innovación y Universidades, Instituto de Salud Carlos III (PI22/01046, PI23/00734, RICORs2040 RD24/0004/0030, CIBER-CV), Spain. The presenting author is a predoctoral fellow of MCIM (FPU22/00397).

Protective effects of new SGK1 inhibitors on angiotensin II-induced cardiovascular damage

Wilfrido Arrúa[1], Naoual Boukich[1], Enrique Madruga[2,3], Eduardo Oliver[2,4,5], Isabel Lastres-Becker[3,6,7], Ana Martínez[2,3], Ana García-Redondo[1,5,7], Ana M. Briones[1,5,7]

[1]Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain; [2]Centro de Investigaciones Biológicas Margarita Salas (CIB), CSIC, Madrid, Spain; [3]Ciber Enfermedades Neurodegenerativas, Madrid, Spain; [4]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; [5]Ciber Cardiovascular, Madrid, Spain; [6]Instituto de Investigaciones Biomédicas "Sols-Morreale" UAM-CSIC, Madrid, Spain; [7]Instituto De Investigación Hospital Universitario La Paz (IdiPAZ), Madrid, Spain

E-mail: wilfrido.arrua@uam.es

The serum and glucocorticoid inducible kinase 1 (SGK1) is a member of the serine/threonine kinase gene family that plays an important role in cardiovascular diseases through complex signaling pathways by activating fibrotic, inflammatory, and oxidative pathways in the heart, vascular organs and kidney (1,2). Since SGK1 regulates the activity of transporters and ion channels and affects blood pressure, its inhibition could be a valuable target for pharmacological approaches aimed at preventing cardiovascular alterations caused by hypertension. Despite the validated biological functions of SGK1, only a few small-molecule SGK1 inhibitors have been developed, and none have advanced to clinical trials because most SGK1 inhibitors have insufficient potency and complete kinome selectivity (3,4). Therefore, the objective of this study was to evaluate the effect of the administration of EMM3.20 or EMM3.43, novel selective SGK1 inhibitors (5), on cardiovascular and kidney damage caused by angiotensin II (AngII). In this study we used aorta, small mesenteric arteries (MRA), perivascular adipose tissue (PVAT), heart and kidney were taken from C57BL/6J male mice, infused or not with AngII (1.44 mg/kg/day, 14 days) in presence or absence of EMM3.20 or EMM3.43 (15mg/kg/day) starting 1 day before AngII infusion. Systolic blood pressure was measured in the animals using tail-cuff plethysmography. Thoracic aorta and mesenteric artery function or structure was analyzed using wire or pressure myography. RNA-seq, qPCR, and histological staining techniques were used to determine the cardiovascular and renal damage caused by AngII. Our results demonstrated that preventive treatment with EMM3.20 and EMM3.43 partially avoided AngII-induced hypertension. The compound EMM3.20 improved endothelial function in the aorta and MRA, while EMM3.43 improved it only in the aorta. Furthermore, both inhibitors improved vascular remodeling in the aorta and MRA, inflammation in PVAT, cardiac hypertrophy and fibrosis, and markers of AngII-induced kidney damage. Specifically, the SGK1 inhibitors decreased markers of immune cells infiltration and proinflammatory cytokines, and extracellular matrix proteins in the aorta, heart, and kidney, increased by AngII. Finally, we can conclude that SGK1 inhibitors prevent AngII-induced cardiovascular and renal damage by reducing mediators of inflammation and fibrosis.

Key words: angiotensin II, SGK1, inflammation, endothelial dysfunction.

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Dapagliflozin reduces proinflammatory and profibrotic mediators in human cardiomyocytes exposed to lipotoxic stress

Sacramento Martínez-Albaladejo[1,3], Nazaret Martín-Gálvez[1], José Tuñón[1,2], Marcelino Cortés[2], Oscar Lorenzo[1,3]

[1]IIS-Fundación Jiménez Díaz, Autónoma University, Madrid
[2]Department of Cardiology, Fundación Jiménez Díaz, Madrid
[3]Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM) Network, Madrid.

E-mail: Oscar.lorenzo@uam.es

BACKGROUND: Sodium–glucose cotransporter-2 inhibitors (SGLT2i) were originally developed for the treatment of type 2 diabetes mellitus (T2DM), a major risk factor for heart failure. Interestingly, these drugs also improve cardiac structure and function, suggesting potential direct effects on cardiomyocytes that warrant further investigation.

MATERIAL AND METHODS: In a previous clinical study, patients with T2DM were treated with dapagliflozin for six months, and cardiac structure, function, and circulating biomarkers were assessed. In the present work, human AC16 cardiomyocytes were exposed to a high concentration of sodium palmitate (90 µM, 24 h) to induce lipotoxic stress. Additional cells were pretreated with dapagliflozin (20 µM), sotagliflozin (20 µM), or cariporide (20 µM), used respectively as SGLT2, dual SGLT2/1, and NHE-1 inhibitors. Total RNA and protein were extracted for RT-qPCR and Western blot analyses.

RESULTS: Dapagliflozin treatment in patients reduced left ventricular mass, improved global longitudinal strain, shortened isovolumetric relaxation time, and decreased plasma levels of creatine kinase and atrial natriuretic peptide. *In vitro*, palmitate exposure markedly upregulated pro-inflammatory and pro-fibrotic genes (IL-1β, IL-6, NLRP3, COL3A1, FN1) and activated P70-S6K, SGK1, and NF-κB-p65, while inhibiting AMPK. All three transporters, SGLT2, SGLT1, and NHE-1, were expressed in AC16 cardiomyocytes, and pretreatment with dapagliflozin, sotagliflozin, or cariporide attenuated these lipotoxic effects in a similar manner.

CONCLUSION: Dapagliflozin exerts direct cardiomyocyte-protective effects by modulating key mediators of inflammation, fibrosis, and metabolic stress, likely through inhibition of SGLT2 and NHE-1 pathways. These mechanisms may contribute to the cardiovascular benefits observed in diabetic patients treated with SGLT2 inhibitors.

Intravenous Haloperidol in Older Adults: QT Risk Description and ECG Documentation

Itzel Sofía Falla Morán, Miguel Antonio Tolosa, Alfredo Portilla Pinzón

Fundación Universitaria de Ciencias de la Salud (FUCS). Bogotá D.C - Colombia

E-mail: sofiafallam@gmail.com

Background:
Intravenous haloperidol is associated with a recognized risk of QT interval prolongation and potentially life-threatening ventricular arrhythmias. Although regulatory agencies recommend electrocardiographic (ECG) monitoring when intravenous administration is unavoidable, real-world ECG monitoring practices in high-risk populations remain poorly characterized.
Objective:
To characterize the risk profile for QT prolongation and describe ECG monitoring practices among older adults treated with intravenous haloperidol.
Methods:
A retrospective cross-sectional study was conducted using electronic health records of patients aged ≥65 years who received intravenous haloperidol between January 2022 and October 2024 at a tertiary-care hospital. Clinical and pharmacological risk factors associated with QT prolongation were collected. Risk stratification was performed using the Tisdale score, while available variables from the RISQ-PATH model were also recorded. Documentation of ECG assessment before and after the first intravenous haloperidol administration was reviewed. Data were analyzed descriptively.
Results:
A total of 244 patients were included. The median Tisdale score was 9 (IQR 8–11), indicating a population at predominantly moderate-to-high risk for QT prolongation. Nevertheless, numerical QTc values were documented in only 5 patients (2.1%) before and 6 patients (2.5%) after intravenous haloperidol administration. Concomitant use of multiple QT-prolonging medications was common. Intravenous haloperidol was most frequently administered in Intensive Care Units and was commonly indicated for delirium. During hospitalization, 23.8% of patients died and 3.6% experienced major cardiac events; however, these outcomes were reported descriptively and should not be interpreted as causally associated with haloperidol exposure.
Conclusions:
Despite the use of intravenous haloperidol in a high-risk population, QTc documentation was uncommon. The results highlight a discrepancy between the recognized arrhythmogenic risk of this medication and routine ECG monitoring practices, emphasizing the importance of implementing more systematic cardiac safety measures in clinical care.

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Session 2: New Therapeutic Modalities

Organizers: Association of SEF Young Researchers

Moderator:

Adrián Gironda Martínez (Universidad de Alcalá)

Invited speaker:

9:00-9:30 Lara García Varela (Instituto de Investigación Sanitaria de Santiago)
Radiopharmaceuticals: From Molecular Imaging to Targeted Radiotheranostics

9:30-10:00 Ferran José Duran (IRB Barcelona)
Targeted Protein Degradation: a novel paradigm in drug development

10:00-10:30 Alfonso Alba Bernal (IdiPAZ/CNIO)
Development of innovative CAR-T cell therapies to treat pediatric rhabdoid tumors

Radiopharmaceuticals: From Molecular Imaging to Targeted Radiotheranostics

Lara García-Varela

Molecular Imaging Group. Health Research Institute of Santiago de Compostela (IDIS). Clinical University Hospital of Santiago de Compostela. Spain.

E-mail: lara.garcia.varela@usc.es

Radiopharmaceuticals are compounds that combine a molecular or biological vector with a radionuclide, enabling the non-invasive diagnosis and targeted treatment of a wide range of diseases. Their applications depend on the physical properties of the radionuclide. Gamma radiation is used for diagnostic imaging using single-photon emission computed tomography (SPECT) and positron emission tomography (PET). In contrast, α- and β^--emitting radionuclides are used for therapy because of their ability to deliver high radiation doses to target tissues. From the vector perspective, radiopharmaceuticals were initially developed using small molecules. Their rapid kinetics can reduce residence time in the body and, consequently, radiation exposure to the patient. One of the best-known examples is [^{18}F]FDG, a glucose analog used to assess glucose metabolism. Because cancer, infection, inflammation, and neuronal dysfunction can alter glucose metabolism, [^{18}F]FDG is used for cancer diagnosis and monitoring, as well as for detecting inflammation, infection, and neurodegenerative diseases. However, because many diseases alter glucose metabolism, [^{18}F]FDG lacks disease specificity. This limitation has led to the development of more specific small-molecule radiopharmaceuticals, including [^{18}F]FES for estrogen receptor imaging in breast cancer, [^{68}Ga]Ga-PSMA-11 for PSMA-targeted imaging in prostate cancer, and [^{18}F]florbetaben and [^{18}F]flortaucipir for the detection of amyloid-β and phosphorylated tau, respectively, in Alzheimer's disease. Nevertheless, small-molecule radiopharmaceuticals may still exhibit nonspecific binding and generate radiometabolites that can interfere with accurate target assessment. To overcome these limitations, radiopharmaceuticals based on peptides and immunoglobulins have been developed, offering greater specificity and selectivity toward their molecular targets. PET imaging using radiolabeled antibodies, known as immunoPET, enables the non-invasive characterization and quantification of molecular targets across primary and metastatic lesions without the need for invasive biopsies. Examples include [^{89}Zr]Zr-trastuzumab for imaging HER2-positive breast cancer and [^{89}Zr]Zr-atezolizumab for assessing PD-L1 expression and potentially predicting response to immunotherapy. Finally, a promising approach that has gained renewed attention is radiotheranostics. This concept combines diagnosis and treatment using the same vector labeled with different radionuclides: one for imaging and target characterization, and another for delivering therapeutic radiation to target cells. A prominent example is PSMA-targeted radiotheranostics, in which [^{68}Ga]Ga-PSMA-11 is used to identify PSMA-positive disease, followed by treatment with [^{177}Lu]Lu-PSMA-617. Overall, this talk will provide an overview of radiopharmaceuticals used for diagnosis and therapy, highlighting their evolution from small-molecule to highly specific biological vectors, as well as the emerging potential of immunoPET and radiotheranostics in precision medicine.

Targeted Protein Degradation: a novel paradigm in drug development

Ferran José-Duran, Marc Aragó, and Cristina Mayor-Ruiz

IRB Barcelona (Barcelona, Spain)

E-mail: ferran.jose@irbbarcelona.org

Targeted protein degradation is a novel pharmacological paradigm that enables the chemical depletion of disease-relevant proteins, including those that were previously considered "undruggable". This strategy leverages small-molecule drugs referred to as “degraders” which are classified as (i) molecular glues (e.g., thalidomide), or (ii) proteolysis-targeting chimeras (PROTACs). Both induce spatial proximity between a target protein and components of the cellular proteolytic machinery (typically an E3 ubiquitin ligase), forming a ternary complex to facilitate ubiquitin transfer and target degradation via the proteasome. Whereas PROTACs have a rational modular design composed of two independent chemical ligands (one binding the target protein, the other engaging an E3) connected by a chemical linker, molecular glue degraders are linker-less compounds that orchestrate highly-cooperative target-E3 interaction. Most available molecular glues have been identified serendipitously or via large-scale screens rather than rational designs.
Overall, degraders have helped widen the druggable proteome, moving from an occupancy-based (inhibitors) to an event-driven catalytic mechanism of action. In addition, degraders offer additional layers of selectivity due to dependency on ternary complex formation and lysine accessibility for ubiquitin transference (e.g., promiscuous kinase inhibitors can be converted into selective kinase degraders).
In this talk, I will discuss the current advances and challenges in the targeted protein degradation field, and will delve into our efforts to facilitate the development of PROTACs.

Acknowledgements

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Development of innovative CAR-T cell therapies to treat pediatric rhabdoid tumors.

Alfonso Alba-Bernal[1], Esther Díaz-Maroto[1], Sara Naharro-González[1], Alicia Martín-Ayuso[1], Andrea Matesanz-María[1], Karima Al-Akioui-Sanz[1], Halin Bareke[1], Estefanía Ayala-Jiménez[1], Laura Clares-Villa[1], Carlos González[2], Diego Plaza[2], Víctor Galán[2], Javier Saceda[3], Cristina Aguirre-Portolés[1], Jordi Minguillón[1,4], Antonio Pérez-Martínez[1,2]

[1]CIBERER-ISCIII, IdiPAZ-CNIO Translational Research Unit in Pediatric Hemato-Oncology, La Paz University Hospital Research Institute, Spanish National Cancer Center, Madrid, Spain
[2]Pediatric Hemato-Oncology Department, La Paz University Hospital, Madrid, Spain
[3]Pediatric Neurosurgery Department, La Paz University Hospital, Madrid, Spain
[4]Advanced Therapies Mixed Unit, CIEMAT/IIS-FJD, Madrid, Spain

E-mail: aalbab@cnio.es/alfonsoalbabernal@gmail.com

Background. Rhabdoid tumors (atypical teratoid/rhabdoid tumor, ATRT, and extracranial malignant rhabdoid tumor, eMRT) are aggressive SMARCB1-deficient pediatric cancers with poor survival and no curative standard. B7-H3 (CD276) is widely expressed in these tumors and is a strong CAR-T target; however targeting a single antigen leaves room for antigen escape. We designed four anti-B7-H3 CAR constructs, compared their cytotoxicity in vitro across multiple healthy donors, and tested whether targeted drugs raise CAR-T target antigens (B7-H3, ST2 and ST6, coded names) to enable a combinational therapy.
Methods. Four constructs were generated: one second-generation CAR (CAR-B7-H3) and three fourth-generation variants (sIC1, TCE, and switch-receptor SR, coded names), alongside an untransduced control (UT). Each was produced by lentiviral transduction of T cells from eight healthy donors. Cytotoxicity was assessed in co-culture against BT-12, CHLA-266 (ATRT) and G401 (eMRT) at effector-to-target ratios (E:T) of 2:1, 1:1, 1:2.5 and 1:5, from 24 to 144 h, by a luciferase-based assay. In parallel, surface B7-H3, ST2 and ST6 markers (coded) were measured by spectral flow cytometry after 72 h of sub-lethal panobinostat (2.1 nM), trametinib (2.1 nM) or tucatinib (16 nM), and on cells surviving CAR-T attack to determine whether the persisting tumor population upregulates targetable antigens
Results. Cytotoxicity was strongly dependent on ratio and time, reaching the highest values between 72 and 96 h. In ATRT cell lines, CAR-B7-H3 and sIC1 constructs reached 84.3 - 91.7% specific lysis at 96 h (BT-12: CAR-B7-H3 84.3 ± 9.5%, sIC1 91.7 ± 4.9%; CHLA-266: CAR-B7-H3 84.6 ± 10.1%, sIC1 88.4 ± 8.0%, mean ± SEM). The TCE construct was intermediate (BT-12 55 - 71%, CHLA-266 67 - 77% across 72 and 96 h), while SR showed the lowest cytotoxicity of all constructs. The G401 cell line (eMRT) was more resistant; in this setting, sIC1 clearly performed best, reaching 59.0 ± 5.9% at 72 h and 62.3 ± 5.5% at 96 h, in comparison with 39.4 ± 9.6% and 46.3 ± 9.3% for CAR-B7-H3, with TCE and SR near background. On the other hand, both the CAR-T cell therapy and the three selected drugs increased CAR-T target antigens on the surviving cells. B7-H3 MFI fold-change rose up to 1.62 in BT-12 after CAR-T and up to 1.52 in G401 after targeted therapy. ST2 and ST6 were induced de novo in ATRT cell lines: after CAR-T therapy, ST2-positive cells increased from 4% and 1% to 80% and 84% in BT-12 and CHLA-266, respectively, compared with untreated tumor cells. ST2-positive cells increased from 47% to 85% in G401. A similar increase was observed for ST6, rising from 2% and 11% to 76% and 71% in BT-12 and CHLA-266. Regarding targeted therapy, panobinostat produced the strongest modulation: ST2-positive cells reached 74.4% in G401 from 47%, and ST6-positive cells rose from 3% to 51%

and from 12% to 63% in BT-12 and CHLA-266, respectively, after HDAC inhibition.
Conclusions. Across eight donors, the fourth-generation sIC1 and the second-generation CAR-B7-H3 constructs were the most active constructs against rhabdoid cell lines, including the resistant eMRT model, whereas the switch-receptor SR showed the lowest cytotoxicity. Because sub-lethal targeted therapy raises the same antigens that CAR-T pressure induces (ST2 and ST6), these data support combinational therapies as a promising approach to treat rhabdoid tumors.

Acknowledgements (Times New Roman 9)

We are deeply grateful to the patients and their families, whose generosity and courage make this research possible and give it its true meaning. We would especially like to thank Sara, Manuel's mother. Proyecto Manuel carries her son's name in his memory, and her trust, strength, and support have accompanied and guided this work from the beginning. We are honoured to carry it forward.
We gratefully acknowledge the CRIS Cancer Foundation for believing in and funding Proyecto Manuel, and for its ongoing commitment to advancing research into childhood cancers that are still too often left without answers.
Finally, we thank all our colleagues, past and present, for their help, generosity, and companionship. Their scientific input, technical support, and day-to-day dedication have been essential at every stage, and we feel fortunate to be part of such a committed team.

Session 3: Pain and Neural Pharmacology

Moderator:

Raquel Abalo (Universidad Rey Juan Carlos)

Invited speaker:

15:00-15:30 Enrique Cobos (Universidad de Granada)
Digital Researchers: The Evolution of Preclinical Behavioral Pharmacology (With a Focus on Pain Research)

Selected oral communications:

15:30-15:45 Amada Puerto-Moya (Universidad de Granada)
Soluble epoxide hydrolase inhibition and opioid analgesia: a preclinical approach to postoperative pain

15:45-16:00 Aitziber Mendiguren (Universidad del País Vasco)
Involvement of the EP4 receptor in the development of cellular tolerance to opioids in locus coeruleus neurons

16:00:16:15 Neus Calza-Zamora (Universidad de Barcelona)
Allosteric modulations within the heteromeric complex between the muopioid receptor and the adenosine 1 receptor

16:15-16:30 Pedro Marín (Universidad de Murcia)
Influence of Administration Route on Ketoprofen Pharmacokinetics in Goats

Digital Researchers: The Evolution of Preclinical Behavioral Pharmacology (With a Focus on Pain Research)

Rafael González Cano, Miguel Ángel Tejada, Mª Carmen Ruiz Cantero, Álvaro Luque Jiménez, Enrique José Cobos

Department of Pharmacology and Neurosciences Institute (Biomedical Research Center), Universidad Granada and ibs.GRANADA, Granada, España.

E-mail: ejcobos@ugr.es

Animal behavioral assessment in preclinical research faces critical methodological challenges. The massive time investment required for manual assays leads to physical and cognitive fatigue in researchers, which can alter evaluation criteria over time. This fatigue, combined with the inherent subjectivity in interpreting complex behavioral parameters, results in an alarming lack of inter-observer reproducibility (Chesler et al., 2002; Zumbusch et al., 2024), ultimately hampering scientific progress and clinical translation. To overcome these limitations, we have developed Artificial Intelligence (AI)-based solutions, custom-trained by our group, that act as "digital researchers": tireless automated observers capable of analyzing thousands of hours of video with objective, consistent criteria, entirely free from human bias.

We focus on the application of these tools to pain and behavioral research through two platforms that we have developed. First, the Face Analyzer utilizes frontal-view recordings to objectively quantify pain by automatically "reading" facial expressions in experimental animals, thereby standardizing the evaluation of novel analgesics. Notably, pharmacological responses to standard analgesics (e.g., morphine or ibuprofen) and experimental compounds (such as sigma receptor, P2X3, or CGRP receptor antagonists) differ significantly depending on whether pain-induced facial expressions or mechanical thresholds—using the von Frey test—are evaluated (Santos-Caballero et al., 2024 and 2025). Consequently, these metrics provide valuable, non-overlapping data that complement conventional testing. Second, we introduce the PhenoScreener, a top-down recording system designed for comprehensive automated behavioral phenotyping. By capturing video from above, this device is uniquely suited for assessing complex behavioral movements involving the entire body rather than just the face. This technology is able to detect and quantify low-frequency responses in long-term recordings that typically elude human observation, such as paw-licking in neuropathic animals. We also validated this device by quantifying scratching behavior in morphine-treated animals. Together, these digital researchers establish a universal quality standard that drastically reduces preclinical variability and ensures scientific data integrity in behavioral pharmacology.

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Soluble epoxide hydrolase inhibition and opioid analgesia: a preclinical approach to postoperative pain

Amada Puerto-Moya[1,2], María Robles-Funes[1,2], María del Carmen Ruiz-Cantero[2,3], José Manuel Entrena[1,2], Laura Salvans[3], Santiago Vázquez[3], Enrique José Cobos[1,2]

1. University of Granada and Institute of Neurosciences (Granada, España)
2. Biosanitary Research Institute ibs.Granada (Granada, España)
3. University of Barcelona (Barcelona, España)

E-mail: amadapm@ugr.es

Postoperative pain is often inadequately managed [1]. Soluble epoxide hydrolase (sEH) metabolizes epoxyeicosatrienoic acids (EETs), which are eicosanoids produced by cytochrome P450 (CYP450) during the inflammatory response [2]. The accumulation of EETs produces analgesia. In fact, EC5026, a selective sEH inhibitor, is currently being developed as a treatment for neuropathic pain. The inhibition of sEH increases the analgesia of central endogenous opioid peptides (EOPs) [3]. EOPs can also be produced peripherally, particularly by immune cells, such as those that migrate to the surgical wound. It hasn`t yet been studied whether sEH inhibition is capable of potentiating peripheral opioid analgesia produced by immune-derived EOPs or by opioid drugs such as morphine. The aim of this study was to evaluate the effect of EC5026, alone or in combination with morphine, in laparotomized mice.

A transverse laparotomy was performed on CD1 mice. The mechanical threshold was evaluated using von Frey filaments. The drugs or their vehicles were administered subcutaneously.

The administration of morphine (0.125–1 mg/kg) and EC5026 (1.25–5 mg/kg) reversed postoperative allodynia in a dose-dependent manner, producing the maximum effect 2 hours post-administration. The maximum effect of morphine was superior to that of EC5026. The anti-allodynic effect of both drugs was reversed by both the opioid antagonist naloxone and its peripheral analog, naloxone methiodide (NxM; 2 mg/kg). This indicates that both compounds exert their effects through the activation of peripheral opioid receptors.

MSPPOH (20 mg/kg), an inhibitor of CYP450 epoxygenase activity, inhibited the effect of EC5026 without altering that of morphine, indicating that EC5026 acts through EETs to produce its analgesic effect. Furthermore, this effect was also reversed by the inhibition of neutrophil recruitment through the in vivo administration of an anti-Ly6G antibody. Taken together, these data indicate that EC5026 acts on sEH and potentiates EOPs of neutrophilic origin.

The association of an inactive dose of EC5026 (1.25 mg/kg) with either inactive doses of morphine (0.125 mg/kg) or doses that induce a mild anti-allodynic effect (0.25 mg/kg) showed a synergistic (supra-additive) anti-allodynic effect that was sensitive to NxM. This suggests that sEH inhibition can be used to potentiate the peripheral opioid analgesia of opioid drugs.

The inhibition of sEH, both alone and in combination with morphine, modulates peripheral opioid analgesia, showing potential as a possible treatment for postoperative pain.

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Involvement of the EP4 receptor in the development of cellular tolerance to opioids in locus coeruleus neurons

Irati Rodilla, Aitziber Mendiguren, Joseba Pineda

University of the Basque Country (UPV/EHU), Leioa, Spain

E-mail: aitziber.mendiguren@ehu.eus

Prostanoid system activation contributes to the reduction of opioid analgesic effects, whereas its inhibition enhances opioid-induced analgesia (Trang et al., 2005). PGE2, which is the most expressed prostanoid in the CNS (López and Ballaz, 2020), is involved in opioid-induced hyperalgesia and withdrawal (Dunbar et al., 2006; Li et al., 2018). However, the influence of prostaglandins receptors (EP receptors) on opioid-induced effects remains to be elucidated. The locus coeruleus (LC), the main noradrenergic nucleus in the CNS, expresses MOR and EP receptors and it is involved in opioid-induced effects. The purpose of this study was to investigate the involvement of EP4 receptors in the development of cellular tolerance to opioids in LC neurons by single-unit extracellular recordings *ex vivo*. Treatment with morphine (200 mg/kg/72 h, 3 d, s.c.) reduced the inhibitory effect of ME on the firing rate of LC neurons, revealing morphine-induced opioid tolerance in LC neurons. In morphine-treated rats, the concentration-effect curve for rivenprost (0.01 – 100 nM) was shifted to the left by 4-fold as compared to that in the sham-treated group (EC50 in sham group: 2.57 nM, $n = 7$ *vs* EC50 in morphine group: 0.70 nM, $n = 9$; $P < 0.05$). In rats co-treated with morphine (200 mg/kg/72 h, s.c.) and the vehicle of ONO-AE3-208 (saline; s.c.), the concentration-effect curve for ME was shifted to the right by 2-fold as compared to its control group. In animals co-administered with morphine (200 mg/kg/72 h, s.c.) and the EP4 receptor antagonist ONO-AE3-208 (0.3 mg/kg/24 h, s.c.), the concentration-effect curve for ME was also shifted by 2-fold to the right, but it was not different from that of its control group. However, perfusion with ONO-AE3-208 (3 and 30 nM) over the slice preparation elicited a 5 and 20-fold rightward shift of the concentration-effect curve for rivenprost (0.1 – 300 nM), respectively, confirming its EP4 receptor antagonistic profile (EC50 in control group: 2.22 nM, $n = 11$ *vs* EC50: 11.23 nM and 44.51 nM in the presence of ONO-AE3-208 3 nM and 30 nM, $n = 5$ and $n = 7$; $P < 0.05$). Subchronic morphine treatment enhances the stimulatory effect of an EP4 receptor agonist on the firing activity of LC neurons, but *in vivo* administration of an EP4 receptor antagonist fails to prevent the morphine-induced development of cellular tolerance. The present work may contribute to increase the understanding of cellular mechanisms underlying inflammatory pain and opioid tolerance.

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Allosteric modulations within the heteromeric complex between the mu-opioid receptor and the adenosine 1 receptor

Neus Calza-Zamora, Cèlia Rodríguez-Casas, Natalia Llopart, Verònica Casadó-Anguera, Vicent Casadó

University of Barcelona and Institute of Biomedicine of the University of Barcelona, Barcelona, and Nanomedicine and Molecular Neuropharmacology Research Group, Barcelona.

E-mail: neus.calza.zamora@ub.edu

Chronic pain is a pathological condition that affects almost 26% of the Spanish population1, representing a major social and economic challenge. Currently, mild to moderate pain is treated with non-steroidal anti-inflammatory drugs, whereas severe pain is mainly managed with opioids. Opioids effectively alleviate pain by acting on mu opioid receptors (MOR)2, but their use is associated with side effects, including hyperactivity and restlessness, commonly observed during withdrawal syndrome and also characteristic of restless legs syndrome (RLS)3,4. Previous studies have shown that co-administration of MOR and adenosine A1 receptor (A1R) agonists induces a synergistic antiallodynic effect5. Both receptors are expressed in striosomal neurons of the direct pathway of the basal ganglia, where they display intricate functional interactions. Moreover, they belong to the superfamily of G protein-coupled receptors (GPCRs), which can form oligomeric complexes with distinct pharmacological and functional properties6,7. Therefore, the main aim of this project was to determine the presence of a MOR-A1R receptor complex and characterize its molecular fingerprint. Using biochemical, biophysical, pharmacological, radiochemical, and immunological approaches, we performed ex vivo and in vitro experiments in rodent brain slices, striatal membranes, and transfected cells. We confirmed the presence of MOR-A1R heteromers by immunohistochemical assays performed ex vivo on mouse brain slices. In addition, radioligand competitive binding assays in striatal tissues, together with G protein activation, β-arrestin recruitment, and cAMP accumulation assays in transfected cells co-expressing MOR and A1R, were performed to investigate the allosteric modulations derived from oligomer formation. The results showed a reduced response of both receptors when being co-activated by their respective agonists, demonstrating a negative cross-talk between MOR and A1R. Moreover, a bidirectional cross-antagonism within the heteromer was detected, in which antagonists of one receptor impaired agonist-mediated activation of the partner receptor. Our findings may provide the basis for the development of innovative therapeutic strategies targeting MOR-A1R heteromers, potentially enabling safer and more effective pain management by reducing addiction liability and opioid withdrawal-associated restlessness, while also alleviating symptoms related to RLS.

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Influence of Administration Route on Ketoprofen Pharmacokinetics in Goats

Pedro Marin[1], Duygu Durna Corum[2], Kamil Uney[3], Devran Coskun[4], Fátima Martín-Sánchez[1], Elena Badillo[1], María Teresa Yuste[1], Fatma Akın[4], Mustafa Hitit[5], Ahmet Levent Bas[3], Orhan Corum[2]

[1] University of Murcia, Murcia, Spain.
[2] University of Hatay Mustafa Kemal, Hatay, Türkiye.
[3] University of Selcuk, Konya, Türkiye.
[4] University of Siirt, Siirt, Türkiye.
[5] Prairie View University, Texas, USA.

E-mail: pmarin@um.es

Ketoprofen is a nonsteroidal anti-inflammatory drug, marketed as a racemic mixture of the propionic acid family, with analgesic, anti-inflammatory, and antipyretic properties. The objective of this study was to compare the pharmacokinetic profile of ketoprofen in goats after single-dose administration by IV, IM, subcutaneous (SC), and oral routes. The study was conducted in four phases according to a crossover pharmacokinetic design with a 14-day washout period between treatments. Ketoprofen (Vilprofen®, 100 mg/mL) was administered as a single dose of 3 mg/kg via IV (left jugular vein), IM (between the semitendinosus and semimembranosus muscles), SC (axillary region), and oral (via ororuminal tube). Blood samples were collected at different times until 24 hours. The concentration-time data were analyzed by non-compartmental pharmacokinetic methods. Ketoprofen analysis was performed using a validated HPLC-UV method. Table 1 shows the pharmacokinetic parameters expressed as geometric mean (minimum-maximum value) of ketoprofen in goats (n = 8) following IV, IM, SC and oral administration of a single dose of 3 mg/kg.

Parameter	IV	IM	SC	ORAL
$t_{1/2\lambda z}$ (h)	0.71 (0.64-0.78)^{d}	0.96 (0.86-1.06)^{c}	1.16 (1.09-1.24)^{b}	1.59 (1.39-1.71)^{a}
$AUC_{0\text{-last}}$ (h*µg/mL)	16.69 (13.77-19.46)^{c}	13.20 (11.71-14.67)^{b}	8.74 (7.37-9.82)^{a}	7.61 (6.19-11.03)^{a}
$AUC_{0\text{-}\infty}$ (h*µg/mL)	16.75 (13.81-19.50)^{c}	13.27 (11.78-14.77)^{b}	8.84 (7.46-9.92)^{a}	7.72 (6.28-11.12)^{a}
AUC_{extrap} (%)	0.31 (0.19-0.47)	0.53 (0.37-0.77)	1.15 (0.94-1.52)	1.39 (0.84-1.69)
$MRT_{0\text{-}\infty}$ (h)	0.65 (0.54-0.80)^{d}	1.26 (1.11-1.43)^{c}	1.71 (1.58-1.85)^{b}	2.48 (2.44-2.58)^{a}
MAT (h)	-	0.61 (0.57-0.64)^{c}	1.05 (1.02-1.07)^{b}	1.83 (1.74-1.90)^{a}
Cl_T (L/h/kg)	0.18 (0.15-0.22)	-	-	-
V_{dss} (L/kg)	0.12 (0.09-0.16)	-	-	-
C_{max} (µg/mL)	-	10.81 (9.32-12.95)^{b}	5.25 (4.19-6.50)^{a}	2.82 (2.38-3.45)^{a}
T_{max} (h)	-	0.50 (0.17-0.75)	0.42 (0.33-0.75)	0.33 (0.25-1.00)
F (%)	-	79.26 (75.73-85.26)^{c}	52.79 (50.00-75.17)^{b}	46.11 (41.97-57.04)^{a}

^{a,b,c,d}: Different letters indicate statistically significant differences between groups within the same row ($p<0.05$).

Ketoprofen showed a low volume of distribution after IV administration, suggesting limited tissue distribution and a predominance within the vascular compartment in goats. Bioavailability differed significantly among extravascular routes, being highest after IM administration (79.3%), followed by SC (52.8%) and oral administration (46.1%), indicating that IM administration provides the most efficient systemic exposure after IV dosing.

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Session 4: Natural Products Pharmacology

Moderator:

Carmen Montesinos (Universitat de Valencia)

Invited speaker:

15:00-15:30 Marina Sánchez Hidalgo (Universidad de Sevilla)
Extra virgin olive oil and systemic lupus erythematosus prevention: Mechanistic insights and therapeutic potential

Selected oral communications:

15:30-15:45 Javier Cano Lou (Universidad San Jorge)
Phytochemical characterization, multifunctional bioactivity, and toxicological evaluation of apple (Malus domestica Borkh.) leaves: revalorization of by-products with pharmaceutical applications

15:45-16:00 Jessica Bravo (Universidad Diego Portales; Chile)
From Chilean Native Plants to Veterinary Topical Formulations: Antibacterial Candidates for Canine Pyoderma

16:00:16:15 Ana María Sánchez-Tévar (Universidad de Málaga)
Influence of the vascular antioxidant effects of minor compounds from extra virgin olive oil on prostanoid production under acute hyperglycemic conditions in acute hyperglycaemia conditions

16:15-16:30 Luis Rodríguez-Santos (Universidad de Santiago de Compostela)
Exploring Neuro-Epithelial Toxicity and Serotonergic Dysregulation Induced by Diarrheic Shellfish Toxins in an In Vitro Co-Culture Model

Extra virgin olive oil and systemic lupus erythematosus prevention: Mechanistic insights and therapeutic potential

Marina Sánchez-Hidalgo[1,2]

1 Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla,41013 Seville, Spain
2 Faculty of Pharmacy, University of Seville, 41012 Seville, Spain.

E-mail: hidalgosanz@us.es

Mediterranean diet rich in plant-based foods such as whole grains, legumes, fruit, vegetables, and extra virgin olive oil (EVOO), might have the potential to contribute to well-being and has protective effects against cardiovascular, neurodegenerative disease, and cancer. One of the Mediterranean diet characteristic habits is the daily consumption of 25–50g/day of EVOO. Specifically, EVOO consumption in the diet leads to protective effects in the Mediterranean population. In fact, it is well-known that EVOO contains significant amounts of monounsaturated fatty acid (MUFA) (oleic acid) and other minor but highly bioactive components including triterpenic alcohols, sterols, hydrocarbons, vitamins, β-carotene, phytosterols and numerous functional phenolic compounds (e.g., hydroxytyrosol, oleocanthal and oleacein). In this context, we have demonstrated the functionalism of EVOO and its phenolic compounds in several chronic autoimmune inflammatory diseases, including systemic lupus erythematosus (SLE), ulcerative colitis and rrheumatoid arthritis. Particularly, EVOO and its phenols improved vascular dysfunction and revealed anti-inflammatory and immunomodulatory potential in an experimental model of pristane-induced SLE via activation of nuclear-factor-erythroid-2-related factor 2/hemeoxigenase-1 (Nrf-2/HO-1) antioxidant pathway and suppression of Janus kinase/signal transducer and transcription activator of transcription (JAK/STAT), nuclear factor kappa (NF-κB), inflammasome and mitogen-activated protein kinase (MAPK) activation. Additionally, we have also described the beneficial effects of fractions from EVOO (unsaponifiable fraction (UF) and phenolic fraction (PE)) and its phenolic compounds in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages and provided the first evidence that PE modulates cytokine production and attenuates induced T-cell activation from patients with SLE, probably through NF-κB signalling pathway. Interestingly, intervention combining EVOO and a multicomponent health promotion and physical exercise program improved disease activity, cardiovascular risk factors and changed epigenetic profile (miRNAs) in SLE patients. On the other hand, it is well-known that skin is involved in ~75% of SLE patients. In this sense, we also described that EVOO phenols afford protection from inflammation in primary human keratinocytes by interference with the NF-κB pathway. All these observations reported are promising and suggest that EVOO and its minor components play a remarkable role in these healthy benefits in SLE, influencing the course of the experimental disease, ameliorating the symptoms and severity and downregulating the inflammation at molecular level. Nevertheless, further research is needed to understand the contribution and efficacy of EVOO and its polyphenols in humans and validate the epigenetic mechanisms involved in the context of SLE disease.

Phytochemical characterization, multifunctional bioactivity, and toxicological evaluation of apple (Malus domestica Borkh.) leaves: revalorization of by-products with pharmaceutical applications

Javier Cano-Lou[1], Gema Casado-Hidalgo[1], Cristina Moliner[1], Carlota Gómez-Rincón[1,2], Francisco Les[1,2], Ana Pina[2], Víctor López[1,2]

1-Universidad San Jorge, 50830 Villanueva de Gállego (Zaragoza), España.
2- Instituto Agroalimentario de Aragón IA2, Universidad Zaragoza-CITA, Zaragoza, España.

E-mail: jcanol@usj.es

Apple leaves from Malus domestica Borkh., an abundant agricultural by-product routinely removed during harvesting processes, have gained increasing attention as a promising source of phenolic compounds, extending their value beyond traditional agricultural practices1. Polyphenols are bioactive compounds that through pleiotropic actions can help to prevent or treat a wide range of metabolic disorders2. In addition, the development of New Approach Methodologies (NAMs) for hazard identification has become a growing priority; while in vitro cytotoxicity assays have limit utility, whole-organism models such as Caenorhabditis elegans provide valuable insights into neurotoxicity and oral intake exposure3.
Leaves from the commercial cultivars Reineta, Verde Doncella and Royal Gala were used for polyphenol extraction, employing ethanol as a green solvent and ultrasound-assisted extraction (UAE). The individual phenolic compounds were analyzed by HPLC- MS/MS. Subsequently, antidiabetic activity was evaluated through the inhibition of α-glucosidase, α-amylase, dipeptidyl peptidase-4 (DPP-4), and the formation of advanced glycation end products (AGEs). Hypolipidemic activity was assessed by measuring the inhibitory effects on HMG-CoA reductase and pancreatic lipase, whereas antihypertensive activity was evaluated via angiotensin-converting enzyme (ACE) inhibition. Cell viability was assessed by MTT in Caco-2 and HepG2 cells, and locomotor activity in C. elegans using the WMicroTracker One system.
The main phenolic compounds identified were chlorogenic acid, phlorizin, phloretin, isoquercitrin, and delphinidin 3,5-diglucoside. Verde Doncella showed higher antioxidant, anti-glycation, and ACE inhibitory activities, while Reineta exhibited stronger antidiabetic (α-glucosidase and DPP-4) and HMGR inhibition, both outperforming Royal Gala. In terms of cytotoxicity, Verde Doncella and Reineta markedly reduced HepG2 viability (>70%) with limited effects on C. elegans at 1000 μg mL^{-1}, whereas Royal Gala showed cell line–specific effects only. None of them affect the viability of Caco-2 cells. In conclusion, apple leaves represent a matrix as a promising renewable resource for food, nutraceutical, and pharmaceutical applications.

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From Chilean Native Plants to Veterinary Topical Formulations: Antibacterial Candidates for Canine Pyoderma

Martina Jacobs[1,2], Noelia Valdivia[1], Martín Varas[1,2], Paola Ramos[1], Flavia Bruna[1,3,8], Gabriela Valenzuela-Barra[1,4], Olomina Correa[1,4], Antonia Díaz[1,9], Gabriela Maturana[1], Irene Martínez[2], Francisco Abusleme[5,7], Belén Rivera[7], María Olga Bargsted[5], Daniela Siel [5,6,] *, Jessica Bravo [1,*]

1 *Laboratory of Bioactive Natural Products, School of Medicine, Center for Biomedical Research, University Diego Portales, Ejército 141, Santiago 8370007, Chile*

2 *Department of Chemical Engineering, Biotechnology and Materials, Faculty of Physical and Mathematical Sciences, University of Chile, Beauchef 851, Santiago 8370458, Chile*

3 *Hormone and Cancer Biology Laboratory, Institute of Experimental Medicine and Biology of Cuyo (IMBECU), CONICET CCT Mendoza – National University of Cuyo (UNCuyo), Mendoza 5500, Argentina*

4 *Department of Pharmacological Chemistry and Toxicology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Carlos Lorca Tobar 964, Santiago 8380492, Chile*

5 *School of Veterinary Medicine, Faculty of Medicine and Health Sciences, Mayor University, Camino La Pirámide 5750, Santiago 8580000, Chile*

6 *Center for Biomedicine, Mayor University, Camino La Pirámide 5750, Santiago 8580000, Chile*

7 *Oftaderm Veterinary Clinic, Av. Francisco Bilbao 7341, La Reina, Santiago 7850003, Chile*

8 *Center of Odontological Research (CIO) from the Faculty of Odontology, National University of Cuyo (UNCuyo), Mendoza 5500, Argentina*

9 *School of Biotechnology, Faculty of Sciences, Engineering and Technology, Mayor University, Camino La Pirámide 5750, Santiago, Chile*

E-mail: Presenting author jessica.bravo@mail.udp.cl

Canine pyoderma is one of the most common dermatological conditions in dogs and is mainly associated with *Staphylococcus pseudintermedius*, an opportunistic pathogen with increasing antimicrobial resistance. The growing prevalence of multidrug-resistant strains has highlighted the need for new topical alternatives capable of reducing the use of conventional antibiotics in veterinary dermatology. Chilean native plants represent a valuable source of bioactive natural products with potential pharmacological applications.

This study aimed to evaluate the antibacterial potential, formulation feasibility, and preliminary safety of topical prototypes based on essential oils obtained from *Cryptocarya alba* and *Laureliopsis philippiana* for the management of canine pyoderma. Essential oils were obtained by steam distillation and chemically characterized by GC–MS. Their antibacterial activity was assessed against clinical isolates of *S. pseudintermedius* using disk diffusion and microdilution assays. Subsequently, essential oil-loaded topical formulations were developed and evaluated for physicochemical stability, organoleptic characteristics, microbiological safety, and preliminary dermal tolerance in a murine model. In addition, preliminary *in vivo* evaluations in canine patients with pyoderma were conducted to explore their potential clinical applicability.

Both essential oils showed antibacterial activity against *S. pseudintermedius*, supporting their selection as active natural ingredients for topical formulation development. The prototypes displayed suitable

physicochemical characteristics and acceptable preliminary stability under the evaluated conditions. Dermal safety studies showed no evident signs of irritation, erythema, edema, or histological alterations in treated animals. Preliminary observations in canine patients suggested favorable tolerability and potential improvement of clinical signs, although further controlled studies with larger sample sizes are required to confirm therapeutic efficacy.

Overall, these findings support the potential of Chilean native plant essential oils as promising natural-product-based candidates for the development of innovative topical formulations against canine pyoderma.

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Influence of the vascular antioxidant effects of minor compounds from extra virgin olive oil on prostanoid production under acute hyperglycemic conditions.

Ana María Sánchez-Tévar[1], Laura Ortega-Hombrados[1], María Dolores Rodríguez-Pérez[1], María Monsalud Arrebola-Ramírez[2], Esther Martín-Aurioles[3], Sergio Pérez-Burillo[1], Cristina Verdugo-Cabello[1], Rocío Cobos-López[1], José Pedro De La Cruz[1], José Antonio González-Correa[1]

1 Departamento de Farmacología, Facultad de Medicina, Universidad de Málaga, IBIMA Plataforma BIONAND, Instituto de Investigación Biomédica de Málaga, 29010 Málaga, Spain.
2 UGC Laboratorio Clínico, Hospital de la Axarquía, Área de Gestión Sanitaria Este de Málaga-Axarquía, 29740 Málaga, Spain.
3 Distrito Sanitario Málaga-Guadalhorce, UGC La Roca, 29009 Málaga, Spain.

E-mail: amstevar@uma.es

It is well known that maintaining vascular prostacyclin (PGI2) synthesis is important for antithrombotic function, and vascular prostacyclin acts as a vasodilator and antiplatelet agent, playing a key role in the pathophysiology of ischemic cardiovascular disease. In contrast, platelet-derived thromboxane A2 (TxA2) is a vasoconstrictor and proaggregatory prostanoid involved in thrombosis.
This study aimed to compare the in vitro effects of the main minor extra virgin olive oil (EVOO) components on the thromboxane A2/prostacyclin balance and to assess their antioxidant effects in rat aortic tissue under normoglycaemic (100 mg/dL) and hyperglycaemic (300 mg/dL) conditions.
Aortic rings from a total of 84 Wistar rats (42 males and 42 females) were used, and incubated with oleacein, oleocanthal, hydroxytyrosol, tyrosol, dihydroxy fenilglicol, oleanolic acid and maslinic acid.
Under hyperglycaemic conditions, thromboxane production was 1.3 times higher, while prostacyclin production was 40.9% lower than in samples with 100 mg/dL glucose in aortic rings, accompanied by marked oxidative stress (65.6% higher than in samples with 100 mg/dL glucose). The compounds tested inhibited thromboxane production in a concentration-dependent manner, with relative potencies: secoiridoid derivatives (IC50 range: 10^{-6} M) = triterpenes (10^{-6} M) > alcoholic phenols (10^{-5} M for hydroxytyrosol and 10^{-4} M for the rest), while preserving prostacyclin production (5–20% inhibition). All compounds also exerted vascular antioxidant effects, reducing oxidative stress markers and enhancing antioxidant parameters (range: 10^{-6}–10^{-5} M), and these effects were observed under both normoglycaemic (100 mg/dL) and hyperglycaemic (300 mg/dL) conditions.
In conclusion, the main minor components of extra virgin olive oil exert antioxidant effects in the vascular tissue and reduce platelet prostanoid production while preserving vascular prostacyclin synthesis, likely due to their strong antioxidant actions, thereby supporting EVOO acute protective effects.

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Exploring Neuro-Epithelial Toxicity and Serotonergic Dysregulation Induced by Diarrheic Shellfish Toxins in an *In Vitro* Co-Culture Model

Luis Rodríguez-Santos[1], M. Carmen Louzao[1], Eva Cagide[2], Mercedes Alvarez[2], Carmen Vale[1], Natalia Vilariño[1], Mercedes R. Vieytes[3], Manuel Lolo[2], Luis M. Botana[1]

1 Departamento de Farmacología, Facultad de Veterinaria, Universidad de Santiago de Compostela, 27002 Lugo, Spain;
2 Laboratorio Cifga, 27003 Lugo, Spain;
3 Departamento de Fisiología, Facultad de Veterinaria, Universidad de Santiago de Compostela, 27002 Lugo, Spain.

E-mail: luis.rodriguez.santos@usc.es

Diarrhetic shellfish toxins (DSTs) are natural compounds mainly synthesized by dinoflagellates belonging to the genera *Dinophysis* and *Prorocentrum*. This group of toxins includes Okadaic acid (OA), Dinophysistoxin-1 (DTX1), and Dinophysistoxin-2 (DTX2) which can bioaccumulate in filter-feeding seafood, posing a recurrent threat to seafood safety and public health. Although their acute gastrointestinal (GI) effects have been extensively documented, the mechanisms underlying their neuro-intestinal toxicity remain to be fully elucidated. Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in the regulation of multiple physiological processes. Modifications in serotoninergic signalling can disrupt GI motility, secretion and epithelial barrier function contributing to symptoms such as diarrhea and abdominal pain. In this study we investigated the neuro-epithelial toxicity and serotonergic alterations induced by DSTs using an *in vitro* model of intestinal barrier based on a Caco-2/SH-SY5Y co-culture system.

For the experimental setup, Caco-2 cells were seeded onto 1.1 cm2 transwell inserts and allowed to differentiate into enterocyte-like cells over 21 days. The human neuroblastoma cell line SH-SY5Y was cultured in the corresponding underlying 12-well plates. Transepithelial Electrical Resistance (TEER) was monitored throughout the experiment to assess epithelial barrier integrity. DSTs were applied to the apical compartment, and serotonin levels released into the basolateral compartment were quantified using a specific ELISA assay.

The results revealed that all DSTs significantly reduced TEER values, indicating increasing epithelial permeability. Among the toxins tested, DTX1 exhibited the greatest potency, inducing a decrease in barrier integrity that persisted up to 24 hours. Furthermore, DTX1 was detected in the basolateral compartment at higher concentrations than OA and DTX2, suggesting a greater capacity to cross the epithelial barrier. Neurotransmitter analysis revealed that 24 h exposure to all three DSTs resulted in a reduction in extracellular serotonin levels.

In conclusion, all investigated DSTs compromise epithelial barrier function, with DTX1 displaying the stronger effect. In addition, these toxins impair neuro-epithelial serotonergic signalling, highlighting a mechanism that may contribute to the intestinal dysmotility associated with Diarrhetic Shellfish Poisoning (DSP). These findings provide new insights into the pathophysiology of DSP and may help advance the understanding of the mechanisms responsible of the gastrointestinal manifestations.

Acknowledgements The research leading to these results has received funding from the following grants. From Ministerio de Ciencia e Innovacion, Grant CPP2021-008447 funded by MCIN/AEI/10.13039/501100011033 and by The European Union Next Generation EU/PRT. From Ministerio de Ciencia, Innovacion y Universidades, la Agencia Estatal de Investigacion y FEDER PID2024-1594180B-I00, PID2023-149618OB-I00. From Conselleria de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia, GRC (GI-1682-2025). From Interreg EAPA-0032/2022 – BEAP-MAR and EAPA_0130/2024-REVALGAE (cofounded by the EU), and from European Union HORIZON-CL6-2023-CIRCBIO-01 COMBO – 10113543.

Session 5: Pharmacogenetics and Personalized Medicine

Moderator:

Lucía Gandía (Universidade Autónoma de Madrid)

Invited speaker:

16:30-17:00 Francisco Abad Santos (Universidad Autónoma de Madrid)
Clinical Implementation of Pharmacogenetic Biomarkers in Routine Practice: From Evidence to Personalized Prescribing

Selected oral communications:

17:00-17:15 Luis Sendra (Universidad de València)
Precision Medicine in Pediatric Oncology: A Pharmacogenetic Approach

17:15-17:30 Ángela Jiménez Ortas (Universidad de Granada)
TNAP haplodeficiency leads to dysregulation of bile acid homeostasis and increased susceptibility to clavulanic acid–induced cholestasis

Clinical Implementation of Pharmacogenetic Biomarkers in Routine Practice: From Evidence to Personalized Prescribing

Francisco Abad-Santos

Clinical Pharmacology Department, Hospital Universitario de La Princesa, Facultad de Medicina, Universidad Autónoma de Madrid

E-mail: francisco.abad@uam.es

Interindividual variability in drug response remains a major challenge in clinical practice, leading to therapeutic failure and adverse drug reactions. Pharmacogenetics has emerged as a key tool to optimize drug selection and dosing, contributing to the development of precision medicine. Over the last two decades, several pharmacogenetic biomarkers have been progressively incorporated into routine clinical practice, supported by robust scientific evidence and international guidelines.

This presentation reviews the main pharmacogenetic markers currently used in daily clinical practice, focusing on their clinical utility, level of evidence, and implementation strategies. Relevant examples include thiopurine methyltransferase (TPMT) and NUDT15 for thiopurines, HLA-B*57:01 for abacavir hypersensitivity, CYP2C19 for clopidogrel response, DPYD for fluoropyrimidine toxicity, CYP2D6 for opioids and psychotropic drugs, CYP2C9 for drugs such as siponimod and anticoagulants. and SLCO1B1 for statins.

In addition, pharmacogenetic testing has been formally incorporated into the portfolio of services of the Spanish National Health System (SNHS), reflecting its clinical relevance and impact on patient care. This catalog includes a defined set of genes and drug-gene pairs for which testing is recommended or required before treatment or when an adequate response is not achieved. The inclusion of these biomarkers in the SNHS service portfolio aims to ensure equitable access to pharmacogenetic testing, standardize clinical practice, and promote the safe and effective use of medicines across healthcare settings.

These biomarkers illustrate different clinical scenarios, including prediction of severe toxicity, identification of non-responders, and dosage individualization. In some cases, genotyping has become mandatory prior to treatment initiation, while in others it provides actionable recommendations that improve efficacy and safety. The integration of these results into electronic health records, together with clinical decision support systems, enables real-time guidance for prescribers.

Despite these advances, the widespread implementation of pharmacogenetics still faces several challenges, such as cost, turnaround time, complexity of interpretation, and limited awareness among clinicians. Emerging strategies, including pre-emptive genotyping panels and integration into healthcare systems, are expected to overcome these barriers and increase clinical adoption.

In conclusion, pharmacogenetic biomarkers are already part of routine care for selected drugs and clinical situations. Their broader implementation will require multidisciplinary collaboration, education of healthcare professionals, and further evidence demonstrating clinical and cost-effectiveness. Pharmacogenetics represents a paradigm shift towards more personalized, safe, and effective pharmacotherapy.

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Precision Medicine in Pediatric Oncology: A Pharmacogenetic Approach

Gladys G. Olivera Pasquini[1,2], Enrique Gabriel Zucchet[1], Luis Sendra[2], Julia Balaguer[3], María del Mar Andrés Moreno[3], María Teresa Tormo[3],María José Herrero Cervera[1,2], Adela Cañete Nieto[3]

1. Pharmacogenetics and Gene Therapy Unit, La Fe Health Research Institute, Valencia, Spain.
2. Department of Pharmacology, Universitat de València, Valencia, Spain
3. Pediatric Hematology-Oncology Unit. Hospital Universitario y Politécnico La Fe, Valencia, Spain

E-mail: luis.sendra@uv.es

Introduction. Cancer is the leading cause of childhood mortality in developed countries. Although survival rates are increasing, the adverse effects of chemotherapy remain a major challenge. In this context, pharmacogenetics contributes to improving treatment efficacy and reducing adverse effects. While pharmacogenetics has already been implemented in clinical practice, further research is needed on variants for which evidence is still insufficient, particularly in the field of pediatric oncology. The main objective of this study was to analyze these variants in order to clarify their clinical relevance and provide new data supporting their potential clinical utility.

Materials and Methods. Patients were recruited from the Pediatric Oncology Unit at Hospital Universitario La Fe. Two cohorts were studied, Cohort A (n = 95) was genotyped using the Neuroblastoma pharmacogenetics panel (96 SNPs), while Cohort B (n = 215) was genotyped using the "VIP Onco" panel (63 SNPs). MassARRAY technology (Agena Bioscience) and the Titan-Axiom Array Spanish BioBank platform were used. Clinical data regarding treatment efficacy and toxicity were obtained from electronic medical records according to CTCAE v4.03 criteria. Statistical analyses were performed using penalized logistic regression models, including Elastic Net and LASSO, as well as Cox regression models.

Results. For neuroblastoma, variants associated with severe toxicities were identified in the genes *XPC, SEMA3C, ERCC2, MTHFR, SLCO1B1, SLIT1, and SLC22A16*. Variants associated with response to induction treatment were found in *ABCB1, ABCC2, CBR3, MAP3K1, NQO2, SLCO1B1, VDR*, and *VEGFA*. Survival was mainly associated with variants in the *MTHFR* gene, improving prognostic stratification when combined with clinical factors such as *MYCN* status. In addition, several genes were associated with event-free survival, including *ABCB1, SOD2, NR1I2, ABCC1,* and *VDR*. In Cohort B, variants associated with hematological, gastrointestinal, and infectious toxicities, among others, were identified following administration of polychemotherapy cycles in genes such as *CYP2C19, XRCC1, ESR1*, and *MTHFR*. Novel findings of treatment-related toxicities were identified in genes including *CYP3A4, ENOSF1, ESR1, DYNC2H1, MTRR*, and *SLCO1B1*. Variants in *MTHFR* and *SLC19A1* were associated with overall survival, while variants in *ERCC1* and *PTEN* were associated with relapse.

Conclusion. These findings support the value of pharmacogenetic research in pediatric oncology to advance toward safer and more personalized clinical treatments.

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TNAP haplodeficiency leads to dysregulation of bile acid homeostasis and increased susceptibility to clavulanic acid–induced cholestasis

Ángela Jiménez Ortas[1], Sonia Sánchez Campos[2,3], Victoria García Mediavilla[2,3], Esther Nistal González[2,3], Marta Moreno Torres[4], Fermín Sánchez de Medina[5], Ramiro Jover[4], Olga Martínez Augustin[1]

[1]Department of Biochemistry and Molecular Biology II, CIBEREHD, ibs.GRANADA, University of Granada
[2]Department of Biomedical Sciences, University of León
[3]Instituto de Investigación Biosanitaria de León (IBIOLEON)
[4]Departament of Biochemistry and Molecular Biology, CIBEREHD, Universitat de València
[5]Department of Pharmacology, CIBEREHD, ibs.GRANADA, University of Granada

E-mail: ajimenezo@ugr.es

Clavulanic acid, widely used in combination with β-lactam antibiotics, is one of the leading causes of drug-induced liver injury (DILI), frequently associated with an idiosyncratic cholestatic phenotype. However, the mechanisms underlying differential susceptibility to this hepatotoxicity remain poorly understood. Tissue-nonspecific alkaline phosphatase (TNAP) may play a role in modulating hepatic bile acid homeostasis and the response to liver injury.

In this study, we evaluated the effects of oral clavulanic acid administration in wild-type (WT) and TNAP$^{+/-}$ mice under acute (24 h) and subchronic (7 days) exposure conditions. Hepatic gene expression analysis at 24 h revealed activation of bile acid sensing pathways, as demonstrated by increased expression of *Nr1h4* (FXR) and its downstream target *Nr0b2* (SHP). This activation was significantly more pronounced in TNAP$^{+/-}$ mice compared to WT animals, indicating a genotype-dependent response. Despite this activation of the FXR–SHP regulatory axis, TNAP$^{+/-}$ mice showed a marked increase in *Cyp7a1* expression, the rate-limiting enzyme in bile acid synthesis, contrasting with the expected FXR-mediated repression. This response suggests a dysregulation of bile acid homeostasis, characterized by an impaired negative feedback loop controlling bile acid synthesis. Consistent with this, plasma bile acid (BA) levels were measured and revealed high interindividual variability across treated animals. Despite this heterogeneity, partial TNAP deficiency was associated with a markedly increased susceptibility to severe cholestasis, with TNAP$^{+/-}$ mice exhibiting approximately a fivefold higher risk of developing severe BA accumulation (BA > 1000 µM) compared to WT animals.
Overall, these results indicate that TNAP deficiency leads to a dysregulated hepatic response to clavulanic acid, characterized by impaired bile acid homeostasis and increased risk of severe cholestasis. This model provides a valuable platform for investigating mechanisms underlying idiosyncratic DILI and susceptibility factors.

Session 6: Teaching Innovation in Pharmacology

Moderator:

Josep Eladi Baños (Universitat de Vic)

Invited speaker:

16:30-17:00 Ricardo A. Peña Silva (Asociación Colombiana de Farmacología; ACF)
Innovación en educación en farmacología: metacognición e inteligencia artificial

Selected oral communications:

17:00-17:15 Isabel García-Arnandis (Universidad de València)
AI-Generated comics as a tool for health communication training in pharmacy students

17:15-17:30 Inmaculada Bellido Estévez (Universidad de Málaga)
Impact of an AI-generated virtual patient in the setting of an interactive structured objective clinical examination on therapeutic decision

Innovación en educación en farmacología: metacognición e inteligencia artificial

Ricardo A. Peña Silva MD PhD

Asociación Colombiana de Farmacología; ACF

Email: rpena@uniandes.edu.co

Introducción:
La enseñanza de la farmacología enfrenta retos derivados del crecimiento y la complejidad del conocimiento biomédico, y la necesidad de promover un aprendizaje significativo y sostenible. Aunque la inteligencia artificial ofrece nuevas oportunidades para transformar los procesos educativos, su implementación debe integrarse con estrategias pedagógicas fundamentadas en la evidencia. El objetivo de este trabajo es presentar un conjunto de innovaciones educativas desarrolladas para favorecer un aprendizaje más efectivo en farmacología mediante la integración de metacognición, simulación, analítica de datos e inteligencia artificial.

Métodos:
Se implementó un enfoque de innovación educativa basado en intervenciones progresivas dentro de cursos de farmacología. Las estrategias incluyeron el diseño de rutas de aprendizaje y matrices de actividades para hacer explícita la progresión curricular; simuladores virtuales y actividades de comunicación científica para diversificar las experiencias de aprendizaje; clínicas de memoria y diarios reflexivos para fortalecer la metacognición; y herramientas de inteligencia artificial y analítica de datos para analizar bienestar, hábitos de estudio y patrones de aprendizaje de los estudiantes.

Resultados:
Las estrategias implementadas facilitaron una mayor claridad sobre la estructura de la área y el curso, promovieron la reflexión sobre los propios procesos de aprendizaje y favorecieron experiencias activas centradas en la resolución de problemas. La incorporación de diarios fue percibida favorablemente por los estudiantes, quienes reportaron beneficios en la organización del tiempo, el establecimiento de metas y la reflexión sobre su aprendizaje. Adicionalmente, la integración de analítica de datos permitió explorar patrones de bienestar y desempeño con potencial para orientar intervenciones educativas personalizadas.

Conclusiones:
La combinación de principios de aprendizaje basados en evidencia, estrategias metacognitivas e inteligencia artificial representa una aproximación factible para fortalecer la educación en farmacología. Pequeñas innovaciones implementadas de manera sistemática pueden contribuir a un aprendizaje más inteligente, centrado en el estudiante y soportado en datos, proporcionando información útil para optimizar tanto los procesos de enseñanza como el acompañamiento académico.

Acknowledgements Estudiantes de farmacología

AI-Generated comics as a tool for health communication training in pharmacy students

Isabel García-Arnandis[1,2], Isabel Andújar[1], M. Carmen Recio[1,2], M. Luisa Ferrándiz[1,2]

[1]University of Valencia, Burjassot, Spain, [2]IDM, Valencia, Spain

E-mail: isabel.garcia-arnandis@uv.es

The incorporation of artificial intelligence (AI) into higher education is opening new possibilities for active learning and the development of communication skills in health sciences. The aim of this study was to describe an educational activity based on the use of generative AI for creating health-related comics and to explore students' perceptions of its usefulness in pharmacy education.

For this purpose, a descriptive educational experience was conducted with undergraduate pharmacy students. Participants designed comics aimed at communicating health information to a general audience using generative AI tools. After the activity, students completed an anonymous questionnaire with Likert-scale items (1–5) assessing satisfaction, perceived learning, usefulness for communication, and attitudes toward AI. Data were analysed using descriptive statistics, including frequencies and percentages.

Students reported a positive perception of the activity, particularly regarding its interest and usefulness for developing health communication skills. The perceived contribution to knowledge acquisition was more moderate. Most participants considered AI a helpful tool to support learning and expressed willingness to use it in future educational activities. However, confidence in the reliability of AI-generated outputs was limited, and students generally did not perceive AI as a substitute for teaching staff. The importance of using AI in a critical and responsible manner was widely acknowledged. These findings suggest a favorable but cautious student perspective toward the integration of AI in teaching.

These findings suggest that the use of generative AI for comic creation appears to be a feasible and adaptable approach to support communication skills and digital competence in pharmacy students. Its educational value may benefit from structured, guided, and context-specific implementation in higher education settings.

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Impact of an AI-generated virtual patient in the setting of an interactive structured objective clinical examination on therapeutic decision.

Inmaculada Bellido Estevez[1], Alejandro Barroso González[1,2], Aida Raigón Ponferrada[1,3], José Luis Guerrero Orriach[1,3], María Victoria Bellido Estevez[2], Aurelio Gómez Luque[1,3], Encarnacion Blanco-Reina[1]

[1]Department of Pharmacology, School of Medicine, University of Malaga. IBIMA.
[2]Service of Anesthesiology, Resuscitation and Pain Therapy. Regional University Hospital, Malag
[3]Service of Anesthesiology, Resuscitation and Pain Therapy. Virgen de la Victoria University Hospital, Malaga. Spain

E-mail: ibellido@uma.es

Introduction: The Objective Structured Clinical Examination (OSCE) is a widely used methodology for assessing clinical competencies in health sciences education. However, its implementation often requires substantial human resources, time, and financial investment. Generative artificial intelligence (AI) offers new opportunities to transform OSCEs into more interactive, adaptive, and student-centered learning experiences.
Objective: To evaluate the impact of an AI-generated virtual patient integrated into an interactive OSCE (AI-OSCE) on communication, clinical reasoning, pharmacological learning, and therapeutic decision-making skills.
Methods: A prospective educational study was conducted with medical students enrolled in Anaesthesia and podiatry students enrolled in Pharmacology. Students worked in groups of up to seven participants. Each group completed a 5–10-minute AI-OSCE based on a virtual patient ("Maria") presenting an allergy to amoxicillin-clavulanic acid. Students interacted with the patient through natural language, obtaining information about medical history, symptoms, diagnostic tests, treatments, and clinical evolution while searching for supporting evidence. The AI generated adaptive responses according to students' actions: relevant information was only provided when appropriate questions were asked; omitted questions regarding medical history or medication were not spontaneously answered. Failure to recognize anaphylaxis within 5 minutes triggered progressive clinical deterioration (SpO_2 88%, blood pressure 80/50 mmHg), requiring reassessment and urgent intervention. After completion, each group presented its management strategy to the class, allowing comparison of different clinical approaches derived from interactions with the same AI-generated patient. Student satisfaction was assessed through a voluntary survey.
Results: Thirty-five students participated (71.4% women; mean age 20 ± 2.5 years). Five AI-OSCE scenarios were completed and discussed. The average time required for case resolution and presentation was 1.2 ± 0.5 hours. The activity was associated with improved performance in the final course assessment. Overall satisfaction reached 96.5%. Students reported that the experience enhanced communication skills, clinical reasoning, and understanding of how different interactions with the same patient can lead to distinct therapeutic decisions and outcomes.
Conclusions: The incorporation of a virtual patient created using generative AI in the scenario of an interactive objective structured clinical examination (OSCE) improved participation, active learning, self-reflection, communication, acquisition of clinical skills and exam scoring for medical and podiatry students.

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Session 7: Cardiovascular & Renal Pharmacology

Moderator:

Ana Briones Alonso (Universidad Autónoma de Madrid)

Invited speaker:

9:00-9:30 Mª Dolores Sánchez Niño (Hospital Universirtario Fundación Jiménez Díaz; Universidad Autónoma de Madrid)
Pharmacological modulation of the kidney gerosuppressive function

Selected oral communications:

9:30-9:45 Carla Maya López (Instituto de Investigación Sanitaria Fundación Jiménez Díaz)
SCaMC-1 deficiency aggravates acute kidney injury severity

9:45-10:00 Mª José Jiménez Aragón (Universidad de Granada)
Transfer of aged gut microbiota induces vascular and immune aging features in germ-free mice

10:00:10:15 Isabel Sánchez Pérez (Universidad Autónoma de Madrid)
Reduced Vascular and Endothelial Toxicity of Iodido Platinum(Ii) Complexes Compared with Cisplatin: Cellular and Functional Insights

10:15-10:30 Cristina Arce Recatalá (Universitat de València)
Community Pharmacy as a Frontline for Detection of Chronic Kidney Disease Risk and Medication Optimizatio

Pharmacological modulation of the kidney gerosuppressive function

María Dolores Sánchez Niño[1,2,3]

[1] *Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain*
[2] *RICORS2040, Madrid, Spain*
[3] *IIS-Fundacion Jimenez Diaz, Madrid, Spain*

E-mail: mariadolores.sanchez@uam.es; mdsanchez@fjd.es

Chronic kidney disease is the fastest-growing global cause of death and is projected to become the fifth leading cause of death by 2040. The phenotype of chronic kidney disease has been likened to accelerated biological aging. A better understanding of the mechanisms that mediate accelerated biological aging can help in designing new treatments that increase the survival of kidney patients.

Klotho is a protein originating in kidney tubules that has gerosuppressor (anti-aging), anti-inflammatory, and antifibrotic properties. When chronic kidney disease progresses, Klotho production is the first renal function to be lost, before the glomerular filtration rate declines. Since the tubular structure is essentially normal when Klotho production is lost, we postulated that there are mediators that decrease Klotho production in tubular cells that still could express Klotho. In recent years, our laboratory has investigated the mediators that regulate Klotho production during kidney damage. Both albuminuria, which is often the earliest marker of chronic kidney disease, and local inflammation decrease Klotho production by renal tubular cells. Specifically, cytokines such as TNFα and TWEAK decrease tubular Klotho production in an NFκB-dependent manner and through epigenetic mechanisms such as histone acetylation. Furthermore, several NFκB regulatory proteins modulate its impact on renal Klotho production. We have characterized a set of external ligands, intracellular signaling mechanisms, and transcription factors that modulate kidney Klotho expression. For example, GDF15 increases Klotho production and protects the kidneys. The transcription factor Fosl1 has a similar effect; it increases during acute kidney injury in an adaptive manner to preserve Klotho and limit the severity of the injury. From a clinical perspective, nephroprotective drugs such as SGLT2 inhibitors also preserve renal Klotho production. Developing strategies to maintain the gerosuppressive function of the kidneys may not only slow the loss of kidney function but also limit the systemic impact on biological aging.

Acknowledgements: ISCIII FIS/Fondos FEDER PI24/00079, Universidad Autónoma de Madrid.

SCaMC-1 deficiency aggravates acute kidney injury severity

Carla Maya López[1,2], Aranzazu Pintor Chocano[1,2], Alberto Ortiz[1,2,3], María Dolores Sánchez Niño[1,2,3]

[1] *IIS-Fundacion Jimenez Diaz, Madrid, Spain*
[2] *RICORS2040, Madrid, Spain*
[3] *Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain*

E-mail: carla.maya@iis-fjd.es

Acute kidney injury (AKI) represents an important global health issue, affecting around 13.3 million people worldwide and accounting for nearly 1.7 million deaths each year (1). Due to the limited availability of early diagnostic tools and the lack of effective therapeutic strategies, AKI remains a major clinical need and a key priority for biomedical research.

Slc25a24 encodes the SCaMC-1 protein, a mitochondrial carrier involved in ATP-magnesium/inorganic phosphate transport and in buffering calcium levels in the mitochondria matrix, protecting cells against oxidative stress-induced cell death (2). However, the expression and function of Slc25a24 in the kidney have not been characterized. We explored the role of Slc25a24 in AKI.
In a transcriptomics array of murine nephrotoxic AKI, *Slc25a24* was identified as one of the most upregulated genes encoding mitochondrial proteins. The functional implications of *Slc25a24* upregulation were explored in mice and in cultured murine proximal tubular cells.

Slc25a24 expression was increased during murine nephrotoxic AKI induced by cisplatin or a folic acid overdose and in human AKI. *Slc25a24* overexpression in AKI was validated at the mRNA and protein levels and localized mainly to proximal tubules. In cultured murine proximal tubular cells, the pro-inflammatory cytokine TWEAK increased Slc25a24 expression.

Slc25a24-deficient mice developed more severe AKI (folic acid or cisplatin) as characterized by lower kidney function, more severe kidney inflammation and cell death and the nephroprotective gene Klotho downregulation. In cultured murine proximal tubular cells, *Slc25a24* downregulation induced by specific small interfering RNA resulted in increased the proinflammatory response and cell death and downregulated Klotho and in the sensitization to inflammation induced by TWEAK or TWEAK/TNFα/interferon-γ.

In conclusion, our findings identify Slc25a24 as a potential nephroprotective factor in AKI, highlighting it as a promising candidate for future therapeutic strategies.

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Transfer of aged gut microbiota induces vascular and immune aging features in germ-free mice

María Jiménez-Aragón[1,*], Sofía Miñano[1,*], Cristina González-Correa[1,2], Cristina Luque-Jiménez[1], Elena Gálvez-Gutierrez[1], Juan Duarte[1,2,3], Néstor de la Visitación[1], Marta Toral[1,2,3]

[1]Department of Pharmacology, University of Granada, 18071 Granada, Spain. [2]Instituto de Investigación Biosanitaria de Granada, ibs.GRANADA, Granada, Spain. [3]Ciber de Enfermedades Cardiovasculares (CIBERCV), Spain
**These authors contributed equally as first authors.*

*E-mail: mariajimenez@ugr.es*Abstract

Background Aging is associated with endothelial dysfunction, hypertension, chronic low-grade inflammation, and T-cell senescence. Age-related alterations in gut microbiota composition have emerged as potential contributors to these processes, although their causal role in vascular aging remains poorly understood.

Objective To determine whether aged gut microbiota promotes vascular dysfunction and T-cell senescence.

Design Germ-free mice were colonized with fecal microbiota from young (3-month-old) or aged (22-month-old) donor mice and maintained for six weeks. Vascular, and immune parameters were subsequently evaluated.

Results Recipients of aged microbiota developed higher blood pressure. Aged microbiota impaired endothelium-dependent relaxation in both aorta and mesenteric resistance arteries. In the aorta, impaired acetylcholine-induced relaxation was improved by incubation with the pan-NOX inhibitor VAS2870 or the Rho kinase inhibitor Y27632, suggesting the involvement of redox-sensitive pathways previously linked to immune-mediated vascular injury. In mesenteric resistance arteries, aged microbiota impaired both nitric oxide- and endothelium-derived hyperpolarization-dependent components of acetylcholine-induced relaxation. Moreover, aged microbiota promoted T-cell senescence, as evidenced by increased PD-1 and KLRG1 expression in mesenteric lymph node T lymphocytes.

Conclusion Transfer of aged gut microbiota is sufficient to induce hypertension, endothelial dysfunction, and hallmarks of T-cell senescence in germ-free mice. These findings demonstrate that age-associated gut dysbiosis transfers a vascular aging phenotype and support a central role for the microbiota–immune–vascular axis in age-related cardiovascular dysfunction.

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Reduced Vascular and Endothelial Toxicity of Iodido Platinum(Ii) Complexes Compared with Cisplatin: Cellular and Functional Insights

Jorge Melones-Herrero[1,4] Ana Cuadrado-Gómez[1], Alicia Villacampa[1], Sofía Figueiras[1], Oscar Lorenzo [3], Carlos F. Sánchez-Ferrer[1], Concepción Peiró[1], Isabel Sánchez-Pérez[1,2,4]

[1] Facultad Medicina. UAM, Madrid, Spain; [2.]Instituto de Investigaciones Biomédicas Sols-Morreale (CSIC-UAM), Madrid, Spain; [3.]Instituto de Investigaciones Sanitarias-Fundación Jimenez Díaz,CIBERDEM, UAM, Madrid, Spain; [4.]IRYCIS, Madrid, Spain

E-mail: is.perez@uam.es

Platinum-based chemotherapy remains a mainstay in the treatment of gastrointestinal cancers, although toxicity frequently limits its clinical use. We previously identified the iodido platinum (II) complexes ***trans***-[PtI_2(isopropylamine)$_2$] (I5) and ***cis***-[PtI_2(isopropylamine)$_2$] (I6) as promising antitumor agents with potent activity against gastrointestinal cancer models and reduced systemic toxicity in vivo (1). Since cardiovascular toxicity and endothelial dysfunction represent major complications associated with platinum-based therapies (2), this study aimed to characterize the cardiovascular safety profile of these compounds.

Human cardiomyoblasts (AC16) and human umbilical vein endothelial cells (HUVECs) were exposed to cisplatin (CDDP), I5 or I6. Cell viability, clonogenicity, oxidative stress, DNA damage and senescence were evaluated by MTS assays, colony formation, dihydroethidium fluorescence, γH2AX staining and senescence-associated β-galactosidase activity. Stress and inflammatory signaling pathways were analyzed by Western blot and RT-qPCR. Endothelial migration was assessed using wound-healing assays, while microvascular function was examined *ex vivo* in isolated mouse mesenteric arteries using wire myography.

Compared with CDDP, I5 and I6 displayed substantially lower toxicity, with higher IC_{50} values in both AC16 cardiomyoblasts (CDDP: ~5 µM; I5: ~12 µM; I6: ~13 µM) and HUVEC endothelial cells (CDDP: ~21 µM; I5: ~31 µM; I6: ~34 µM), while preserving long-term proliferative potential. I6 exhibited minimal effects across all experimental conditions. I5 induced moderate reactive oxygen species production in AC16 cells associated with partial reduction of clonogenicity, suggesting adaptive redox signaling rather than overt toxicity. Both compounds caused limited DNA damage and minimal induction of senescence compared with CDDP. Mechanistically, CDDP activated p53/p38 stress signaling and inflammatory pathways, whereas I5 and I6 induced a restricted adaptive response characterized by p16 upregulation without relevant induction of inflammatory mediators. Functionally, I5 induced only transient effects on endothelial migration while preserving endothelial-dependent vascular relaxation ex vivo (90 ± 4% versus 68 ± 6% for CDDP).

These findings further support a more favorable safety profile of iodido platinum(II) complexes and identify preservation of endothelial function together with reduced cardiovascular toxicity as additional advantages for their future translational development as anticancer agents.

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Community Pharmacy as a Frontline for Detection of Chronic Kidney Disease Risk and Medication Optimization

Gema Escribá Martí[1], Cristina Arce Recatalá[1], Luis Salar Ibañez[2]

[1]Universitat de València, Burjassot, Spain,
[2]CEU Universidad Cardenal Herrera, Valencia, Spain

E-mail: Cristina.arce@uv.es

Chronic Kidney Disease (CKD) is a highly prevalent and underdiagnosed public health problem, often described as a "silent epidemic", which is strongly associated with aging, multimorbidity, and polypharmacy. Early detection through estimated glomerular filtration rate (eGFR) assessment and optimization of pharmacotherapy are essential to prevent disease progression and nephrotoxicity [1]. Community pharmacies represent a strategic healthcare setting for CKD screening and nephroprotective interventions due to their accessibility and capacity to identify potentially inappropriate prescriptions (PIPs) [2, 3]. The aim of this study was to evaluate the effectiveness of a community pharmacist intervention focused on nephroprotection and optimization of PIPs in patients at risk of CKD.

A prospective, multicenter, analytical study was conducted in 16 Spanish community pharmacies. Patients with CKD risk factors were included. Renal function was assessed using creatinine measurements obtained through previous laboratory tests or point-of-care testing (StatSensorXpress®), and eGFR was calculated using the CKD-EPI 2009 equation. Patients with eGFR <60 ml/min/1.73 m² underwent a structured pharmacotherapeutic review to identify PIPs, including unadjusted doses, nephrotoxic drugs, and high-risk drug combinations. Pharmaceutical interventions consisted of dose adjustment recommendations or drug withdrawal proposals communicated to primary care physicians.

A total of 818 patients were included (mean age 72.9 years; 6.7 drugs/patient), showing a high prevalence of polypharmacy. The prevalence of eGFR <60 ml/min/1.73 m² was 34.8% (n=285), and impaired renal function was significantly associated with advanced age and increased medication burden. Among patients with eGFR <60 ml/min/1.73 m², 40.7% presented at least one PIP, with prevalence increasing according to CKD stage. A total of 195 pharmaceutical interventions were performed, mainly involving dose adjustments (71.8%) rather than drug withdrawal (28.2%). The most frequently involved medications included metformin, olmesartan, hydrochlorothiazide, and fenofibrate. Primary care physicians accepted 28.9% of the pharmacist's recommendations, with significantly higher acceptance rates for drug withdrawal compared with dose adjustments.

Reduced renal function is highly prevalent among older patients attending community pharmacies and is closely associated with polypharmacy. Community pharmacists play a key role in the early detection of impaired renal function, identification of PIP, and implementation of nephroprotective strategies including referral to primary care for dose adjustment or withdrawal medications recommendations.

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Session 8: Pharmacology of ion channels

Moderator:

Ricardo Borges (Universidade de La Laguna)

Invited speaker:

9:00-9:30 Antonio Vicente Ferrer Montiel (Universidad Miguel Hernández)
TRP channels in chemotherapy induced peripheral neuropathy

Selected oral communications:

9:30-9:45 Ricardo Caballero (Universidad Complutense de Madrid)
A novel FKBP12 Ligand (AHK) shortens cardiac action potential duration and inhibits L-type Ca^{2+} and Na^{+}-Ca^{2+} exchanger currents

9:45-10:00 Leandro Zúñiga (Universidad de Talca; Chile)
Rational Design and Functional Evaluation of Novel Peptide Inhibitors Targeting the Oncogenic Potassium Channel TASK-3

10:00:10:15 Carmen Nanclares (Universidad Autónoma de Madrid)
Involvement of P2X7 receptors in the pathophysiology of ALS and their characterization as a potential therapeutic target in $SOD1^{G93A}$ mice

10:15-10:30 Pablo Aránguiz (Pontificia Universidad Católica de Valparaíso; Chile)
Blockade of HCN Channels by Ivabradine Mitigates Myocardial Fibrosis through Inflammasome Modulation

TRP channels in chemotherapy induced peripheral neuropathy

Antonio Ferrer Montiel

Instituto de Investigación en Biotecnología y Salud (IDiBE). Universidad Miguel Hernández. Elche

E-mail: aferrer@umh.es

Abstract

Chemotherapy-induced peripheral neuropathy (CIPN) is a highly prevalent and debilitating complication of cancer treatment, affecting up to 90% of patients receiving certain chemotherapeutic agents. It is characterized by sensory disturbances including paresthesia, dysesthesia, spontaneous and burning pain, and mechanical and thermal hypersensitivity, predominantly in the hands and feet. While symptoms resolve after treatment cessation in some patients, they can persist for more than a year in up to 40% of cases and frequently require chemotherapy dose reduction or discontinuation. To investigate the mechanisms underlying CIPN, we developed a long-term in vitro nociceptor model that enabled the study of sensory neuron sensitization and recovery following exposure to chemotherapeutic drugs. Exposure to paclitaxel or oxaliplatin increased spontaneous neuronal activity and repetitive action potential firing, indicating enhanced excitability of nociceptors. This sensitization was reversible, peaking approximately 48 hours after treatment and gradually resolving after drug withdrawal. Mechanistically, paclitaxel increased the activity and expression of TRPV1 and TRPM8 channels, whereas oxaliplatin enhanced TRPV1 and TRPA1 function and expression. Repeated paclitaxel exposure further demonstrated a key role for thermoTRP channels in the persistence of CIPN during cumulative chemotherapy cycles. These findings identified TRPV1 as a promising therapeutic target. Using the capsaicin pharmacophore as a scaffold, we developed AG1529, a non-pungent TRPV1 antagonist with micromolar potency and limited activity at related thermoTRP channels. Additional chemical modifications were introduced to minimize systemic exposure and reduce the risk of adverse effects such as hyperthermia. A topical anhydrous formulation containing 0.075% AG1529 was subsequently evaluated in a randomized, double-blind pilot clinical trial involving 142 cancer patients treated with taxanes and/or platinum-based drugs across nine hospitals. The treatment reduced both the incidence and severity of CIPN, supporting the concept that modulation of epidermal thermosensory pathways protects nociceptive terminals. Overall, these results demonstrate that peripheral targeting of sensory receptors represents a promising strategy for preventing and treating chemotherapy-induced neuropathy.

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DECA-11 Restores Nav1.5 and Kir2.1 Channel Expression in the Membrane

Sara Pérez-Martín[1], Josu Rapún[1], Alba De La Iglesia[1], Teresa Crespo-García[1], Luis Loya[1], Gorka San José[2], Begoña López[2], Aránzazu González-Miqueo[2], Eva Delpón[1], Ricardo Caballero[1]

1. Department of Pharmacology and Toxicology. School of Medicine. Universidad Complutense de Madrid. Instituto de Investigación Gregorio Marañón. CIBERCV, Instituto de Salud Carlos III, Madrid, Spain
2. Program of Cardiovascular Diseases, CIMA Universidad de Navarra and IdiSNA. CIBERCV, Instituto de Salud Carlos III, Pamplona, Spain
3.

E-mail: edelpon@ucm.es

Ventricular arrhythmias and ventricular fibrillation are the main cause of death in patients with heart failure (HF), who are estimated to be around 15 million in Europe. HF with reduced ejection fraction (HFrEF) induces an electrical remodeling characterized by downregulation of Nav1.5 and Kir2.1 channels (encoded by *SCN5A* and *KCNJ2* genes, respectively) at the cardiomyocyte membrane, reducing the Na^+ (I_{Na}) and inward rectifier K^+ (I_{K1}) current densities, thereby impairing ventricular excitability and delaying repolarization, factors that together constitute a highly arrhythmogenic substrate. We designed a gene therapy encoding an 11-mer peptide named DECA-11 (WO2025068623), capable of selectively increasing I_{Na} and I_{K1} in heterologous expression systems, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), and mouse ventricular cardiomyocytes that reduces VA risk in a mouse model of HF. In the present study, we aim to explore the mechanisms responsible of the effects of DECA-11. In spontaneously beating hiPSC-CMs, DECA-11 did not augment the late I_{Na} nor modify the amplitude and duration of intracellular Ca^{2+} transients or the contraction and relaxation speed at three frequencies (0.5, 1, and 1.8 Hz). Furthermore, DECA-11 did not modify the biophysical properties of Nav1.5 and Kir2.1 channels, as it had no effect on their conductance (γ), open probability (P_o) and kinetics characterized in single-channel experiments (cell-attached configuration of the patch-clamp technique). However, DECA-11 produced a pro-transcriptional effect on the promoter of the human *KCNJ2* gene (P=0.006) and increased *KCNJ2* mRNA expression levels (P=0.0003), while it did not modify the *SCN5A* gene transcription or mRNA expression levels. Flow cytometry experiments showed that DECA-11 augmented the number of fluorescent cells and their fluorescence intensity, which reflects an increase in the number of Nav1.5 and Kir2.1 channels at the cell membrane. Importantly, DECA-11 increased sarcolemmal and total expression of Nav1.5 and Kir2.1 channels ($P \leq 0.0001$) in ventricular samples of mice subjected to transverse aortic constriction. In conclusion, DECA-11 increases I_{Na} and I_{K1} by augmenting the sarcolemmal Nav1.5 and Kir2.1 channel expression without affecting their biophysical properties.

Acknowledgements

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Rational Design and Functional Evaluation of Novel Peptide Inhibitors Targeting the Oncogenic Potassium Channel TASK-3

Constanza Maldonado-Morales, Leslie contador, Rafael Zúñiga, Leandro Zúñiga

Laboratorio de Fisiología Molecular, Center for Nanomedicine, Diagnostic, and Drug Development (ND3), Facultad de Medicina, Universidad de Talca, Talca, Chile. Center for the Development of Nanoscience and Nanotechnology (CEDENNA), Proyecto CIA250002, Facultad de Medicina, Universidad de Talca, Talca, Chile,

E-mail: lzuniga@utalca.cl

The potassium channel TASK-3 (KCNK9) has emerged as a promising therapeutic target in cancer due to its overexpression and functional contribution to tumor progression, survival, and resistance mechanisms. This study aimed to develop and evaluate novel peptide inhibitors targeting TASK-3 through an integrated computational and experimental approach. A recently resolved Cryo-EM structure of TASK-3 (PDB: 8K1Z) was incorporated to refine the structural model used for peptide design and interaction prediction. Structural and physicochemical criteria were applied to identify candidate peptide sequences with potential affinity for the TASK-3 binding site. Candidate peptides were selected through phage display biopanning using HEK-293 cells heterologously expressing TASK-3 channels. Preliminary molecular docking analyses were performed to predict peptide–channel interactions, and functional effects were evaluated using electrophysiological assays in heterologous expression systems.

Two initial peptide candidates showed inhibitory effects on TASK-3 currents in HEK-293 cells, reducing channel activity by approximately 40% at 100 µM. Docking analyses supported the potential interaction of these peptides with the predicted binding region of the channel. In parallel, optimization of phage display selection conditions enabled the identification of seven additional peptide clones, which are currently undergoing sequencing, electrophysiological evaluation, and pharmacological characterization.

Overall, these findings establish a robust platform for the rational discovery and functional validation of TASK-3 inhibitory peptides. The integration of Cryo-EM-guided modeling, phage display screening, and electrophysiological characterization provides a promising framework for the development of novel anticancer therapeutics targeting oncogenic potassium channels.

Keywords: TASK-3, potassium channels, peptide inhibitors, phage display, electrophysiology, Cryo-EM, cancer pharmacology.

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Involvement of P2X7 receptors in the pathophysiology of ALS and their characterization as a potential therapeutic target in SOD1^{G93A} mice

María Cruz-Lavado[1], Adrián Gironda-Martínez[2]; Ricardo de Pascual[1], Sahil Adriouch[3], Luis Gandía[1], Carmen Nanclares[1]

1.-Depto. de Farmacología y Terapéutica, Universidad Autónoma de Madrid, Madrid, Spain.
2.-Depto. de Ciencias Biomédicas, Universidad de Alcalá de Henares, Madrid, Spain
3.-Normandie Univ, UNIROUEN, INSERM U1234, Pathophysiology, Autoimmunity and Immunotherapy(PanTHER), Rouen, France.

E-mail: carmen.nanclares@uam.es

Amyotrophic Lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disease characterized by progressive degeneration of upper and lower motor neurons, leading to muscle weakness, paralysis, respiratory failure and death within a few years after diagnosis1. Current treatments are limited to modestly slow disease progression, which highlights the urgent need for new therapeutic approaches2. In recent years, the purinergic receptor P2X7 (P2X7R) has been shown to be an important key contributor to ALS-associated neuroinflammation, making it an attractive target3. The aim of this study is to evaluate whether P2X7R modulation can influence disease progression in the SOD1^{G93A} mouse model by using a vector encoding nanobody-based construct:13A7, a P2X7R-blocking nanobody4. Wild type and SOD1^{G93A} mice will receive either saline or the nanobody-treatment, and disease progression will be monitored through body weight, neurological score, RotaRod and inverted screen test. Additionally, once the animals reach the humane endpoint, they will be euthanized under anesthesia, and the spinal cord will be extracted to determine the number of viable motor neurons (using ChAT staining) and to quantify neuroinflammatory markers (Iba-1 for microglia and GFAP for astrocytes) by means of Western blot and immunofluorescence techniques. We expect that P2X7R blockade will reduce disease-associated inflammation and preserve motor function, thereby slowing ALS progression and providing preclinical support for this receptor to be considered as a therapeutic target.

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Blockade of HCN Channels by Ivabradine Mitigates Myocardial Fibrosis through Inflammasome Modulation

Pablo Aránguiz

Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.

E-mail: pablo.aranguiz@pucv.cl

Background: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, particularly the HCN2 and HCN4 isoforms, contribute to cardiac automaticity, and their blockade with ivabradine (IVA) has shown potential antifibrotic effects. Cardiac fibroblasts (CFs) play a central role in myocardial fibrosis following ischemia/reperfusion (I/R) injury by activating the NLRP3 inflammasome, which subsequently releases profibrotic cytokines.

Aim: To evaluate the effects of IVA-mediated HCN channel inhibition on the fibrotic activity of CFs and its modulation of inflammasome activation.

Methods: CFs isolated from neonatal rat hearts were subjected to simulated I/R conditions (6 hours of ischemia, 16 hours of reperfusion) in the presence or absence of IVA (10 µM). NLRP3 inflammasome components were assessed by RT-qPCR and western blot. LPS (10 µM) and ATP (100 µM) served as positive controls for inflammasome activation.

Results: IVA treatment significantly reduced NLRP3 inflammasome activation, as evidenced by the decreased expression of NLRP3 and caspase-1 compared to the untreated I/R group. Notably, the expression of HCN2 and HCN4 was upregulated under ischemic conditions, which was prevented by IVA pretreatment. Additionally, IVA reduced collagen-I and alpha-SMA expression, indicating the inhibition of CF differentiation into myofibroblasts.

Conclusions: HCN channel blockade with IVA attenuates the profibrotic response in CFs, potentially through the downregulation of inflammasome activation. This highlights the potential of IVA as a therapeutic strategy to mitigate adverse cardiac remodeling and fibrosis post-I/R injury.

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Session 9: Digestive and Respiratory Pharmacology

Moderator:

Ana Mª Sahagún (Universidad de León)

Invited speaker:

15:00-15:30 Julio Gálvez (Universidad de Granada)
Bridging Experimental Research and Clinical Practice: Pharmacology of Thymus serpyllum extract (360GUT) in Functional Gastrointestinal Disorders

Selected oral communications:

15:30-15:45 Fernando de la Cuesta (Universidad Autónoma de Madrid)
Comparative analysis of two sources of biological nanovesicles with potential in Pharmacology: mesenchymal stem cells vs. Plants

15:45-16:00 Patrice Marques (Universidad de Valencia)
Platelet activation as a predictor of 3-year lung function decline in early-stage COPD

16:00:16:15 Carmen de la Fuente-Gómez (Universidad de Valencia)
Antifibrotic potential of BP-2, a pan-PPAR agonist, in metabolic dysfunction-associated steatohepatitis

16:15-16:30 Juan José Enguix-Huete (Universidad de Granada)
Effect of Endogenous and Exogenous Glucocorticoids on the Intestinal Barrier in Response to an In Vivo Model of COVID-19 Cytokine Storm

Bridging Experimental Research and Clinical Practice: Pharmacology of Thymus serpyllum extract (360GUT) in Functional Gastrointestinal Disorders

Julio Gálvez

Department of Pharmacology; University of Granada

E-mail: jgalvez@ugr.es

Functional gastrointestinal disorders, such as irritable bowel syndrome (IBS), involve complex pathophysiology encompassing gut-brain axis dysregulation, visceral hypersensitivity, impaired epithelial barrier integrity, and mucosal low-grade inflammation associated with gut dysbiosis. Addressing the need for safe, multi-targeted therapeutic options, we present a translational pharmacological trajectory, from basic research to clinical validation, evaluating a standardized extract of wild thyme (*Thymus serpyllum*), commercially designated as 360GUT. In rodent models of inflammatory bowel disease, post-inflammatory IBS, and high-fat diet-induced metabolic syndrome, oral administration of the extract demonstrated significant mucosal anti-inflammatory, visceral analgesic, gut barrier-protective, and prebiotic-like microbial-modulating properties. Translating these findings into a multicenter, randomized, double-blind, placebo-controlled human trial involving 169 subjects with persistent functional gastrointestinal discomfort, daily supplementation with 600 mg of the extract for 8 weeks resulted in a statistically significant reduction in overall gastrointestinal symptom burden assessed by the validated Gastrointestinal Symptom Rating Scale (GSRS), with marked improvements in indigestion, constipation, and diarrhea domains. In parallel, improvements were observed in health-related quality of life assessed by the FDDQL questionnaire, particularly in stress coping and disease control. Furthermore, shotgun metagenomic analysis revealed targeted remodeling of gut microbiota composition and functional metabolic pathways, favoring intestinal mucosal health. With an outstanding safety profile, this standardized *Thymus serpyllum* extract represents a scientifically validated, multi-targeted botanical intervention that successfully bridges preclinical pharmacology and clinical reality for the management of functional digestive discomfort.

Comparative analysis of two sources of biological nanovesicles with potential in Pharmacology: mesenchymal stem cells vs. plants

Miriam Morales-Rodríguez de Lope[1,2], Alan Rivera-Tenorio[1,2], Ángel Moreno-Luque[1,2], Ainara González-Moro[1,2], Estela Herranz[1,2], Milagros Castellanos[3], Álvaro Somoza[3], Carlos F. Sánchez-Ferrer[1,2], Concha Peiró[1,2]; Fernando de la Cuesta[1,2]

1. *Department of Pharmacology and Therapeutics. Universidad Autónoma de Madrid.*
2. *Instituto de Investigación Hospital Universitario La Paz (IdiPaz), Madrid.*
3. *IMDEA Nanociencia. Madrid, Spain.*

E-mail: fernando.delacuesta@uam.es

Extracellular vesicles (EVs) constitute innovative biological therapeutic agents and serve as vehicles for a plateau of drugs and molecules. Human mesenchymal stem/stromal cell-derived EVs (MSC-EVs) have demonstrated a great anti-inflammatory and pro-regenerative potential with minimum cytotoxicity. The main issues for EVs to reach approval from FDA and EMA are the manufacturing heterogeneity and scalability bottlenecks and a lack of validated potency assays. Besides, allogeneic use of human material implies important regulatory complications. As an alternative to human EVs, plant EVs and nanovesicles or NVs (which include intracellular vesicles), from edible vegetables, have arisen as a novel source of biological vesicles with anti-inflammatory effects, as well as biocompatible nanoparticles for drug delivery.

In this work, we aimed to develop optimized GMP-compliant scaled-up methods to produce MSC-EVs and plant NVs, performing a comparative analysis of their yield, reproducibility, stability, functionalization capacity and bioactivity. For MSC-EVs, immortalized MSCs were cultured in a hollow-fibre bioreactor. Liquid chromatography was scaled-up in a FPLC for both MSC-EVs and tea leaf NVs (TL-NVs). Vesicle purity was assessed calculating the vesicle/soluble protein ratio. Stability assays by nanotracking analysis (NTA) were performed over a period of 6 months to test conservation at -80 ºC in different buffers with the presence/absence of sugars (sacarose, trehalose) and human albumin. Bioactivity was tested setting up the following potency assays: wound-healing, tube formation and MTT in MSCs and human aortic endothelial cells (HAEC). Besides, potential as drug delivery vehicles was tested functionalizing these vesicles with quercetin (a serotherapeutic drug) and aptamers.

Although both MSC-EVs and TL-NVs were isolated with great purity, TL-NVs displayed greater yield and vesicle/soluble protein ratio. Both sources showed similar bioactivity in the potency assays with no signs of cytotoxicity up to 800,000 particles/cell. MSC-EVs demonstrated greater stability over time, even without any cryopreserving agent, while plant NVs required a conservation buffer. Besides, functionalization with quercetin was much more efficient in MSC-EVs, while TL-NVs required more aggressive permeabilization methods. Nonetheless, recovery of plant NVs after functionalization was superior.

In summary, we have been able to scale-up production of MSC-EVs and TL-NVs in a GMP-like environment with considerable reproducibility and each of these therapeutic agents and drug carriers have shown differential advantages for future clinical use.

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Platelet activation as a predictor of 3-year lung function decline in early-stage COPD

Patrice Marques[1,2,3], Irene Bocigas[1,4], Elena Domingo[2], Vera Francisco[2], Julia Tarrasó[2,4], Laura Piqueras[2,5,6], Jaime Signes-Costa[1,2,4], Cruz González[2,4], Maria-Jesús Sanz[1,2,6]

[1]Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain
[2]INCLIVA Biomedical Research Institute, Valencia, Spain
[3]CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Madrid, Spain
[4]Pneumology Unit, University Clinic Hospital of Valencia, Valencia, Spain
[5]Department of Pharmacology, Faculty of Pharmacy, University of Valencia, Valencia, Spain
[6]CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Madrid, Spain

E-mail: patrice.gomes@uv.es

Background: Chronic obstructive pulmonary disease (COPD) at an early stage (GOLD 1) remains scarcely characterized in terms of biological predictors of disease progression. We have previously demonstrated, in a cross-sectional baseline analysis, increased platelet hyperreactivity and upregulation of the CXCL16/CXCR6 axis in GOLD 1 individuals compared with healthy smokers and never-smokers [1, 2]. In this longitudinal follow-up study, we aimed to correlate these initial findings with pulmonary function tests performed three years later to identify potential biomarkers of disease progression and clinical outcomes.

Material and methods: A longitudinal study was conducted in 27 long-term smokers with normal lung function (pre-COPD) and 27 patients with mild COPD (GOLD 1). Eight baseline circulating inflammatory mediators related to platelets and the CXCL16/CXCR6 axis were previously selected [1, 2]. Pulmonary function tests were repeated after 3 years. Associations were first explored using Spearman/Pearson correlations and further analysed using univariable linear regression models. Finally, multivariable linear regression adjusted for age, sex, pack-years, and GOLD stage was performed to identify independent predictors of lung function.

Results: After Spearman/Pearson and univariable regression analyses, 7 and 2 baseline inflammatory biomarkers were significantly associated with DLCO and FEV1, respectively, suggesting a close link between systemic inflammation and both early parenchymal injury and airflow obstruction. However, after adjustment for age, sex, pack-years, and baseline GOLD stage, only platelet integrin $\alpha_{IIb}\beta_3$ (PAC-1+) expression remained an independent predictor of lung function decline at 3 years ($\beta1$ = -0.06466; p = 0.031; IC95%: [-0.1231 ; -0.0062]).

Conclusions: Platelet integrin $\alpha_{IIb}\beta_3$ emerges as an independent predictor of airflow obstruction at 3 years. These findings highlight platelet activation as a potential mechanistic link and a promising biomarker for early prediction of lung function decline in early COPD.

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Antifibrotic potential of BP-2, a pan-PPAR agonist, in metabolic dysfunction-associated steatohepatitis

Carmen de la Fuente-Gómez[1,2], Patrice Marques[1,2,3], Elena Domingo[1], Vera Francisco[1], Álvaro Gómez-Martín[1,2], Laura Vila[1], Laura Piqueras[1,4,5], Diego Cortes[1,4], Nuria Cabedo[1,4], María-Jesús Sanz[1,2,5]

[1] *INCLIVA Biomedical Research Institute, Valencia, Spain*
[2] *Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain*
[3] *CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Madrid, Spain*
[4] *Department of Pharmacology, Faculty of Pharmacy, University of Valencia, Valencia, Spain*
[5] *CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Madrid, Spain*

E-mail: delafue8@alumni.uv.es

Background: Metabolic dysfunction-associated steatohepatitis (MASH), the hepatic manifestation of metabolic syndrome (MS), is characterized by liver steatosis, hepatocellular damage and inflammation, often progressing to liver fibrosis, a condition for which effective pharmacological treatments are currently lacking. BP-2, a pan-PPAR agonist synthesized by our group, has previously been demonstrated to exert metabolic benefits and inflammation reduction in a murine model of MS. Therefore, in the present study, we evaluated the therapeutic potential of BP-2 in ameliorating liver steatosis, fibrosis, and inflammation in a murine model of MASH.

Material and methods: Six-week-old C57BL/6 male mice were either fed with a standard chow diet or a MASH-inducing diet (choline-deficient high-fat diet) for 12 weeks. At 8 weeks, MASH animals received subcutaneous administration of either vehicle solution or BP-2 (3 or 10 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. Expression of protein markers of interest was evaluated by Western blot after protein extraction from liver tissue. Immunophenotyping of hepatic immune cells was performed by flow cytometry.

Results: While BP-2 had no observable effect on liver steatosis at the two dosses assayed, the dose of 10 mg/kg/day significantly reduced liver collagen content and α-SMA protein expression, attenuating liver fibrosis in MASH mice. These effects were associated with reduced leukocyte infiltration in the liver, increased regulatory T lymphocytes infiltration, and shifted macrophage polarization towards an anti-inflammatory M2-like phenotype.

Conclusion: Our data support the potential of BP-2 as a therapeutic candidate for reducing liver fibrosis development in MASH, a condition for which pharmacological treatments are currently lacking. Nevertheless, further transcriptomic, proteomic and histological analyses are needed to explore the underlying mechanisms and signaling pathways.

Acknowledgments: This work was supported by the Spanish Ministry of Science, Innovation and Universities [PID2023-152677OB-I00]; the *Generalitat Valenciana* [CIPROM/2022/45, CIGE/2023/073, AICO/2024/321], the ISCIII [PI21/00220, CP21/00025, CD22/00045, PI25/001315, DTS25/00163] and co-funded by the European Union, and the University of Valencia and INCLIVA [VLC-Bioclinic Program; AP-2023-014]. The authors acknowledge Inés Descalzo Arenas and Jose Luis Aparicio Collado, from the Institute of Health Research INCLIVA, for their valuable contributions to this project.

Effect of Endogenous and Exogenous Glucocorticoids on the Intestinal Barrier in Response to an In Vivo Model of COVID-19 Cytokine Storm

Juan José Enguix-Huete[1],Diego Ceacero-Heras[2], Ángela Jiménez-Ortas[2], Guillermo Ruiz-Henares[1], Mireia Tena-Garitaoinaindia[2], Olga Martínez-Augustin[2], Fermín Sánchez de Medina[1]

1 *Department of Pharmacology, Faculty of Pharmacy, University of Granada, ibs.GRANADA, CIBERehd, Granada, Spain*
2 *Department of Biochemistry and Molecular Biology II, Faculty of Pharmacy, University of Granada, ibs.GRANADA, CIBERehd, Granada, Spain*

E-mail: jenguix@ugr.es

COVID-19 can lead to a 'cytokine storm', an intense inflammatory response that disrupts the intestinal barrier, contributing to systemic damage. While glucocorticoids are effective anti-inflammatory agents, they may also impair intestinal epithelial integrity. This study aims to evaluate the role of endogenous and exogenous glucocorticoids in an in vivo cytokine storm model and their impact on the intestinal barrier.

C57BL/6J mice of both sexes were used, including a group with inducible epithelial-specific deletion of the *Nr3c1* gene (glucocorticoid receptor) and a wild-type group. Mice were divided into two treatment conditions: dexamethasone (100 µg) or vehicle (PBS). Animals received an intraperitoneal injection of IL-6 (0.5 µg), IL-1β (0.5 µg), and TNF (1 µg), while controls received phosphate-buffered saline with 0.1% bovine serum albumin. Weight and temperature were monitored for five days. Following necropsy, RT-qPCR, Western blot, and enzymatic activity assays were performed on various tissues. In addition, corticosterone levels were measured by ELISA in plasma and colon explants.

Cytokine-treated mice developed diarrhea and significant weight loss. In intestinal epithelial *Nr3c1* knockout mice, we observed reduced expression of pro-inflammatory genes and altered levels of tight junction proteins compared to wild-type controls. At the systemic level, both lung and liver displayed a protective phenotype in the knockout mice. Finally, treatment with dexamethasone led to a consistent decrease in inflammatory markers compared to PBS-treated groups.

Our preliminary findings suggest that epithelial glucocorticoid receptor deletion confers a protective phenotype not only against cytokine-induced intestinal inflammation, but also systemic involvement, with variable effects on barrier function markers. Dexamethasone treatment further reduced inflammatory responses, supporting the role of glucocorticoid signaling in modulating systemic and mucosal immunity.

Acknowledgements:
Juan José Enguix Huete is supported by a predoctoral fellowship (FPU22) from the Spanish Ministry of Science, Innovation and Universities.
This work was supported by the EPICORT project funded by the Instituto de Salud Carlos III (ISCIII, PI21/00952)

Session 10: Pharmacology of Senescence & Aging

Moderator:

Concha Peiró (Universidad Autónoma de Madrid)

Invited speaker:

15:00-15:30 Guillermo Díaz Araya (Universidad de Chile)
Cardiac fibroblast senescence and its impact on tissue remodeling and fibrosis: a pharmacological perspective

Selected oral communications:

15:30-15:45 Jaime Rubio (Universidad de Castilla-La Mancha)
Role of ER stress in topotecan antitumoral treatment

15:45-16:00 Ana Cuadrado-Gómez (Universidad Autónoma de Madrid)
Vasoprotective Effects of Canagliflozin in Murine Models of Endothelial Dysfunction Induced by Ageing and Inflammation

16:00:16:15 Laura Rey (Universidad de Santiago de Compostela)
Evaluation of the impact of the senescence-associated secretory phenotype of peripheral endothelial cells on the blood brain barrier

16:15-16:30 Mabel Catalán (Universidad de Chile)
Targeting age-related metabolic decline: Mitochondrial uncoupling by TPP+C10 induces visceral adipocyte browning

Cardiac fibroblast senescence and its impact on tissue remodeling and fibrosis: a pharmacological perspective

Guillermo Díaz-Araya

Laboratorio de Farmacología Molecular, Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.

E-mail: gadiaz@ciq.uchile.cl

Abstract

Aging and chronic cellular stress are determinant risk factors in the development and progression of cardiovascular diseases, where cellular senescence emerges as a key pathophysiological mechanism.

In myocardial tissue, the cardiac fibroblast plays a fundamental role as a sentinel cell, responsible for sensing damage signals and initiating the inflammatory and reparative responses necessary to preserve cardiac architecture. Following tissue injury, transient senescence of these fibroblasts acts as a physiological control mechanism that limits excessive proliferation and modulates initial healing. However, the chronic persistence of senescent cardiac fibroblasts in the myocardium profoundly alters tissue homeostasis. Senescent cells arrest their cell cycle but remain metabolically active, acquiring the so-called senescence-associated secretory phenotype (SASP). Through the SASP, fibroblasts continuously secrete pro-inflammatory factors, chemokines, and cytokines (such as IL-6. IL-1β, IL-5, TNF and MCP-1). This microenvironment alters extracellular matrix equilibrium and perpetuates a state of low-grade sterile inflammation that stimulates the phenotypic transition toward activated myofibroblasts (α-SMA+), exacerbating anomalous collagen deposition and consolidating cardiac fibrosis.

From a pharmacological perspective, unraveling the transduction pathways involved in this process—such as the p38MAPK/NF-FkB axis and the activation of the NLRP3 inflammasome induced by damage stimuli—opens a critical window for therapeutic intervention. Currently, scientific interest focuses on the use of senolytic compounds, aimed at inducing selective apoptosis in senescent cells, and senomorphic (or senostatic) modulators, oriented to mitigate the deleterious components of the SASP. The use of Navitoclax or the combination of Dasatinib + Quercetin has shown a reduction in senescent cardiac myofibroblasts induced by TGF-β1, highlighting the pharmacological strategies that can be utilized to attenuate the deleterious effects of senescent cardiac myofibroblasts.

Along this line, specialized pro-resolving lipid mediators, such as Resolvin D1 (RvD1) and Resolvin E1 (RvE1), have demonstrated a promising capacity to attenuate premature senescence and the secretion of pro-fibrotic mediators in vitro and in vivo, positioning themselves as cutting-edge therapeutic strategies against adverse myocardial remodeling.

Acknowledgements (Agencia Nacional de Investigación y Desarrollo, ANID, Proyecto FONDECYT 1250183)

Role of ER stress in topotecan antitumoral treatment

Jaime Rubio, Valentín Ceña V, Mª Dolores Pérez-Carrión, Inmaculada Posadas

CIBER, Instituto de Salud Carlos III, Madrid, Spain; Unidad Asociada Neurodeath, INAMOL, Universidad de Castilla-La Mancha, Albacete, Spain

E-mail: jaime.rubio@uclm.es

Topotecan (Topo) is a topoisomerase I inhibitor clinically approved for the treatment of solid tumours including high-risk neuroblastoma and gliomas, although it has demonstrated modest clinical effect due to low penetration to CNS and the development of drug resistance (1), (2). The endoplasmic reticulum (ER) has been described as a potential sensor capable of triggering both adaptive and pathological signalling. Initially, ER stress activates adaptive responses that promote cell survival; however, persistent ER stress has also been associated with tumour progression and resistance to antitumoral therapies by enhancing cellular mechanisms involved in survival and stress adaptation (3).

In the present work, we investigated the role of ER stress in modulating the antitumor efficacy of Topo. Our hypothesis is that ER stress contributes to Topo-induced resistance in tumour cells, and ER stress modulators may increase tumour sensitivity.

To test this hypothesis, we evaluated cell viability by measuring the percentage of LDH release and MTT transformed, analysed protein expression via western blot, and measured oxidative stress and caspase activity by fluorometry.

Our preliminary results suggest that ER stress plays a dual role in tumour sensitivity to Topo. At low concentrations of the antitumoral drug, co-treatment with salubrinal (Sal), an inhibitor of GADD34 that prevents eIF2α dephosphorylation, decreased cell viability compared with Topo treatment alone. However, at higher concentrations of Topo, Sal seems to reverse the cytotoxic effect of the antitumoral drug suggesting that, under moderate stress conditions, ER stress activation may contribute to tumour cell survival and resistance, whereas under severe cytotoxic conditions, modulation of this route may alter the balance between adaptive and apoptotic signalling pathways.

In conclusion, our findings suggest that ER stress plays a dual role in the tumoral response to topotecan treatment depending on the intensity of the insult. These results highlight the complexity of ER stress signalling in cancer therapy and support further studies to better define the role or therapeutic potential of ER stress modulation in combination with topotecan.

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Vasoprotective Effects of Canagliflozin in Murine Models of Endothelial Dysfunction Induced by Ageing and Inflammation

Ana Cuadrado-Gómez[1,2], Alicia Villacampa[1,2], Diego Berlanas[1,2], Carmen M. Parra-Moreno[1,2], Jorge F. Gómez-Cerezo[3], María Posada[4], Concepción Peiró[1,2], Carlos F. Sánchez-Ferrer[1,2]

1. *Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, España.*
2. *Instituto de Investigación Sanitaria del Hospital La Paz (IdiPAZ), Madrid, España*
3. *Department of Internal Medicine, HU Infanta Sofía, Madrid, Spain*
4. *Department of Surgery, HU Fundaciín Jiménez Díaz, Madrid, Spain*

E-mail: ana.cuadrado@uam.es

Canagliflozin is an inhibitor of the sodium-glucose cotransporter 2 (SGLT2) that, beyond its established glucose-lowering efficacy, provides significant cardiovascular benefits. Although widely used in patients with diabetes mellitus (DM), the specific cellular mechanisms underlying its direct vasoprotective actions which reduce cardiovascular risk, remain to be fully elucidated. Therefore, this study aims to determine whether canagliflozin can prevent or improve endothelial dysfunction in mesenteric microvessels using murine models of ageing and hyperinflammation.

Different approaches evaluating the vascular reactivity via wire myography of small vessels were performed using mesenteric arteries harvested from C57/BL6 mice. First, the preventive *ex vivo* effect of canagliflozin (1 µmol/L) on vascular impairment induced by interleukin-1 beta (IL-1β, 2.5 ng/mL) was assessed in control untreated mice. Second, the *ex vivo* effect of canagliflozin (1 µmol/L) was evaluated in established pathophysiological models, including aged mice (22 month) and streptozotocin (STZ, 200 mg/kg)-induced diabetic mice (DM STZ-induced).

Our results demonstrated that canagliflozin pretreatment successfully prevents IL-1β *ex vivo*-induced endothelial impairment, while acute incubation significantly improves endothelium-dependent relaxation in microvessels from both aged animals and STZ-induced diabetic models. Overall, these findings indicate that canagliflozin exerts direct local vasoprotective effects *ex vivo*, preventing inflammation-induced damage and reversing established endothelial dysfunction associated with ageing and diabetes. Ongoing experiments *in vivo* are being conducted to further elucidate the molecular mechanisms underlying these protective vascular effects.

Keywords: Canagliflozin, endothelial dysfunction, SGLT2 inhibitors, interleukin-1 beta, mice mesenteric arteries.

Acknowledgements. This work is part of the research project titled “The NLRP3 inflammasome at the crosstalk of inflammatory vascular aging: a new target for senomorphic drugs” funded by the National R&D Plan (PID2023-147378OB-I00 supported by MCIN/AEI/10.13039/501100011033/ FEDER, UE), a grant agreement for the 'Knowledge Generation Projects' with reference PID2023-147378OB-100 and actions for the training of predoctoral research staff PREP2023-001312 associated with said project. In addition, Community of Madrid 2023 Call for Applications Co-financed by the European Social Fund Plus PEJ-2023-AI/SAL-GL-28071 and PEJ-2023-AI/SAL-GL-28347 and Postdoctoral Fellowship Sara Borrel (CD24/00217).

Evaluation of the impact of the senescence-associated secretory phenotype of peripheral endothelial cells on the blood brain barrier

Laura Rey[1], Aitor Picos[1], Nuria Seoane[1], Manuel Campos-Toimil[1], Dolores Viña[1]

[1]*Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela, Santiago de Compostela, Spain.*

E-mail: laura.rey.romay@rai.usc.es

Aging is the primary risk factor for the development of chronic diseases in developed countries. Over time, the body undergoes age-related physiological changes that increase the likelihood of developing various pathologies, including cardiovascular and neurodegenerative disorders. Among the mechanisms involved in neurodegenerative diseases, dysfunction of the blood–brain barrier (BBB) stands out as a key process in the onset and/or progression of these conditions. Despite advances in our understanding of the BBB, little is still known about how alterations in the peripheral vascular system may contribute to its deterioration [1].

During aging, peripheral blood vessels accumulate senescent cells, which acquire a pro-inflammatory senescence-associated secretory phenotype (SASP) capable of exerting systemic effects. Among the therapeutic strategies aimed at modulating the effects of the SASP, rapamycin has emerged as a promising senomorphic drug. As an inhibitor of the mTOR complex, rapamycin has been shown in animal models of aging to reduce BBB permeability and extend lifespan [2].

The aim of this study was to evaluate the impact of the peripheral endothelial SASP on the structure and function of the BBB and to assess the potential protective effect of rapamycin. To this end, conditioned media obtained from HUVEC cells at different stages of aging, treated or untreated with rapamycin, were applied to an in vitro BBB model based on the bEnd.3 cell line cultured on Transwell® inserts. Cell viability, oxidative stress, inflammatory profile (IL-6), phosphorylated endothelial nitric oxide synthase (p-eNOS) levels, transendothelial electrical resistance (TEER), and the expression of tight junction proteins were evaluated.

Our results showed that conditioned medium from untreated senescent HUVECs (containing SASP-associated factors) induced structural and functional alterations in the in vitro BBB model. Notably, these effects became progressively more pronounced as the degree of HUVEC senescence advanced. In contrast, rapamycin treatment delayed the onset of senescence in HUVECs and consequently reduced the damage induced in the BBB by conditioned medium from late-passage HUVECs, highlighting its potential as a promising therapeutic agent for mitigating the effects of vascular aging on neurovascular dysfunction.

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Targeting age-related metabolic decline: Mitochondrial uncoupling by TPP+C10 induces visceral adipocyte browning

Mabel Catalán[1], Cristian Suárez-Rozas[2], Raúl Vivar[1], Diego García-Díaz[1], Gladys Tapia[1].

1. *Universidad de Chile, Santiago, Chile.*
2. *Universidad Bernardo O'Higgins, Santiago, Chile.*

E-mail: mabelcatalan@u.uchile.cl

Aging is characterized by a generalized metabolic decline, strongly linked to the loss of thermogenic capacity and the dysfunctional expansion of visceral white adipose tissue (vWAT). This phenomenon contributes to the development of cellular senescence, insulin resistance, and chronic systemic inflammation (inflammaging). Reactivating the browning process in the vWAT represents a disruptive therapeutic strategy to counteract age-related metabolic decline. In this study, we evaluated the potential of TPP+C10, a mitochondriotropic lipophilic cation derived from gallic acid designed to act as a mild mitochondrial uncoupler.

In vitro evaluations used differentiated 3T3L1 cells incubated with TPP+C10. We evaluated, by western blot and RT-qPCR, the levels of PGC-1α and UCP1 for browning determination, and AMPK activation by western blot. Oxygen consumption by seahorse analysis. In vivo evaluations were performed in C56BL6 mice. Animals were fed a high-fat diet for 12 weeks and a normal diet for 12 weeks. After mice were obese (in an animal model of age-dependent metabolic decline), TPP+C10 at 10 mg/kg was administered for 6 weeks. vWAT was evaluated by hematoxylin/eosin staining and immunofluorescence against UCP1. Browning adipose tissue was evaluated as in vitro model.

In vitro results showed that treatment with 1 µM TPP+C10 in adipocytes increased oxygen consumption rate (OCR) immediately, activating the AMPK energy signaling pathway and promoting mitochondrial fission, without compromising cell viability. In vivo results showed that the administration of 10 mg/kg of TPP+C10 significantly attenuated visceral adiposity, improved glucose tolerance, and protected against hepatic steatosis. Fundamentally, histological and immunohistochemical analyses revealed a remarkable conversion of aged unilocular adipocytes into multilocular cells with robust expression of uncoupling protein 1 (UCP1) in vWAT.

These findings demonstrate that TPP+C10 is a potent pharmacological agent that rescues metabolic homeostasis during aging by reprogramming bioenergetics and rejuvenating visceral adipose tissue function.

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Session 11: Neuropharmacology

Moderator:

Dolores Viña Castelao (Universidade de Santiago de Compostela)

Invited speaker:

9:00-9:30 Manuela García López (Universidad Autónoma de Madrid)
NRF2 induction as a strategy for neuroinflammatory-related diseases

Selected oral communications:

9:30-9:45 Victoria Maneu (Universidad de Alicante)
Potential involvement of Pannexin 2 in Retinal Degeneration

9:45-10:00 María Álvarez-Rubal (Instituto de Investigación Sanitaria Hospital Universitario la Princesa)
Role of the NLRP3 inflammasome in Blood-Brain Barrier Maintenance During Ischemic stroke

10:00:10:15 Christian Griñán-Ferré (Universidad de Barcelona)
FLAV-27 Restores Synaptic Function in BTBR Autism Model via G9a Inhibition

10:15-10:30 Abraham B Torregrosa (Universidad Miguel Hernández)
Sex-dependent modulation of binge-like ethanol intake by the MAGL inhibitor MCH11

NRF2 induction as a strategy for neuroinflammatory-related diseases

[1]Manuela G. López, [1]Eric del Sastre, [1]Lucía Viqueira, [1]Cristina Fernández-Mendivíl, [1]Ángela Gómez-Mediavilla, [2]Ángel J. García-Yagüe, [1]Elsa Cortés-Montero, [1]Silvia Santamaría, [2]Antonio Cuadrado, [3]Renzo Mancuso, [4]Rafael León, [4]María Isabel Rodríguez-Franco (*Tau-NeuroDiscovery Consortium*)

[1]NeuroprotectionLab, Dept. Pharmacology. Universidad Autónoma de Madrid. Spain.
[2]Department of Biochemistry. Universidad Autónoma de Madrid and Instituto Biomédicas Sols-Morreale CSIC. Spain.
[3]VIB-UAMTWERP Center for Molecular Neurology. Belgium. [4]Instituto de Química Médica-CSIC. Spain

E-mail: manuela.garcia@uam.es

Nuclear factor erythroid 2–related factor 2 (NRF2) is a transcription factor that regulates cellular redox homeostasis and inflammatory responses; mechanisms known as major contributors to many neurodegenerative diseases. NRF2 inducers in clinic are electrophilic compounds, making them highly reactive molecules that can provoke off-target effects Therefore, NRF2-KEAP1 protein-protein inhibitors (PPI) could be a safer alternative. After a drug discovery program, we have identified ND525 as PPI lead compound. Here we study its mechanism of action and its protective effect in neuroinflammatory and Alzheimer models.

The NRF2 inducing mechanism has been evaluated in AREc32-Luc cells, by Superficial plasmon resonance, GSH conjugation and lC-IT-MS análisis, and by using $NRF2^{KO}$ and $KEAP1^{KO}$ MEFs. Efficacy has been evaluated in *in vitro* and *in vivo* models of LPS-induced inflammation, tauopathy and Alzheimer disease.

ND523 is a non-electrophilic compound that induces NRF2 by inhibiting the NRF2/KEAP1 interaction. It showed a good anti-neuroinflammatory profile in a microglial murine cell line, in human induced pluripotent stem cells (hiPSC)-derived microglía and *in vivo*. ND523 also reduced tau pathology and ameliorated cognitive deficits in *in vivo* models of tauopathy.

ND523 is a first in class protein-protein inhibitor of NRF2/KEAP1 with potential therapeutic interest in neurodegenerative diseases.

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https://www.neurodiscovery-ndd.com/

Potential involvement of Pannexin 2 in Retinal Degeneration

Mateo Ruiz-Conca[1,2], Enola Missonnier[1], Estela Tébar-Garcerán[1], Marta Estalrich[1], Oksana Kutsyr[3], Eva Tudurí[1,2], Lorena Vidal-Gil[1], Natalia Martínez-Gil[1,2], Laura Fernández Sánchez[1,2], Victoria Maneu[1,2]

[1] *University of Alicante, Alicante, Spain*
[2] *Alicante Institute for Health and Biomedical Research (ISABIAL), Alicante, Spain.*
[3] *University of Colorado, CO, USA.*

Email: vmaneu@ua.es

Pannexin channels are a family of three glycoproteins that form large-pore channels in the cell membrane, allowing the passage of ions, small molecules, and metabolites of up to approximately 1.5 kDa. One of their main roles is in purinergic signaling, a key process involved in the regulation of the central nervous system activity. Even though their physiological importance is increasingly recognized, their contribution to human disease and their potential as therapeutic targets are still not fully understood. This is especially the case for Pannexin-2 (Panx2), which remains the least studied member of the pannexin family and is still relatively poorly characterized. Our main objective was to explore whether Panx2 might contribute to retinal neurodegenerative diseases. By using flow cytometry, we analyzed retinal expression of Panx2 in the P23H mouse model of retinitis pigmentosa and in matched healthy C57BL/6J mice. We show that healthy retinas exhibit a very low population of cells expressing Panx2 channels, whereas this population is increased in the retinas of P23H mice, especially when the disease is advanced. Panx2 exhibited co-expression with the purinergic receptors P2X7R, but not with P2X4R. This result was reinforced by the reanalysis of RNA-seq data from GEO functional genomics data repository, which points to an increased Panx2 mRNA expression in P23H mice in intermediate stages of the disease, compared to control animals. Moreover, functional enrichment analysis of Panx2-associated pathways suggests an upregulation of P2X7R and P2X4R in the P23H retina. We also observed an increased proportion of cells expressing P2X4R but not P2X7R in this model. This finding differs from our previous results in other retinal degeneration models, probably reflecting model-specific differences in microglial activation and purinergic signaling. Overall, our results suggest that Panx2 may play a pivotal role in retinal degeneration that has not been recognized before, raising interesting questions for further investigation.

Acknowledgements
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Role of the NLRP3 inflammasome in Blood-Brain Barrier Maintenance During Ischemic stroke

María Álvarez-Rubal[1], Kiara Anaide Pinto[1], Esther Fuertes Yebra[1], Laura Ávila Poza[1], Clara Martín Fernández[1], Inés Valencia[1], Céline Decouty-Pérez[1], Javier Egea[1]

[1]Laboratorio de Neuroinflamación Molecular y Plasticidad Neuronal, Unidad de Investigación, Hospital Universitario Santa Cristina. Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, España.

E-mail: maria.alvarezrubal@gmail.com

Acute brain injuries, including strokes and traumatic brain injuries, are leading causes of disability worldwide. Specifically, ischemic stroke accounts for 80–85% of all stroke cases and is characterized by a reduction in blood flow to a specific area of the brain, triggering an inflammatory response and subsequent cell death. Acute inflammation occurs as a response mechanism to damage caused by cerebral ischemia, disrupting the different cellular components that constitute the blood–brain barrier (BBB). A key player in this process is the NLRP3 inflammasome, a multiprotein complex responsible to produce pro-inflammatory cytokines (IL-1β and IL-18), and whose involvement in BBB maintenance has previously been shown to be time-dependent in ischemic stroke.

Our research group hypothesizes that protecting the BBB and reducing the inflammatory response post-ischemia are crucial for maintaining communication and functionality within the neurovascular unit, thereby minimizing inflammation-induced damage. Therefore, we aim to investigate the interaction between pericytes and endothelial cells, two critical components in maintaining BBB permeability after cerebral ischemia.

BBB status was analysed in mice subjected to a transient middle cerebral artery occlusion model (tMCAO). Changes were assessed using genetic and pharmacological inhibition of the NLRP3 inflammasome, with labelling of the different components of the neurovascular unit, placing particular emphasis on endothelial cells and pericytes. At 24 hours after ischemia, wild-type animals (WT) exhibited a reduction in pericyte coverage. In contrast, NLRP3 knockout animals (NLRP3 KO) showed an early reduction in pericyte coverage at the onset of reoxygenation, which persisted at 24 hours. Furthermore, animals treated with the inflammasome inhibitor MCC950 one hour after ischemia maintained pericyte coverage throughout the reoxygenation period. Based on these findings, responses to inflammatory stimuli and hypoxia were evaluated “in vitro” using a three-dimensional culture model in which human cerebral endothelial cells and pericytes were co-cultured. It was observed that cultures receiving the MCC950 prior to hypoxia showed an early degradation of the cultures compared with the ones treated with the inhibitor following hypoxia. Therefore, we sought to evaluate this inflammatory and hypoxic process exclusively in pericytes. Immunofluorescence analysis revealed ASC specks expression under both conditions, which decreased following MCC950 treatment. A similar trend was observed in the expression of pericyte-specific proteins (CD13) and the NLRP3 inflammasome.

Variations in the maintenance of pericyte coverage depend on inhibition of the NLRP3 inflammasome, leading to altered expression of pericyte markers, suggesting that the inflammasome may represent a potential tool for assessing BBB integrity in ischemic stroke.

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FLAV-27 Restores Synaptic Function in BTBR Autism Model via G9a Inhibition

Núria Pérez-Salvador[1,2], Muhammad Ali[3], Aina Bellver-Sanchis[1,2], Carmen Escolano[4,5], Malak Hajar[6], Petrilla Jayaprakash[6], Mercè Pallàs[1,2,7], Bassem Sadek[6], Christian Griñán-Ferré[1,2,7]*

1-Dept. Pharmacology and Therapeutic Chemistry, Institut de Neurociències-Universitat de Barcelona, Barcelona, Spain; 2-Institute of Neurosciences of the University of Barcelona, University of Barcelona, Barcelona, Spain; 3-Functional Informatics Lab, National Center for Bioinformatics, Quaid-i-Azam University, Islamabad, Pakistan; 4-Laboratory of Medicinal Chemistry. Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain; 5-Institute of Biomedicine of the University of Barcelona (IBUB), Universitat de BarcelonaBarcelona, Spain; 6-Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab. Emirates University, Al Ain, UAE.; 7-Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain

E-mail: christian.grinan@ub.edu

Background: Autism spectrum disorder (ASD) involves intricate genetic, epigenetic, and circuit disruptions. Currently, no drugs specifically target its core behavioral symptoms. A major epigenetic change is the dysregulation of histone H3K9 dimethylation, mainly caused by the G9a/EHMT2 methyltransferase, which can impair synaptic function and neurodevelopment. FLAV-27 is a new inhibitor that competes with SAM to inhibit G9a activity, aiming to reduce abnormal H3K9me2 levels and reestablish transcriptional pathways associated with ASD.
Methods: We used an integrative approach that combined multiple omics methods to examine the molecular and spatial effects of FLAV-27 in BTBR T+tf/J mice (BTBR), a well-established model of idiopathic ASD. The study included behavioral tests, bulk RNA sequencing, ATAC-seq, and 10x Visium spatial transcriptomics of cortical and hippocampal regions. Additionally, we aligned the data with structural MRI and compared it to human ABIDE-II datasets to evaluate transcriptional relevance.
Results: FLAV-27 notably reduced repetitive behaviors in BTBR mice in a dose-dependent way. RNA-seq showed upregulated genes associated with synaptic plasticity and neurotransmission, along with decreased mitochondrial and stress-response activities. ATAC-seq indicated widespread chromatin remodeling, with increased accessibility at neuronal regulatory sites. In the hippocampus, western blot results confirmed lower H3K9me2 levels and FLAV-27–induced changes in ERK and SNAP25, reinforced by validation of key synaptic and transcriptional markers. Combining RNA-seq and ATAC-seq data revealed that over 7,000 genes were regulated in concert, including loci associated with ASD. Spatial transcriptomics indicated a restoration of cortical and hippocampal organization, while MRI–transcriptome alignment identified a treatment-affected ventral tegmental area with elevated dopaminergic gene expression.
Conclusions: FLAV-27 led to enhanced behavioral, transcriptional, epigenetic, spatial, and imaging outcomes. It improved synaptic function while reducing mitochondrial overactivity and oxidative stress. These results indicate that G9a inhibition triggers early epigenetic processes that support broad neural recovery, positioning FLAV-27 as a promising, mechanism-based treatment candidate for ASD.

Acknowledgements This study was supported by the Ministerio de Economía, Industria Economía, Industria y Competitividad (Agencia Estatal de Investigación, AEI) and European Union NextGenerationEU/PRTR (PID2022-139016OA-I00, PDC2022-133441-I00 ,to CGF and MP; PID2022-1380790B-I00, to C.E.), MICIU/AEI/10.13039/501100011033 and FEDER, UE and PDC2022-133441-I00 MICIU/AEI /10.13039/501100011033 Europea Next GenerationEU/ PRTR), Generalitat de Catalunya (2021 SGR 00357). This study was co-financed by Secretaria d'Universitats i Recerca del Departament d'Empresa i Coneixement de la Generalitat de Catalunya 2023 (Product 0092; Llavor 005 and Llavor 007 to CGF). Also, the authors are grateful to the United Arab Emirates University for the award of the intramural research grants (12R207 and 12M274 to Bassem Sadek).

Sex-dependent modulation of binge-like ethanol intake by the MAGL inhibitor MCH11

Abraham B. Torregrosa[1,2,3], **María Salud García-Gutiérrez**[1,2,3]

[1] *Instituto de Neurociencias, Universidad Miguel Hernández-CSIC, Avda. de Ramón y Cajal s/n, San Juan de Alicante, 03550, Alicante, Spain;*
[2] *Red de Investigación en Atención Primaria de Adicciones, Instituto de Salud Carlos III, MICINN and FEDER, Madrid, Spain;*
[3] *Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante, Spain.*

E-mail: a.bailen@umh.es

Monoacylglycerol lipase (MAGL) inhibition has emerged as a potential strategy for regulating alcohol-related behaviours by modulating the endocannabinoid system. Here, we investigated the effects of MCH11, a novel monoacylglycerol lipase inhibitor, on binge-like ethanol intake in male and female C57BL/6J mice exposed to the drinking-in-the-dark paradigm (DID). After 3 weeks of binge drinking, mice received acute (day 4, DID-3) or repeated (days 1-4, DID-4) administration of MCH11 (20 and 40 mg/kg, i.p.). Quantitative PCR measured tyrosine hydroxylase (Th) in the ventral tegmental area (VTA); dopamine receptor 2 (Drd2) in the prefrontal cortex (PFC) and nucleus accumbens (NAc); and mu-opioid receptor (Oprm1), cannabinoid 1 (Cnr1), and cannabinoid 2 (Cnr2) in the NAc.

The results revealed higher ethanol intake in females than in males throughout the DID paradigm. MCH11 reduced ethanol consumption in both sexes after acute administration at 40 mg/kg; however, repeated administration produced stronger effects in females than in males. Ethanol exposure was associated with sex-dependent alterations in dopaminergic, opioid and cannabinoid-related gene expression within the mesocorticolimbic regions. In more detail, ethanol increased Th in the VTA and Cnr1 in the NAc, mainly in males, and showed opposite regulation of Drd2 in the NAc between sexes. Oprm1 increased in the NAc for both sexes, more in males, while Cnr2 in the NAc and Drd2 in the PFC decreased similarly. Notably, MCH11 modulated these alterations, aligning with behavioural findings.

Overall, these findings indicate that MCH11 attenuates binge-like ethanol intake and suggest the existence of sex-dependent molecular mechanisms underlying its effects.

Session 12: Pharmacology of Inflammation

Moderator:

Mª Jesús Sanz (Universitat de Valencia)

Invited speaker:

9:00-9:30 Mª Angeles Moro (Centro Nacional de Investigaciones Cardiovasculares, CNIC))
Immunothrombosis, a central mechanism in cerebrovascular disease

Selected oral communications:

9:30-9:45 Elena Domingo (Instituto de Investigación Sanitaria-INCLIVA)
Time-course of atherogenic lesion formation in apoE-/- mice subjected to an atherogenic diet: determination of circulating CC chemokines.

9:45-10:00 Mª Carmen Carceller (Universitat de València)
C-Jun N-terminal Kinase (JNK) Inhibition impairs epidermal barrier integrity and modulates inflammatory responses in psoriatic-like human skin equivalents

10:00:10:15 Álvaro Gómez-Martín (Instituto de Investigación Sanitaria – INCLIVA)
Tirzepatide, a novel dual agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors, attenuates endothelial dysfunction and vascular inflammation

10:15-10:30 Alicia Villacampa (Instituto de Investigación Hospital Universitario La Paz – IDIPAZ)
Basigin mediates SARS-CoV-2 spike protein-induced inflammasome activation and endothelial dysfunction

Immunothrombosis, a central mechanism in cerebrovascular disease

María Ángeles Moro, Alicia García-Culebras, María Isabel Cuartero, Carolina Peña-Martínez, Ignacio Lizasoain

Centro Nacional de Investigaciones Cardiovasculares (CNIC), Instituto de Investigación Hospital 12 de Octubre (i+12), and Departamento de Farmacología, Facultad de Medicina, Universidad Complutense de Madrid (UCM)

E-mail: mamoro@cnic.es

Ischemic stroke and vascular cognitive impairment are major causes of death, disability, and dementia worldwide. Although reperfusion therapies have transformed acute stroke care, clinical outcomes remain highly variable, underscoring the need to better understand the mechanisms that regulate tissue injury, repair, and long-term brain dysfunction after cerebrovascular disease.

Increasing evidence identifies immunothrombosis, the coordinated activation of immune and thrombotic pathways, as a central process in this context. In particular, neutrophil-platelet interactions and the formation of neutrophil extracellular traps (NETs) contribute to microvascular obstruction, inflammation, impaired perfusion, and progression of ischemic injury. In this presentation, we will discuss experimental and clinical evidence supporting immunothrombosis as a key driver of cerebrovascular pathology. Our work has shown that circadian rhythms critically shape neutrophil function and NET formation after stroke, generating time-of-day-dependent neutrophil states that influence collateral circulation, microvascular perfusion, and infarct evolution. These findings support that the timing of stroke onset and immune activation may have important consequences for acute tissue damage and therapeutic responsiveness. Beyond the acute vascular lesion, immunothrombosis may also influence longer-term brain dysfunction. NET- and platelet-derived mediators have the potential to affect brain homeostatic systems involved in cognition, including perivascular and extravascular territories. These mechanisms may help link acute cerebrovascular injury to vascular cognitive impairment and post-stroke cognitive decline.

Together, these findings support immunothrombosis as a pathophysiological axis connecting vascular injury, inflammation, impaired brain homeostasis, and cognitive outcome. Understanding this continuum may open new opportunities for therapeutic strategies aimed at improving cerebrovascular recovery from the acute phase of stroke to long-term brain function.

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Time-course of atherogenic lesion formation in *apoE*$^{-/-}$ mice subjected to an atherogenic diet: determination of circulating CC chemokines.

Elena Domingo[1], **Aida Collado**[6], **Patrice Marques**[1,2,3], **Miguel Cervera M**[1,2,4], **José T. Real**[1,2,4], **Laura Piqueras**[1,5,6], **María-Jesús Sanz**[1,2,6]

[1] *INCLIVA Biomedical Research Institute, Valencia, Spain*
[2] *Faculty of Medicine, University of Valencia, Valencia, Spain*
[3] *CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Madrid, Spain*
[4] *University Clinic Hospital of Valencia, Valencia, Spain*
[5] *Faculty of Pharmacy, University of Valencia, Valencia, Spain*
[6] *CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Madrid, Spain*
[7] *BioClinicum, Karonlinska Institutet, Stockholm, Sweden*

E-mail: edomingo@incliva.es

Background: 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 first evaluated the impact of an atherogenic diet in the lesion formation in *apoE*$^{-/-}$ *CCR3*$^{+/+}$ and *apoE*$^{-/-}$*CCR3*$^{-/-}$ mice finding increased lesion formation in the latter. Since CCR3 is the unique eotaxin-1/CCL11 receptor, we have investigated the generation and systemic release of this CC chemokine after 1, 2, 4 and 8 weeks exposure to an atherogenic diet.

Material and methods: Male and female *apoE*$^{-/-}$ C57BL/6 mice were either fed with control or an atherogenic diet during 1, 2, 4 or 8 weeks. Lesion formation and eotaxin-1/CCL11 expression were evaluated though immunohistochemical techniques in the lesion and the liver. Plasma levels of the chemokine were measured by ELISA. Statistical significance was determined using a Two-way ANOVA followed by Bonferroni's post hoc test on raw data.

Results: Atherosclerotic plaque area and plasma eotaxin-1/CCL11 levels were greater in male and female *apoE*$^{-/-}$ mice fed with an atherogenic diet for 4 and 8 weeks than their respective controls. Using immunohistochemical techniques, CCL11/eotaxin-1–positive area was detected in the atherosclerotic plaques and the liver of both genders at the same time points. Interestingly, in the liver, an increasing tendency in the expression of this chemokine was already evident after 1 week of atherogenic diet administration.

Conclusion: Although further results are required, our preliminary findings appear to demonstrate a relationship between the liver and the heart that warrants extensive investigation.

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C-Jun N-terminal Kinase (JNK) Inhibition impairs epidermal barrier integrity and modulates inflammatory responses in psoriatic-like human skin equivalents

Anastasia Riske[1,2], Mª. Luisa Ferrándiz[1,2], Diego Pascual-García[1], Valentina Carvajal García[1], Mª. Carmen Carceller[1,2], Isabel García-Arnadis[1,2], Mª. Carmen Montesinos.[1,2]

[1]University of Valencia, Burjassot, Spain, [2]IDM, Valencia, Spain

E-mail: luisa.ferrandiz@uv.es

Psoriasis is a chronic inflammatory skin disorder characterized by abnormal keratinocyte proliferation, epidermal hyperplasia, and sustained immune activation. Previous studies have shown that impaired c-Jun N-terminal kinase (JNK) signaling in psoriatic dermal fibroblasts may reduce their capacity to regulate and resolve inflammation [1]. Therefore, this study investigated the effects of JNK inhibition on skin homeostasis, epidermal barrier function, and inflammatory regulation using psoriatic-like full-thickness human skin equivalents (FTHSE). The aim was to further clarify the role of JNK signaling in the pathophysiology of psoriasis.

Psoriatic-like FTHSE were generated through stimulation with tumor necrosis factor (TNF)-α and interleukin (IL)-22 (10 µg/mL each) and cultured for 14 days in the presence or absence of the JNK inhibitor SP600125 (10 or 50 µM). Skin morphology, epidermal barrier markers, and psoriasis-associated biomarkers were analyzed using immunohistochemistry and immunoassay techniques.

JNK inhibition caused structural abnormalities in FTHSE under both unstimulated and inflammatory conditions, including disturbed keratinocyte organization and altered epidermal thickness. Psoriatic stimulation reduced the expression of barrier-associated proteins, including filaggrin, loricrin, and cytokeratin (CK)-10, while increasing involucrin and CK-6 expression. These effects became more pronounced following JNK inhibition, suggesting further impairment of epidermal barrier integrity. In addition, JNK inhibition reduced the overall expression of psoriasis-related inflammatory biomarkers. IL-8 levels decreased significantly after treatment with 10 µM SP600125 in both unstimulated (from 66.7 ± 17.1 to 24.8 ± 12.9 pg/mg, $p < 0.05$) and stimulated conditions (from 161.9 ± 26.8 to 115.1 ± 11.5 pg/mg, $p < 0.05$). Similarly, the expression of SKALP/Elafin, HS100A7, and hBD2 in stimulated FTHSE was reduced following JNK inhibition.

In summary, JNK inhibition disrupts epidermal architecture and compromises skin barrier function in FTHSE, while also modulating inflammatory signaling pathways associated with psoriasis. These findings indicate that impaired JNK signaling may contribute to chronic inflammation and barrier dysfunction in psoriasis.

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Acknowledgements

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Tirzepatide, a novel dual agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors, attenuates endothelial dysfunction and vascular inflammation

Álvaro Gómez-Martín[1,2], María Abad[2], Patrice Marques[1,3,4], Cristina Arce[2,7], Elena Domingo[1,2], José Tomás Real[5,6], María-Jesús Sanz[1,2,6], Laura Piqueras[2,6,7]

[1]*Department of Pharmacology,: Faculty of Medicine, University of Valencia, Valencia, Spain.*
[2] *INCLIVA Biomedical Research Institute, Valencia, Spain*
[3]*Health Research Institute Hospital La Fe (IIS La Fe), Valencia, Spain.*
[4]*CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Madrid, Spain*
[5]*Endocrinology and Nutrition Service, University Clinic Hospital of Valencia, Valencia, Spain.*
[6]*CIBERDEM: Diabetes and Associated Metabolic Diseases Networking Biomedical Research- ISCIII, Madrid, Spain*
[7] *Department of Pharmacology, Faculty of Pharmacy, University of Valencia, Valencia, Spain*

E-mail: agomez@incliva.es

Background: Endothelial dysfunction and vascular inflammation play a central role in the development and progression of cardiovascular complications associated with metabolic disorders. Recent advances in the pharmacological treatment of metabolic disorders have led to the development of 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. Beyond their metabolic benefits, their ability to modulate endothelial dysfunction and vascular pathophysiology has not yet been fully characterized. This study aimed to evaluate the effects of tirzepatide, a dual GLP-1R/GIPR agonist on endothelial dysfunction and vascular inflammation, and to explore the molecular pathways underlying these effects through *in vitro* experimental approaches.

Methods: The effects of dual GLP-1R/GIPR agonism on TNF-α-induced endothelial dysfunction and vascular inflammation were evaluated *in vitro.* Human umbilical vein endothelial cells (HUVECs) were used. Soluble chemokine quantification, laminar flow chamber assays, western blotting, and immunofluorescence were employed. GLP-1R and GIPR antagonists were employed to confirm tirzepatide-mediated effects.

Results: *In vitro* studies in TNF-α-stimulated HUVECs demonstrated that tirzepatide reduced the release of MCP-1/CCL2 and RANTES/CCL5 ($p<0.05$). Moreover, tirzepatide inhibited TNF-α-induced leukocyte adhesion and downregulated the expression of the vascular adhesion molecules VCAM-1 and ICAM-1 ($p<0.05$). Immunofluorescence and western blot analyses further revealed that tirzepatide suppressed activation of the NF-κB signaling pathway in endothelial cells ($p<0.05$). Most of these effects were partially reversed by pretreatment with either a GLP-1R antagonist or a GIPR antagonist ($p<0.05$), whereas simultaneous blockade of both receptors completely abolished the inhibitory effects of tirzepatide ($p<0.05$).

Conclusions: Tirzepatide confers vascular protection by attenuating endothelial dysfunction.

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Basigin mediates SARS-CoV-2 spike protein-induced inflammasome activation and endothelial dysfunction

Alicia Villacampa[1,2], Ana Cuadrado[1,2], Pilar Dongil[1], Diego Berlanas[1,2], Juan Carlos López Gil[3], Patricia Delgado-Aliseda[1,3], Francisco López Sánchez[4], José Luis Bartha[1,2,4], Fernando de la Cuesta[1,2], Bruno Sainz Jr[3,5], Isabel Sánchez-Pérez[1,3,5], Óscar Lorenzo[1,6], Carlos F. Sánchez-Ferrer[1,2], Concepción Peiró[1,2]

[1]*Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain*
[2]*Instituto de Investigaciones Sanitarias (IdiPAZ), Madrid, Spain*
[3] *Instituto de Investigaciones Biomédicas (IIBM) Sols-Morreale CSIC-UAM, Madrid, Spain*
[4]*La Paz University Hospital, Madrid, Spain*
[5] *Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain*
[6]*Instituto de Investigaciones Sanitarias Fundación Jiménez-Díaz, Madrid, Spain*

E-mail: alicia.villacampa@idipaz.es

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative viral agent for COVID-19. We have previously shown that SARS-CoV-2 spike protein (S) has been shown to activate the NLRP3 inflammasome, increase coagulation factor production, and induce senescence in human endothelial cells despite the absence of angiotensin-converting enzyme 2 (ACE2), the canonical viral receptor (1, 2). This suggests the involvement of alternative receptors mediating the effects of the S protein.

Candidate receptors potentially interacting with the SARS-CoV-2 S protein were evaluated using bioinformatic protein–protein interaction analyses. Basigin (BSG/CD147), a member of the immunoglobulin superfamily and part of the SCARF gene family previously associated with viral entry, was selected for further investigation. Human endothelial cells and *ex vivo* mouse mesenteric arteries were used to evaluate the effects of pharmacological inhibition of BSG with AC73, which prevents BSG dimerization, on S protein-induced inflammatory and vascular responses. Pharmacological inhibition of BSG with AC73 prevented S protein-induced NLRP3 inflammasome priming and activation in human endothelial cells, as studied by western blot and indirect immunofluorescence. In addition, *ex vivo* treatment with AC73 prevented the vascular dysfunction previously observed in mouse mesenteric arteries exposed to the S protein. Both the SARS-CoV-2 S protein and the inflammatory cytokine IL-1β increased BSG mRNA and protein expression levels. Moreover, IL-1β-induced upregulation of BSG was prevented by treatment with Ang-(1-7).

These findings provide evidence that the proinflammatory effects of the SARS-CoV-2 S protein in human endothelial cells are mediated, at least in part, through BSG. Targeting BSG may represent a potential therapeutic strategy to prevent endothelial inflammation and vascular dysfunction associated with SARS-CoV-2.

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POSTER SESSIONS

Cardiovascular Pharmacology

P1

A novel gain of function p.A300T mutation in *PCSK9* gene in a patient with acute myocardial infarction

Ricardo Pan Lizcano, Luis Mariñas Pardo, Álvaro Paramio Duró, Pablo Piñón, Ramón Calviño, José Manuel Vázquez Rodríguez, Manuel Hermida Prieto, Lucía Núñez

1. Unidad de Terapias Celulares. Instituto de Investigación de Enfermedades Raras (IIER). Instituto de Salud Carlos III (ISCIII). Madrid. España.
2. Grupo de Investigación en Cardiología, Instituto de Investigación Biomédica de A Coruña (INIBIC)-CHUAC-UDC, A Coruña, España.
3. Servicio de Cardioloxía, Complexo Hospitalario Universitario de A Coruña (CHUAC)-CIBERCV, Instituto de Investigación Biomédica de A Coruña (INIBIC) A Coruña, España.
4. Departamento de Medicina. Universidade da Coruña. Instituto de Investigaciones Biomédicas de A Coruña

E-mail: lucia.nunez@udc.es

Introduction: Acute myocardial infarction (AMI) remains a leading cause of global morbidity and mortality despite advances in prevention and treatment. Increasing evidence highlights the significant role of genetic factors in modulating individual susceptibility and disease progression. In this context, the application of Next Generation Sequencing is enabling the identification of rare variants and fostering a more precise, personalized approach to cardiovascular risk assessment.

Material and methods: Using Next Generation Sequencing (NGS) technologies, 233 genes associated with MI were sequenced and analyzed in a Spanish patient. The possible effect of the rare variants identified was assessed using five bioinformatics tools: Mutation Taster, SNAP2, SIFT, Polyphen2, and PhD-SNP. Variants considered as pathogenic by, at least, 3 of the 5 programs, were included in an *in vitro* HepG2 Functional Assay Using Flow Citometry to confirm the functional effect.

Results: The c.899C>T variant in the *PCSK9* gene was identified in a 65-year-old man with family history of heart disease during an acute myocardial infarction. The patient had a history of dyslipidaemia and hypertension, as well as three-vessel disease. The patient died 48 hours after the onset of symptoms. The variant, which had not been previously described, results in the amino acid substitution p.A300V. Bioinformatic analysis predicts that this is a potentially deleterious substitution. Functional *in vitro* characterization revealed that the variant was a gain of function variant.

Conclusions: *In silico* and *in vitro* models had showed that the novel variant p.A300V cause a gain-of-function in *PCSK9* gene, leading to high circulating LDL levels that could explain the AMI in this patient.

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P2

Effect of menopause on hypertension-associated cardiovascular damage

Marta Martínez-Casales[1,2,4], Zoe González-Carnicero[1,2,4], Silvia Flaj[1,5], Isabel Ureña[1,5], Ángela Martín[1,2,3,4], Esperanza Herradón[1,5], María T. Barrús[1,2,4], Antonio Gonzalez[1,5], Ana M. Briones[2,3,6], Visitación López-Miranda[1,5], María J. Alonso[1,2,3,4], Raquel Hernanz[1,2,3,4]

[1]*Universidad Rey Juan Carlos (URJC), Alcorcón, Spain*
[2]*Instituto de Investigación Hospital Universitario La Paz (IdiPaz), Madrid, Spain*
[3]*CIBER Cardiovascular, Spain*
[4]*Grupo consolidado en Fisiopatología Cardiovascular, INVASC, URJC, Spain*
[5]*Grupo de alto rendimiento en Farmacología Experimental, Pharmakom, URJC, Spain*
[6]*Universidad Autónoma de Madrid, Spain*

E-mail: marta.martinez@urjc.es

Background: Cardiovascular diseases (CVD) are the leading cause of death among women in Spain, exceeding mortality rates in men. Hypertension is the main modifiable risk factor for CVDs and a key driver of target organ damage, which significantly increases the risk of stroke, heart failure and chronic kidney disease. Early detection of subclinical organ damage is therefore essential for effective hypertension management and for reducing long-term complications. This study aimed to evaluate the impact of menopause on blood pressure increase and the associated target organ damage.

Methods: Adult female Wistar Kyoto (WKY) rats, either ovariectomised (postmenopausal) or sham-operated were used. Animals were studied under control conditions or after induction of hypertension via angiotensin II (AngII) infusion for 2 weeks (200 ng/kg/min).

Results: Menopause did not significantly affect baseline blood pressure. However, postmenopausal rats exhibited vascular endothelial dysfunction and remodelling of resistance vessels without changes in cardiac or renal structure or function. Hypertension induced by AngII infusion was similar in both groups. Nevertheless, postmenopausal rats showed greater endothelial dysfunction and enhanced vasoconstrictor responses, associated with reduced phosphorylated eNOS (p-eNOS) expression. Plasma oxidative stress similarly increased in both AngII-infused groups. Although AngII increased wall thickness in both groups, vascular remodelling patterns differed between the two rat groups: inward remodelling, with reduced lumen diameter in postmenopausal rats, and outward remodelling, with increased vessel diameter in sham animals. Despite this, arterial stiffness increased similarly in both groups. AngII-induced cardiac hypertrophy was observed only in postmenopausal rats, although cardiac function was preserved. No renal hypertrophy was detected despite increased plasma urea nitrogen levels in both groups.

Conclusions: Menopause does not influence blood pressure or hypertension development but increases susceptibility to vascular and cardiac damage under hypertensive conditions, suggesting a higher risk of target organ injury in postmenopausal females.

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P3

New Biomarkers in the Diagnosis and Prognosis of Dilated Cardiomyopathy: Pro-Resolving Lipids and miRNAs

[1]Rafael I. Jaén, [1]Sergio Sánchez-García, [2,3]María Fernández-Velasco, [4]Irene Cuadrado, [4]Beatriz de las Heras, [1,2]Lisardo Boscá* and [1,2,4]Patricia Prieto

1 Biomedical Research Institute "Sols-Morreale" CSIC-UAM, Madrid, Spain; 2 Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain; 3 IdiPAZ/CIBER-CV, La Paz University Hospital, Madrid, Spain; 4 Facultad de Farmacia, Complutense University, Madrid, Spain

E-mail: patriciaprieto@ucm.es

Abstract

Dilated cardiomyopathy is a major cause of heart failure and is one of the most common forms of cardiomyopathy worldwide. Although there has been significant progress in its clinical management, early diagnosis and precise prognosis remain challenging due to the lack of specificity in current biomarkers. As inflammation plays a key role in DCM, we determined the levels of systemic inflammatory markers and specific pro-resolving lipid mediators (SPMs) in a cohort of DCM patients. Our data show that the levels of lipoxin A4 significantly increased in DCM patients (343 + 75.1 pg/mL in controls vs. 482.2 ± 159.1 pg/mL in DCM patients), whereas the opposite was observed for resolving D1 (57.18 ± 32.68 pg/mL in controls vs. 38.55 ± 25.13 pg/mL in DCM patients). These results may indicate that SPMs could be considered new biomarkers related to the progression of this pathology. Moreover, since microRNAs (miRNAs) are also considered potential biomarkers at the molecular level, we conducted comprehensive miRNA expression profiling using a high-throughput array platform in our cohort. Of the differentially expressed miRNAs identified, we chose to focus on two that were significantly upregulated (miR378-3p and miR486-5p; more than two-folds) or downregulated (miR142-3p and miR328-3p < 20% and 40% vs. the control, respectively) in DCM patients, all of them strongly associated with inflammatory pathways. The selected miRNAs showed considerable potential as biomarkers, exhibiting statistical significance after ROC analysis. In fact, improved performance was observed when combining both miR142-3p and miR328-3p, using a LASSO regression model. However, we found no correlation between miRNAs and traditional inflammatory markers or SPMs ruling out the possibility to proposing them as combined biomarkers in this case. The heterogeneity of DCM leads to the need to identify new biomarkers that, either individually or in combination, may improve the prognosis of affected individuals. In our study, we have identified that some of the main SPMs can provide valuable information about disease progression, in addition to the combination of certain circulating miRNAs, which show promising prognostic values in our cohort. Thus, we have identified novel biomarkers that integrate inflammatory profiles with specific circulating miRNA expression patterns is an important step towards more targeted patient stratification in DCM. This approach can improve DCM diagnosis and prognosis, supporting the development of personalized treatments through a multi-parameter panel of biomarkers that can be measured in peripheral blood and used in routine clinical practice. Such a strategy can enable earlier treatment, resulting in better patient outcomes and quality of life.

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P4

New SGK1 inhibitors alleviates pulmonary arterial hypertension and vascular remodeling in a mice model

Natalia Bustos[1], Bertha García-León[1], Yolanda Sierra-Palomares[1], Enrique Madruga[1], Loreto Martínez-González[1,2], Laura de la Bastida-Casero[1], Lola Navarro-Llinares[1], Gerrit Bredeck[1], Isabel Lastres-Becker[2,3], Ana M. Briones[4,5], Ana Martínez[1,2], Eduardo Oliver[1,5,6]

[1]Centro de Investigaciones Biológicas Margarita Salas (CIB), CSIC, Madrid, Spain.
[2]Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED).
[3]Instituto de Investigaciones Biomédicas "Sols-Morreale" UAM-CSIC, Madrid, Spain.
[4]Universidad Autónoma de Madrid, Madrid, Spain.
[5]Centro de Investigación Biomédica en Red Enfermedades Cardiovasculares (CIBERCV).
[6]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.

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

Pulmonary arterial hypertension (PAH) is a rare and devastating disease affecting the pulmonary vasculature, which is characterized by elevated pulmonary arterial pressure followed by right ventricular dysfunction and failure. The pathophysiology of PAH involves pulmonary vascular remodelling, endothelial dysfunction and persistent inflammation, which drive structural vessel changes and progressive impairment of pulmonary hemodynamic. Despite advances in current therapies, available treatments mainly alleviate symptoms and delay disease progression without effectively reversing the underlying pathological processes. Therefore, identifying novel molecular targets involved in disease progression remains a major challenge. In this context, serum and glucocorticoid-regulated kinase 1 (SGK1) has emerged as a key regulator of signalling pathways associated with inflammation and endothelial damage. Previous evidence suggests that SGK1 dysregulation contributes to pathological vascular responses, supporting its potential role in pulmonary vascular disease. Thus, we evaluated the therapeutic potential of two novel SGK1 inhibitors recently developed by our research group in a chronic experimental model of PAH in mice. Hemodynamic, inflammatory and vascular remodelling parameters were assessed to determine the impact of SGK1 inhibition on disease progression. Our findings indicate that pharmacological inhibition of SGK1 ameliorates pulmonary hemodynamic and attenuates pathological features associated with vascular remodelling and inflammation, as evidenced by a significant reduction in right ventricular systolic pressure and a decrease in endothelial damage, cell adhesion molecules and remodelling markers. Overall, these results support SGK1 as a promising therapeutic target in PH and highlight SGK1 inhibition as a potential strategy to counteract key mechanisms underlying disease progression.

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P5

Treatment with a SPM-enriched marine oil supplement improves vascular dysfunction and remodeling in angiotensin II–induced hypertension and obesity

Marta Sastre[1], Irene Pastor[1], Naoual Boukich[1,2,3], Ana García-Redondo[2,3,4], Ana M. Briones[1,2,3]

[1]Departamento de Farmacología, Facultad De Medicina, Universidad Autónoma De Madrid, Spain;
[2]Ciber Cardiovascular, Spain;
[3]Instituto De Investigación Hospital Universitario La Paz (IdiPAZ), Spain;
[4]Departamento De Fisiología, Facultad De Medicina, Universidad Autónoma de Madrid, Spain.

E-mail: marta.sastre@uam.es

Background: Hypertension is the leading risk factor for cardiovascular disease (CVD) worldwide and is characterized by vascular and cardiac alterations, including endothelial dysfunction, vascular remodeling and stiffness, and cardiac hypertrophy (1). It is recognized as a chronic low-grade inflammatory disease in which angiotensin II (Ang II) acts as a key pro-inflammatory stimulus (2). Inflammation is actively resolved by specialized pro-resolving lipid mediators (SPMs), derived from ω-3 and ω-6 polyunsaturated fatty acids, which promote tissue repair and restore homeostasis. Impaired resolution may contribute to chronic inflammation in CVD (2). The **aim** of this study was to evaluate the effects of a marine oil supplement enriched with SPM precursors on Ang II–induced CV damage. **Methodology:** Several groups of male C57BL/6J mice were used: (i) high-fat diet (HFD, 60% kcal, 18 weeks) with Ang II infusion (0.88 mg/kg/day, 4 weeks), and (ii) Ang II infusion (1.44 mg/kg/day, 14 days) both with or without SPM-enriched marine oil (3 µL/g/day, Solutex) starting one day before Ang II infusion, and (iii) control lean mice. Systolic blood pressure was measured by tail-cuff plethysmography. Vascular function and structure were assessed by pressure and wire myography and histological analyses in aorta, carotid and/or mesenteric arteries. **Results:** Obesity combined with low-dose Ang II induced a modest increase in blood pressure and cardiac hypertrophy but marked endothelial dysfunction in aorta and carotid arteries. SPM-enriched marine oil did not affect blood pressure or cardiac hypertrophy but restored endothelial function. Small arteries from obese hypertensive mice showed inward eutrophic remodeling and increased stiffness that was prevented by SPM-enriched marine oil. In the Ang II model, hypertension, cardiac hypertrophy, endothelial dysfunction, increased aortic contractility, and hypertrophic remodeling were modestly reduced by marine oil, without effects in vascular stiffness. **Conclusion:** supplementation with a SPM-enriched marine oil improves endothelial function and vascular remodeling in hypertension with or without obesity, supporting its potential as a therapeutic strategy targeting impaired resolution of inflammation in CVD.

Key Words: angiotensin II, endothelial dysfunction, vascular remodeling, resolution of inflammation.

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Pain and Neural Pharmacology

P6

Direct Allosteric Modulation of G_i Protein Conformational Dynamics by a μ-Opioid Receptor Positive Allosteric Modulator

Pedro Renault and Jesús Giraldo

Universitat Autònoma de Barcelona, Barcelona, Spain

E-mail: jesus.giraldo@uab.cat

The μ-opioid receptor (MOR) is a class A G protein-coupled receptor (GPCR) and is the principal target of opioid analgesics, but its activation by conventional agonists also leads to serious side effects such as respiratory depression, tolerance, and addiction. Positive allosteric modulators (PAMs) have emerged as an attractive alternative because they can enhance the activity of endogenous opioid peptides, thereby offering the potential for effective pain relief with an improved therapeutic index. The MOR PAM BMS-986122, for example, produces analgesia in animal models without directly activating the receptor, instead potentiating the signaling of peptides released during pain [1].

Although NMR experiments already indicated that BMS-986122 stabilizes the active conformation of the MOR [2], a complete mechanistic understanding of allostery requires moving beyond the isolated receptor, as the G protein itself functions as an endogenous PAM, cooperatively stabilizing a high-affinity agonist-bound state. Consequently, the actions of exogenous PAMs can only be fully understood within the complete receptor:agonist:G protein complex [3]. However, the influence of BMS-986122 on G protein conformational dynamics has remained largely unexplored.

To address this, we performed microsecond-scale all-atom molecular dynamics simulations of the MOR:G_i heterotrimer complex, both in the absence and presence of BMS-986122, using the endogenous opioid peptide endomorphin-1 as the orthosteric agonist. Comparative analysis of the trajectories showed that the presence of BMS-986122 increased the distance between the Ras-like and α-helical domains of the Gα subunit relative to the complex lacking the modulator, facilitating GDP release. The data therefore reveal that the PAM directly modulates G protein conformational dynamics, indicating a mechanism of action that extends beyond the previously reported stabilization of active receptor conformations. These findings uncover an additional layer of complexity in MOR allostery. and may inform the design of safer analgesics that more effectively harness the endogenous opioid system.

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P7

Exploring sex-dependent differences in chromaffin cell responses in the CCI rat model of neuropathic pain

Ada Quintero Pérez, Marina Arribas-Blázquez, Mª Victoria Barahona Gomariz, Antonio R. Artalejo, Luis Alcides Olivos-Oré

Department of Pharmacology and Toxicology; Veterinary Faculty. Instituto Universitario de Investigación en Neuroquímica. University Complutense of Madrid

E-mail: adquint@ucm.es

Background: Neuropathic pain remains a critical biomedical challenge, with emerging evidence highlighting significant sexual dimorphism in its underlying pathophysiological mechanisms. The hypothalamic-pituitary-adrenal (HPA) axis and sympathetic neuroendocrine responses play pivotal roles in modulating chronic pain states, yet sex-specific variations in peripheral nerve injury-induced adaptations remain poorly understood. This study aimed to investigate sex differences in mechanical hypersensitivity, adrenal gland morphology, and electrophysiological properties of adrenomedullary chromaffin cells following peripheral nerve injury.
Material and Methods: Adult male and female Sprague Dawley rats were subjected to a chronic constriction injury (CCI) of the sciatic nerve to induce neuropathic pain. Pre- and post-surgery mechanical allodynia was systematically evaluated by using the automated von Frey filament test. Following behavioral characterization, adrenal glands were harvested and weighed to assess macroscopic morphological alterations. Cellular and molecular adaptations were investigated with the patch-clamp technique. Specifically, voltage-dependent sodium (NaV) currents and acetylcholine (ACh)-evoked currents were characterized in isolated chromaffin cells using the whole-cell patch-clamp configuration. Furthermore, real-time catecholamine secretion from adrenal chromaffin cells was evaluated via carbon-fiber amperometry.
Results: Our preliminary findings reveal distinct sex-dependent phenotypes in our peripheral neuropathy model. CCI induced a more pronounced reduction in mechanical withdrawal thresholds in female rats compared to males. This behavioral difference correlated with significant morphological changes, as female rats exhibited a reduction in adrenal gland weight relative to their male counterparts, in which it increased. At the cellular level, whole-cell patch-clamp recordings demonstrated distinct sex-specific shifts in the biophysical properties of NaV currents with as well as an augmentation in current density in female rats; likewise, there was an increase in the amplitude of ACh-evoked currents in male, but not in female rats. Concurrently, amperometric recordings indicated variations in the dynamics of secretion between male and female chromaffin cells under neuropathic conditions.
Conclusions: Our findings suggest that chronic constriction injury of the sciatic nerve triggers distinct behavioral, neuroendocrine, and electrophysiological adaptations in male and female rats, which may underscore the necessity of considering sex in the development of targeted, mechanistic-based therapeutic interventions for neuropathic pain.

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P8

Sigma-1 Receptor Blockade Enhances the Therapeutic Efficacy of NSAIDs in Collagen-Induced Arthritis

Miguel Á. Huerta[1,2,3], Antonia Artacho-Cordón[1,2,3,4], M. Carmen Ruiz-Cantero[4], Amada Puerto-Moya[1,2,3], Enrique J. Cobos[1,2,3,5], Francisco R. Nieto[1,2,3]

1. Department of Pharmacology, University of Granada, 18016 Granada, Spain
2. Institute of Neuroscience, University of Granada, 18016 Granada, Spain
3. Biosanitary Research Institute ibs.GRANADA, 18012 Granada, Spain
4. Department of Pharmacology, Toxicology and Therapeutic Chemistry, University of Barcelona, Spain
5. Teófilo Hernando Institute for Drug Discovery, Madrid, Spain

E-mail: huerta@ugr.es

Introduction: Rheumatoid arthritis (RA) remains a major therapeutic challenge, as many patients experience persistent pain and incomplete disease control despite current treatments. Non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac and naproxen, are widely used to relieve RA-associated pain, but their efficacy is often limited and dose escalation may increase the risk of adverse effects. Sigma-1 receptor (σ1R) antagonism has emerged as a promising analgesic and anti-inflammatory strategy in preclinical models of arthritis (Nieto et al., 2025). However, whether σ1R blockade can enhance the therapeutic efficacy of conventional NSAIDs in inflammatory arthritis remains unknown.
Methods: Collagen-induced arthritis (CIA) was used as a preclinical model of RA in rats. To assess the interaction between σ1R inhibition and NSAID treatment, the effects of diclofenac and naproxen were evaluated in wild-type and σ1R knockout (σ1R-KO) rats, as well as in animals pharmacologically treated with the σ1R antagonist S1RA. Arthritis progression and inflammation were assessed through macroscopic clinical scoring. Pain-related behaviors and functional impairment were also examined using sensory and functional outcome measures relevant to arthritis-associated pain.
Results: Both genetic deletion and pharmacological antagonism of σ1R enhanced the therapeutic effects of NSAIDs in CIA. In σ1R-KO rats, diclofenac and naproxen produced stronger analgesic and antiarthritic effects than those observed in WT animals. Similarly, co-administration of S1RA potentiated the efficacy of both NSAIDs, improving arthritis-associated pain, functional outcomes, and inflammatory signs.
Conclusion: σ1R antagonism potentiates the analgesic and anti-inflammatory effects of diclofenac and naproxen in experimental arthritis. These results support the combination of σ1R antagonists with NSAIDs as a promising therapeutic strategy to improve pain relief and disease control in RA, potentially allowing enhanced efficacy.

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P9

Targeting the Sigma-1 Receptor to Alleviate Arthritis Progression and Pain

Miguel Á. Huerta[1,2,3], Amada Puerto-Moya[1,2,3], Antonia Artacho-Cordón[1,2,3], Aitana Rickert-Llàcer[1,2,3], M. Carmen Ruiz-Cantero[4], Enrique J. Cobos[1,2,3,5], Francisco R. Nieto[1,2,3]

1.-Dept. Pharmacology, University of Granada, Granada, Spain; 2.-Institute of Neuroscience, Biomedical Research Center, University of Granada, Granada, Spain; 3.-Biosanitary Research Institute ibs.GRANADA, Granada, Spain; 4.-Dept. Pharmacology, Toxicology and Therapeutic Chemistry, University of Barcelona, Barcelona, Spain; 5.-Teófilo Hernando Institute for Drug Discovery, Madrid, Spain

E-mail: fnieto@ugr.es

Introduction: Despite the arrival of several new pharmacological treatments in recent years (disease-modifying antirheumatic drugs, DMARDs), therapeutic outcomes for rheumatoid arthritis (RA) remain often unsatisfactory and are sometimes accompanied by adverse effects. Furthermore, a significant number of patients who achieve adequate disease control with current DMARDs continue to experience residual chronic pain, which severely impacts their quality of life. Sigma-1 receptor blockade has demonstrated analgesic efficacy in pain of different etiologies. However, it had never been evaluated in rheumatoid arthritis (RA).
Methods: The efficacy of sigma-1 receptor blockade was evaluated using a collagen-induced arthritis (CIA) model in rats. Peripheral inflammation, joint pathology, functional impairments, and pain behaviors were comprehensively assessed through histology, magnetic resonance imaging, behavioral tests, and flow cytometry. Sigma-1 receptor knockout (KO) rats and pharmacological sigma-1 antagonists (S1RA, BD1063) were compared to saline and standard treatments (pregabalin, methotrexate) administered via subcutaneous osmotic pumps for 7 days (days 11–18 of CIA time course) to wild-type (WT) rats.
Results: Both genetic deletion (KO) and pharmacological blockade of the sigma-1 receptor robustly reduced peripheral inflammation and joint degeneration, with efficacy comparable to the first-line DMARD methotrexate. Moreover, sigma-1 antagonism improved arthritis-associated functional deficits and pain behaviors, demonstrating superior efficacy compared to the first-line neuropathic pain treatment pregabalin or methotrexate. Importantly, the analgesic effects were not solely attributable to reduced inflammation and joint pathology, as acute treatment with sigma-1 antagonists also produced consistent rapid analgesic effects in contrast to DMARD methotrexate.
Conclusion: Sigma-1 receptor blockade emerges as a promising new strategy for the treatment of rheumatoid arthritis with an unprecedented mechanism of action, and with the added benefit of independently alleviating arthritis-associated pain (Nieto et al., 2025).

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P10

Efficacy and safety of transcutaneous capsaicin vs. lidocaine in post herpetic neuropathy of the feet

Inmaculada Bellido Estevez [1], Alejandro Barroso González [1,2], Salvador Romero Molina[3], Luis González Becerra [1], Encarnación Blanco Reina [1]

[1]Department of Pharmacology, School of Medicine, University of Malaga. IBIMA. [2]Service of Anesthesiology, Resuscitation and Pain Therapy. Regional University Hospital, Malaga. [3]Service of Anesthesiology, Resuscitation and Pain Therapy. Virgen de la Victoria University Hospital, Malaga. Spain

E-mail: ibellido@uma.es

Introduction: Post herpetic neuralgia (PHN) is a neuropathic pain that causes a reduction in patients' quality of life. PHN of the feet causes significant pain. Treatment for PHN is preferably transdermal capsaicin, lidocaine, or oral drugs such as antidepressants or antiepileptics. Capsaicin, an agonist of the vanilloid receptor 1 (TRPV1), desensitizes cutaneous nociceptors, producing analgesia. And lidocaine is an amide-class local anaesthetic used to inhibit pain sensations.

Objective: To determine the efficacy and safety of transcutaneous capsaicin versus lidocaine in the treatment of pain secondary to post herpetic neuropathy of the feet.

Materials and Methods: A controlled, longitudinal, randomized, two-arm study comparing an 8% capsaicin patch with a 5% lidocaine patch (700 mg/day), blinded to the analyser, in patients with post herpetic neuropathy of the feet. Inclusion criteria: ≥18 years of age, both genders, with symptoms of post herpetic neuropathy of the feet, ≥4 intensity (VAS scale), ability to perform all tests, and with informed consent. Epidemiological, clinical, treatment, adverse drug reactions (ADRs), and symptom intensity data were evaluated using the following scales: NPSI, RDA, SALSA, POMS-SF, QoL, and FACT-C. Follow-up was conducted pre-treatment and after 1 day, 1 week, and 6 and 12 weeks of treatment. The analgesic effect, VAS and LANSS scores, and adverse reactions were quantified.

Results and discussion: Eighty-four patients were included, 51.2% women, with a mean age of 48.9 ± 23 years. Capsaicin significantly reduced pain intensity, post-herpetic neuropathy symptoms, and restrictions on daily activity at 6 and 36 weeks of treatment compared to lidocaine. Adverse reactions to capsaicin were transient and detected within the first 4 days, while adverse reactions to lidocaine were more persistent due to its daily administration.

Conclusions: Transcutaneous capsaicin was more effective than transcutaneous lidocaine in treating pain and neuropathic symptoms of post herpetic neuralgia in the feet, thus improving patients' quality of life. It had a similar safety profile to lidocaine in the short term, but in long-term treatment the safety profile of capsaicin was better than that of lidocaine.

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P11

Structural characterization of the μ-opioid receptor–adenosine A1 receptor heteromer and identification of selective ligands

Cèlia Rodríguez-Casas, Neus Calza-Zamora, Natalia Llopart, Verònica Casadó-Anguera, Vicent Casadó

University of Barcelona and Institute of Biomedicine of the University of Barcelona, Barcelona, Spain, and Nanomedicine and Molecular Neuropharmacology Research Group, Barcelona, Spain.

E-mail: celia.rodriguezcasas@ub.edu

A 26% of the Spanish population suffers from chronic pain[1], a debilitating condition that severely impacts patients' quality of life and represents a major socioeconomic burden. Although opioids remain the most effective treatment for severe pain[2], their prolonged use is associated with development of addiction and other side effects associated with opioid use[1]. These analgesic effects are predominantly mediated by the μ-opioid receptor (MOR), a G-protein coupled receptor (GPCR) expressed in pain-processing pathways but also in brain regions associated with reward circuitry, dependence, and other adverse effects linked to opioid use. Beyond acting as individual signalling units, GPCRs are known to form high order oligomeric complexes that modify their pharmacological and functional properties compared to the receptors alone[3,4]. This distinct fingerprint makes receptor oligomers promising therapeutic targets for developing new effective and safer drugs. In this context, previous studies have evidenced that a co-administration of MOR and adenosine 1 receptor (A_1R) agonists triggers a synergic antiallodynic effect[5], suggesting both a functional and a physical interaction between these receptors. Moreover, MOR and A_1R are co-expressed in several brain regions, making the formation of MOR-A_1R heteromers possible. Thus, this study aimed to characterize the molecular organization and interaction interface of the MOR-A_1R heteromer, as well as to identify ligands with selective pharmacological properties for this receptor complex, with the ultimate goal of developing opioid-based therapies with reduced side effects. Firstly, saturation BRET and bimolecular luminescence complementation (BiLC) assays were carried out to confirm the heteromerization of MOR-A_1R *in vitro* in mammalian transfected cells. Moreover, we performed additional BiLC assays using cells incubated with interfering peptides with the sequences of the transmembrane domains of each receptor to determine its interface of interaction. After having determined the heteromerization of MOR-A_1R and its structure, we carried out a screening to find heteromer-selective ligands through different protein G-dependent and -independent signaling pathways. Interestingly, in the presence of A_1R, endomorphin-1 and SR17 have a higher activation potency of MOR, while loperamide, fentanyl, PZM and buprenorphine have a worse potency in β-arrestin recruitment. Future investigation will be required to deepen our knowledge on heteromer-selective ligands of MOR-A_1R and to determine the therapeutic potential of targeting this heteromer for the development of more effective opioid analgesics with minimized side effects for the treatment of chronic pain.

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P12

Perioperative pain management in feline surgical procedures

E. Milena Vázquez[1], Beatriz Romero[1], M. Jesusa Martínez[1], Julen Susperregui[2], Raquel Díez[1]

[1]*Pharmacology. IBIOMED. University of León, Leon, Spain*
[2]*Applied Mathematics. University of Leon, Leon, Spain*

E-mail: evaza@unileon.es

Adequate pain assessment and management are essential in surgical patients, as they contribute to improve recovery and reduce postoperative complications (1). Effective pain control in small animals are essential to reduce patient anxiety, discomfort and improve outcomes (2). Despite the availability of standardized analgesic protocol guidelines, their practical application in veterinary clinical practice remains unknown (1). Therefore, an observational and retrospective study was conducted in cats that underwent surgical procedures at the Veterinary Teaching Hospital of the University of León in 2022. Data were collected from GestorVet application, and information processed and analyzed using Microsoft Excel (2019) and IBM SPSS Statistics v.26. A total of 379 cats were eligible for inclusion, many of them were female (69.7%), adult (83.6%) and Common European breed (95.0%). The most common procedures were reproductive surgeries (88.4%), traumatological (4.5%) and dental (3.4%). Among reproductive procedures, all were ovariohysterectomies (74.9%), and orchiectomies (25.1%). Perioperative analgesia was administered to 98.4% of the patients, including opioids (97.6%), non-steroidal anti-inflammatory drugs (NSAID) (93.9%), and local anesthetics (59.4%). NSAIDs and opioids were co-administered in 93.4% of procedures, while all three groups were used in 58.0% of animals. Among opioids, methadone (97.6%) was the most commonly used drug, robenacoxib (75.0%) as NSAID, and bupivacaine from local anesthetics (66.6%). Bupivacaine was the only drug administered off-label (57.7%). Regarding the use of local anesthetics in orchiectomies, they were administered in 13.1% of cases: in 7.1% only one drug, and in 6% two compounds. In ovariohysterectomies, local anesthetics were used in 82.5% of the procedures, either one (44.3%) or two drugs (38.2%). In ovariohysterectomies, the use of local anesthetics was 31.2 times (OR = 31.2; 95% CI: 15.3-63.7; $p < 0.001$) more likely than in orchiectomies, as was the association of two local drugs 9.8 times higher (OR = 9.8; 95% CI: 3.8-25.0; $p < 0.001$).

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Natural Products Pharmacology

P13

Hydrogel Formulation Enhances the Antibacterial Efficacy of Chilean Honey Against *Staphylococcus pseudintermedius*

Andrea Müller, Rocío Soto, Bárbara Campos, Michelle Briceño, Liliam Monsalve

Universidad de O'Higgins, San Fernando, Chile.

*E-mail: andrea.muller@uoh.cl

The close integration of companion animals into human households increases opportunities for transmission of zoonotic microorganisms. Canine pyoderma, the most common dermatological disease in dogs, primarily caused by *Staphylococcus pseudintermedius*, is an opportunistic pathogen with increasing antimicrobial resistance [1]. Because conventional therapy often relies on broad-spectrum antibiotics, new topical strategies able to control bacterial growth while supporting wound management are required. Honey has recognized antimicrobial and wound-healing properties, but its efficacy depends on composition and delivery system [2]. This study evaluated whether the incorporation of Chilean honey into hydrogel matrices enhances *in vitro* antibacterial activity against clinical *S. pseudintermedius* isolates.

Ten raw Chilean honey samples, two artificial honeys, and ten honey-based hydrogels were tested against 14 clinical *S. pseudintermedius* strains isolated from dogs. Antibacterial activity was assessed by the Kirby–Bauer disk diffusion method according to CLSI criteria and compared with ten antibiotics used in veterinary practice. A 17 mm inhibition-zone threshold was used to classify antibacterial response. Honey samples were characterized for diastase index, hydroxymethylfurfural, total polyphenols, antioxidant capacity, color, pH, soluble solids and electrical conductivity. Paired differences between raw honey and hydrogel formulations were analyzed using the Wilcoxon test, and categorical changes using the McNemar test.

Raw honey and honey-based hydrogels showed antibacterial activity, whereas artificial honey produced no inhibition. Hydrogel incorporation increased antibacterial performance compared with raw honey, raising the mean inhibition zone from 11.0 mm to 15.9 ± 2.0 mm (Wilcoxon $p < 0.001$). At the isolate–sample level, hydrogel formulations converted 45 cases initially classified as resistant to raw honey into the sensitive category (>17 mm), a significant transition confirmed by the McNemar test ($p < 0.001$). Although antibiotics produced larger inhibition zones overall, their activity showed marked strain-dependent dispersion. In contrast, hydrogels displayed a more stable antibacterial profile across isolates. Physicochemical analyses indicated that formulation changed the determinants of antibacterial efficacy: in raw honey, inhibition was associated with aging-related variables, including hydroxymethylfurfural and soluble solids, whereas these associations disappeared in hydrogels. Electrical conductivity and antioxidant capacity emerged as more relevant predictors after formulation.

Honey quality parameters alone are insufficient to predict antimicrobial efficacy once honey is incorporated into therapeutic matrices. Chilean honey-based hydrogels enhanced and stabilized antibacterial activity against clinical *S. pseudintermedius* isolates, supporting their potential as complementary topical candidates for veterinary skin infections and antibiotic-sparing tools within a One Health pharmacological strategy.

This work was funded by the National Agency for Research and Development (ANID) grants FONDECYT 11240512 (2023).

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P14

Anti-inflammatory activity of Fupenzic acid through network pharmacology and experimental validation

B. de las Heras[1], E. Fernández-Vega[1], I. Cuadrado[1], S. Hortelano[2], A. Amesty[3], A. Estevez-Braun[3]

[1]Facultad de Farmacia, Universidad Complutense de Madrid (UCM), Madrid, Spain.
[2]Instituto de Investigación de Enfermedades Raras (IIER), Instituto de Salud Carlos III. Madrid, Spain.
[3]Instituto Universitario de Bio-Orgánica Antonio González, Universidad de La Laguna, La Laguna, Tenerife, Spain.

E-mail: lasheras@ucm.es

Fupenzic acid, a pentacyclic triterpene isolated from the leaves of *Crataegus azarolus* L. (Rosaceae), represents a poorly characterized natural compound with potential pharmacological applications (1). Although hawthorn species have long been associated with anti-inflammatory properties, the contribution of non-phenolic constituents such as FA remains largely unexplored. The present study aimed to investigate the anti-inflammatory activity of fupenzic acid and to elucidate its underlying molecular mechanisms using an integrated computational and experimental approach.

Potential molecular targets of this compound were identified through network pharmacology analyses, including GO, KEGG, Reactome, and protein–protein interaction (PPI) network enrichment. Anti-inflammatory activity was evaluated in LPS-stimulated murine peritoneal macrophages by assessing cell viability (MTT assay), nitric oxide (NO) production, expression of inflammatory mediators by RT-qPCR and Western blot, and NF-κB pathway activation. Molecular docking studies were performed to characterize the interaction between the triterpene and NF-κB. In addition, *in silico* ADMET and drug-likeness analyses were conducted to predict pharmacokinetic and toxicity profiles.

Network pharmacology identified 272 potential targets associated mainly with inflammatory response, cytokine signaling, innate immune system regulation, and MAPK pathways. NF-κB emerged as a central hub in the PPI network. Fupenzic acid showed no significant cytotoxicity at concentrations ≤20 µM. In LPS-activated macrophages, it dose-dependently inhibited NO production (IC_{50} = 16.7 µM) and significantly reduced the expression of NOS-2, COX-2, TNF-α, IL-6, IL-12, IL-18, IP-10, and KC at both transcriptional and protein levels. Furthermore, fupenzic acid prevented IκBα/IκBβ degradation and reduced nuclear translocation of NF-κB p65. Molecular docking analyses demonstrated a favorable interaction of the triterpene within the NF-κB DNA-binding site, with a predicted binding energy of −6.08 kcal/mol. ADMET prediction revealed favorable drug-likeness, acceptable oral bioavailability, and low predicted toxicity.

These findings demonstrate that fupenzic acid exerts significant anti-inflammatory effects, at least partially mediated through inhibition of the NF-κB signaling pathway. The combined computational and experimental evidence highlights this compound as a promising lead compound for the development of novel therapeutic strategies against inflammation-related diseases.

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P15

Dehydroisohispanolone Attenuates Doxorubicin-Induced Cardiac Inflammation by Targeting the NLRP3 Inflammasome and Oxidative Stress

Eva Fernández-Vega[a], Sonsoles Hortelano[b], Beatriz de las Heras[a], Paloma Acebo[b], Ana Estevez-Braun[c] and Irene Cuadrado[a].

[a]Universidad Complutense de Madrid, Madrid, Spain; [b]Instituto de Salud Carlos III, Madrid, Sapin; [c]Instituto Universitario de Bio-Orgánica Antonio González (IUBO), La Laguna, Tenerife, Spain.

E-mail: icberrocal@ucm.es

Doxorubicin (DOX) is a highly effective antineoplastic agent, but its clinical use is limited by dose-dependent cardiotoxicity, in which inflammatory signaling and oxidative stress play central roles (1). In this study, we combined network pharmacology with experimental validation to explore the cardioprotective potential of dehydroisohispanolone (DIH), a labdane diterpene, in a model of DOX-induced cardiac inflammation. Putative DIH targets were identified through *in silico* databases and intersected with cardiac inflammation-related genes, yielding 73 common targets. Protein-protein interaction analysis and functional enrichment revealed key hubs and pathways associated with IL-1β production, IL-6 signaling, oxidative stress, and the NLRP3 inflammasome.

Experimental validation was performed in AC16 human cardiomyocytes exposed to DOX. DIH significantly improved cell viability without displaying intrinsic cytotoxicity. Mechanistically, DIH reduced reactive oxygen species generation, decreased caspase-1 expression and activity, and inhibited NLRP3 inflammasome activation. Consistently, DIH attenuated the transcription and secretion of the pro-inflammatory cytokines IL-1β, IL-18, and IL-6, and reduced lactate dehydrogenase release, indicating protection against pyroptotic cell death. Western blot analyses further confirmed that DIH suppressed the activation of key inflammasome components, including cleaved caspase-1, mature IL-1β, and the active N-terminal fragment of gasdermin D. Importantly, DIH did not impair macrophage viability and did not interfere with the anti-tumoral activity of DOX in MCF-7 breast cancer cells.

Overall, these findings show that DIH mitigates DOX-induced cardiac inflammation through a dual mechanism involving the attenuation of oxidative stress and the inhibition of NLRP3 inflammasome-dependent signaling. This work supports DIH as a cardioprotective candidate and highlights the value of integrating network pharmacology with experimental validation to identify multi-target therapeutic strategies against anthracycline-induced cardiotoxicity.

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P16

Antidiabetic potential of traditional medicinal plants used in Benin: *In vitro* assessment of the influence of the plant part and mechanisms of action

Gema Casado-Hidalgo [1], Javier Cano-Lou [1], Eric Agbodjento [2], Victorien Dougnon [2], Cristina Moliner [1], Guillermo Cásedas [1], Víctor López [1]

[1] *Universidad San Jorge, 50830 Villanueva de Gállego (Zaragoza)*
[2] *Universidad de Abomey-Calavi (Benín).*

E-mail: ilopez@usj.es

Diabetes mellitus currently represents one of the major public health challenges worldwide due to its increasing prevalence and associated metabolic complications. In African countries such as Benin, this situation is aggravated by factors including diets rich in rice and refined flours, together with limited access to healthcare systems and conventional treatments. Consequently, medicinal plants remain widely used in traditional medicine for diabetes management, despite the limited scientific evidence regarding their safety, efficacy and mechanisms of action.
In this context, the MINNAGAN project, an Erasmus+ initiative coordinated between Benin, Spain and Finland, was established to modernize traditional medicine in Benin and promote the integration of herbal medicines into healthcare policies through their scientific validation. Within this framework, the present study aimed to evaluate the antidiabetic potential of several plant species traditionally used in Benin, investigating their possible mechanisms of action and the influence of the plant part on biological activity.

Methanolic extracts were prepared from various plant parts (stem, bark, root and leaves) of *Azadirachta indica* A.Juss., *Rauvolfia vomitoria* Wennberg, *Morinda lucida* Benth., *Annona senegalensis* Pers., *Gymnanthemum amygdalinum* (Delile) Sch.Bip., *Phyllanthus amarus* Schumach. & Thonn., *Launaea taraxacifolia* (Willd.) Amin ex C.Jeffrey , *Carica papaya* L., *Momordica charantia* L. and *Mangifera indica* L. using ultrasound-assisted extraction, followed by filtration, evaporation and freeze-drying. The *in vitro* antidiabetic activity of the extracts (up to 1 mg/mL) was assessed using α-glucosidase, α-amylase, dipeptidyl peptidase-4 (DPP-4) and advanced glycation end-product (AGE) formation inhibition assays. Cytotoxicity was also analysed in Caco-2 and HepG2 cells.
The results showed that all parts of *A. senegalensis* except the root, and *M. indica* exhibited the highest inhibition of α-glucosidase, α-amylase and AGEs (IC_{50} < 50 µg/mL), comparable to or even higher than the reference compounds used as positive controls. Furthermore, the leaves and stems of *G. amygdalinum* and all parts of *M. lucida* showed specific activity in inhibiting DPP-4. Although the leaves of all species exhibited higher cytotoxicity, the bioactive concentrations were lower than the cytotoxic ones.
The plant species studied showed promising antidiabetic potential *in vitro*, which partially supports their traditional use in Benin. The results indicate that biological activity varies depending on the part of the plant used, highlighting the importance of selecting plant material appropriately for future studies. Furthermore, the lack of correlation between the various mechanisms of action evaluated suggests that it is necessary to investigate multiple therapeutic targets to characterise their antidiabetic potential more comprehensively.

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P17

Antibacterial activity of sandalwood oil against skin and soft tissue pathogens and assessment of its anti-inflammatory properties

Pilar Cebollada [1], Eleonora Spinozzi [2], Elena Alvarado [3], Cristina Seral [4], Filippo Maggi [2], Víctor López [1,4]

1 Universidad San Jorge, 50830 Villanueva de Gállego (Zaragoza), Spain.
2 University of Camerino, Via Madonna delle Carceri, 62032, Camerino, Italy.
3 Hospital Clínico Universitario Lozano Blesa, Universidad de Zaragoza, Zaragoza, Spain.
4 Instituto Agroalimentario de Aragón-IA2, CITA-Universidad de Zaragoza, 50013 Zaragoza, Spain

E-mail: ilopez@usj.es

The increasing prevalence of antimicrobial resistance among skin and soft tissue pathogens, together with the limited availability of non-prescription topical treatments, highlights the need for novel therapeutic alternatives. Essential oils represent a promising source of bioactive compounds with antimicrobial and anti-inflammatory properties. Among them, sandalwood essential oil obtained from *Santalum album* have been traditionally used for the management of inflammatory and dermatological conditions.

This study aimed to evaluate the antimicrobial and anti-inflammatory potential of *S. album* against clinical isolates associated with skin and soft tissue infections. Antimicrobial activity was assessed against clinical isolates associated with skin and soft tissue infections. Cytotoxicity was evaluated in human skin cell lines, and the effects of the oil on key enzymes involved in the eicosanoid inflammatory pathway were also investigated.

The oil showed potent activity against Gram-positive bacteria, with minimum inhibitory concentrations (MICs) ranging from 15.6 to 62.5 µg/mL and minimum bactericidal concentrations (MBCs) from 31.3 to 125 µg/mL. No significant reduction in cell viability was observed at the tested concentrations. Furthermore, sandalwood oil inhibited 5-lipoxygenase and phospholipase A_2.

These findings demonstrate that sandalwood essential oils combine bactericidal activity against clinically relevant Gram-positive pathogens with anti-inflammatory effects, providing scientific evidence to support their traditional use and highlighting their potential use in the management of skin and soft tissue conditions.

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P18

Antioxidant and neuroprotective activity of anthocyanins from *Lactuca sativa*

Lucía Ventura[1], Cristina Moliner[1], Marta Sofía Valero[1,3], Guillermo Cásedas[1,2],

[1]*Departamento de Farmacia, Facultad de Ciencias de la Salud, Universidad San Jorge, Villanueva de Gállego, Zaragoza, Spain*
[2]*Instituto Agroalimentario de Aragón, IA2, Universidad de Zaragoza-CITA, Zaragoza, Spain*
[3]*Departmento de Farmacología, Fisiología y Medicina Legal y Forense, Facultad de Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain*

E-mail: gcasedas@usj.es

Oxidative stress plays a key role in the development of neurodegenerative, cardiovascular diseases, and cancer. In this way, anthocyanins, flavonoids widely present in the diet, stand out for their high antioxidant capacity.

This study evaluated 12 lyophilized and purified anthocyanin-rich fractions obtained from two types of lettuce: six samples derived from a wild species (*Lactuca homblei*) and six from a commercial variety (*Lactuca Romired*). The plants were subjected to complete or partial water stress, or maintained as controls, in order to assess the effect of drought on anthocyanin content and its relationship with biological activity.

Initially, antioxidant capacity was determined using the biochemical methods DPPH, FRAP, and ORAC. Subsequently, *in vitro* methods were used to evaluate inhibitory activity against enzymes involved in neurobiological and oxidative processes, including acetylcholinesterase, monoamine oxidase A, tyrosinase, and FAAH. Based on the results obtained, the six *Lactuca homblei* samples were selected for exhibiting higher antioxidant activity and greater enzyme inhibitory capacity than the commercial variety.

In a second *in vitro* phase, cytotoxicity and the cytoprotective potential of the selected preparations were assessed in the neuronal cell line SH-SY5Y. In parallel, docking and molecular dynamics studies of the major anthocyanins against the target enzymes were conducted to further investigate their possible mechanisms of action.

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P19

2,4,5-Trimethoxy-2′-trifluoromethylchalcone attenuates inflammatory responses in experimental crystal-induced inflammation

Isabel García-Arnandis[1,2], Laura Catalán[1], M. Carmen Montesinos[1,2], M. Luisa Ferrándiz[1,2], M. Carmen Carceller[1,2]

[1]University of Valencia, Burjassot, Spain, [2]IDM, Valencia, Spain

E-mail: isabel.garcia-arnandis@uv.es

Crystal-induced arthropathies such as gout and calcium pyrophosphate deposition disease (CPPD) are characterized by activation of inflammatory and oxidative pathways involving Nuclear Factor kappa B (NF-κB) signaling, inflammasome-associated responses and reactive oxygen species (ROS) generation. Chalcone derivatives have shown anti-inflammatory and antioxidant properties in different experimental models.

The anti-inflammatory effects of 2,4,5-trimethoxy-2′-trifluoromethylchalcone (CH) were evaluated in two in vivo models related to crystal-induced inflammation: CPPD-induced air pouch inflammation and monosodium urate (MSU)-induced gouty arthritis in mice. Leukocyte migration, myeloperoxidase (MPO) activity and cytokine production were assessed by cell counting, spectrophotometry and ELISA, respectively. Caspase-1 activation and NF-κB phosphorylation were analyzed by Western blot.

CH reduced leukocyte infiltration and MPO activity in CPPD-induced air pouch exudates. In addition, CH decreased interleukin (IL)-1β, IL-18, IL-6 and C-X-C motif chemokine ligand (CXCL)-1 levels, together with lower caspase-1 activation and p65 NF-κB phosphorylation. In the MSU-induced gouty arthritis model, oral administration of CH attenuated paw edema and reduced MPO activity and inflammatory cytokine production in paw homogenates. Lower levels of activated caspase-1 and phosphorylated p65 NF-κB were also detected after CH treatment.

These findings suggest that CH exerts anti-inflammatory effects in experimental crystal-induced inflammation through modulation of oxidative stress-sensitive and inflammasome-associated inflammatory pathways.

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P20

Terpenoids from *Salvia ringens* modulate inflammatory and oxidative responses in RAW 264.7 macrophages

Ioanna Vamvakari[1], Maria Anagnostou[1], Ekaterina-Michaela Tomou[1], Isabel García-Arnandis[2,3], M. Carmen Montesinos[2,3], Helen Skaltsa[1], María del Carmen Carceller Zazo[2,3]

[1]National and Kapodistrian University of Athens, Athens, Greece; [2]University of Valencia, Spain, [3]IDM, Valencia, Spain,

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

Species belonging to the genus *Salvia* L. are recognized as valuable sources of bioactive secondary metabolites with reported antioxidant, anti-inflammatory and cytotoxic properties [1,2]. In the present study, a phytochemical investigation of the aerial parts of *Salvia ringens* Sm. was carried out to isolate terpenoid compounds and evaluate their biological activity.

Phytochemical analysis led to the isolation of five compounds, including one oxygenated diterpene and four triterpenes. The biological activity of the isolated compounds, together with methanolic and cyclohexane extracts, was evaluated in RAW 264.7 murine macrophages.

Cell viability and cytotoxicity were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays, respectively. Anti-inflammatory activity was assessed by evaluating nitrite production in lipopolysaccharide (LPS)-stimulated macrophages, while antioxidant activity was determined by measuring reactive oxygen species (ROS) generation in 12-O-tetradecanoylphorbol-13-acetate (TPA)-stimulated cells using a luminol-dependent chemiluminescence assay.

Methanolic and cyclohexane extracts, as well as oleanolic, ursolic and betulinic acids, showed significant cytotoxic effects, evidenced by increased LDH release. In contrast, lupeol and the isolated diterpene significantly reduced nitrite production in LPS-stimulated macrophages in a concentration-dependent manner while preserving high cell viability.

Both lupeol and the diterpene significantly decreased ROS production in TPA-stimulated cells in a concentration-dependent manner. Significant inhibition was observed at all tested concentrations (12.5-100 μM), whereas lupeol at 50 and 100 μM and the diterpene at 100 μM almost completely abolished ROS generation.

Overall, these findings suggest that terpenoids isolated from *S. ringens*, particularly lupeol and the oxygenated diterpene, exhibit promising anti-inflammatory and antioxidant activities, highlighting their potential as candidates for the development of novel therapeutic agents for inflammatory disorders.

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P21

Chemical characterization, antioxidant, antimicrobial and *in vitro* anti-inflammatory evaluation of *Fuchsia magellanica* Lam. extracts from Valparaíso and Los Lagos Region, Chile

Gabriela Valenzuela-Barra[1], Ulises Norambuena-Jopia[1], Daniela Canales[1], Javier Acevedo-Hernández[1], Diego Rojas[1], Mabel Catalán[1], María Carolina Zúñiga-López[1], Gabriel Fuentes[1], María Paz Barraza[1,2], Miryam Navarro[2], Jessica Bravo[2].

[1]University of Chile, Santiago, Chile. [2]Universidad Diego Portales, Santiago, Chile.

E-mail: gabriela.m.valenzuela@ciq.uchile.cl

Fuchsia magellanica Lam. (Onagraceae), commonly known as "chilco", is a Chilean native species traditionally used as a diuretic, antipyretic and for menstrual disorders. Despite its ethnomedicinal relevance, local evidence on its chemical composition and bioactive properties remains limited[1]*. This study aimed to compare leaf and flower extracts collected from Valparaíso in 2024 and Chiloé, Los Lagos Region, in 2025, focusing on their chemical composition and antioxidant, antimicrobial and in vitro anti-inflammatory activities.*

Leaves and flowers of *F. magellanica* were collected in Valparaíso and Chiloé, Chile, and voucher specimens were deposited at the SQF Herbarium, University of Chile. Serial extracts were obtained with hexane, dichloromethane and ethanol, using acidified ethanol for flowers. Non-polar extracts were analyzed by gas chromatography–mass spectrometry (GC-MS), while ethanolic extracts were evaluated for total phenolic content (TPC), total flavonoid content (TFC), and profiled by high-performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (HPLC-MS/MS) and diode-array detection (HPLC-DAD). Antioxidant capacity was assessed using ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity using fluorescein (ORAC-FL)[2]. Antimicrobial activity was evaluated by agar diffusion against reference strains and clinical isolates of *Helicobacter pylori*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*)[3]. *In vitro* assays in human fibroblasts are being optimized to evaluate cytotoxicity, oxidative stress modulation and inflammatory-related responses [

Regarding the results, leaf ethanolic extracts from both Valparaíso and Chiloé showed high TPC and TFC, with the highest values observed in the Valparaíso samples: 238.9 ± 17.3 mg gallic acid equivalents/g dry extract and 17.6 ± 3.2 mg quercetin equivalents/g dry extract, respectively. HPLC analyses revealed phenolic acids and several flavonoids in leaves, including guajaverin, quercetin derivatives and kaempferol glycosides, which was consistent with their stronger antioxidant activity in DPPH, TEAC and ORAC-FL assays. Valparaíso extracts also showed the most promising antimicrobial profile, including activity against clinical isolates of *Candida albicans* and a noteworthy MIC against *Escherichia coli*, while Chiloé extracts did not show significant antimicrobial activity in this evaluation. In LPS-stimulated human fibroblasts, the Valparaíso flower ethanolic extract significantly inhibited NF-κB phosphorylation at 100 µg/mL, whereas the leaf extract showed no significant inhibition. These findings suggest that the bioactive potential of *F. magellanica* may depend on plant organ, geographical origin and solvent polarity.

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Pharmacogenetics and Personalized Medicine

P22

Optimized OECD H295R Assay for Hormone Profiling–Based Screening of Endocrine Disruptors Affecting Steroidogenesis

Diego Robés, Laura Gómez, Borja Blanco, Geert Daudey, Luz Romero, José M. Brea, Cristina Val, Mabel Loza, Inés Ardao

University of Santiago de Compostela, Santiago de Compostela, Spain.

E-mail: diego.robes.ysart@usc.es

Endocrine disruptors (ED) are substances capable of altering hormonal balance through different mechanisms, such as steroidogenesis [1]. Due to increasing concern regarding human exposure to these compounds in environmental contaminants and consumer products, robust in vitro methods are required for their identification and characterization. In this work, we aimed to expand the H295R steroidogenesis assay described in OECD Test Guideline 456 [2] to the simultaneous analysis of a panel of steroid hormones to evaluate compounds affecting steroid biosynthesis.

Human adrenocortical carcinoma H295R cells were cultured and exposed to test compounds in 96-well plates for 48 h following OECD TG456. Supernatants were collected and cell viability assessed via resazurin assay to exclude cytotoxic conditions. Steroids secreted into the culture medium were extracted by solid-phase extraction (SPE) and quantified using UPLC-MS/MS.

The SPE procedure was optimized by evaluating different conditions, achieving satisfactory recovery percentages (>80%) for most analytes and improving sample cleanup and method robustness. In addition, a UPLC-MS/MS method was developed to allow the simultaneous separation, identification, and quantification of 17 steroid hormones in a single injection.

The optimized assay showed suitable analytical performance for steroid profiling in H295R media. Reference compounds with known effects on steroid production were evaluated in concentration–response curves, showing dose-dependent hormonal profiles. Additionally, alterations in hormone secretion patterns, consistent with endocrine-disrupting activity, were observed for other suspected compounds. The implemented workflow enabled discrimination between compounds with inductive or inhibitory effects on up to 17 steroid hormones.

In conclusion, this work demonstrates the successful implementation and optimization of the expanded H295R assay, coupled with SPE extraction and simultaneous quantification of 17 steroid hormones via UPLC-MS/MS, for endocrine disruptor screening; a technical evolution currently under validation in the OECD Test Guidelines Programme [3]. The developed methodology provides a suitable platform for the identification and characterization of compounds affecting steroid biosynthesis in toxicology and drug discovery applications.

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P23

The influence of underlying hepatic and extrahepatic comorbidities on drug-induced liver injury

Alicia de Vicente-Ortega[1], Inmaculada Medina-Cáliz[2,3], Ismael Álvarez Álvarez[2,3,4], Hao Niu[2,3,4], Judith Sanabria-Cabrera[2,3], José Pinazo-Bandera[1,3,4], Mercedes Robles-Díaz[2,3,4], Raúl J Andrade[1,3,4], M Isabel Lucena[2,3,4], Miren García-Cortés[1,3,4]

[1.] *Servicio de Aparato Digestivo, Hospital de Antequera, Málaga, Spain*
[2] *Servicios de Aparato Digestivo y Farmacología Clínica, Hospital Universitario Virgen de la Victoria, Málaga, Spain*
[3] *Instituto de Investigación Biomédica de Málaga y Plataforma en Nanomedicina-IBIMA. Plataforma BIONAND, Universidad de Málaga, Málaga, Spain*
4. Centro de Investigación Biomédica en Red en el Área Temática de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain

E-mail: imcaliz@uma.es

***Background**:* Idiosyncratic drug induced liver injury (DILI) is a multifactorial entity, with multiple interrelated factors, including underlying medical conditions. This study aimed to evaluate the influence of underlying hepatic and extrahepatic diseases, as well as the overall comorbidity burden on the severity, phenotype and outcomes of DILI episodes.

***Material and methods**:* Data from patients enrolled in the Spanish DILI Registry between 1994 and 2024 were analyzed. Demographic, clinical characteristics and outcome were compared across different comorbidity groups, categorized according to the Charlson Comorbidity Index (CCI).

Results: 932 patients were included, 66.42% with no comorbidity (CCI=0), 29.18% with mild (CCI=1-2) and 4.40% with significant comorbidity (CCI>2). The most prevalent comorbidities were: cardiovascular (27%), endocrine-metabolic (23%) and gastrointestinal (12%) diseases. The intermediate grade of comorbidity was related to higher mean age, whereas women predominated in the group with the lowest comorbidity burden. The greater the comorbidity, the greater the overall mortality, related to a greater number of deaths due to acute liver failure and non-hepatic causes. However, greater comorbidity was not related to greater severity of the DILI episode. A total of 79 patients (8.48%) had baseline liver disease, which was related to a higher comorbidity burden. However, no relationship was observed between underlying liver disease and higher liver-related mortality.

Conclusions: This study demonstrates a significant association between comorbidity and mortality. A higher comorbidity burden, defined by a Charlson index higher than 2, was associated with increased overall mortality, as well as higher rates of liver and non-liver mortality, highlighting the prognostic impact of underlying diseases in DILI patients. We developed a predictive model for mortality and fatal outcomes, integrating multiple variables, including burden of comorbidity.

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Dementia-Related Safety Signals Associated with Commonly Used Analgesics: A Vigibase Disproportionality Analysis

Tania García-Martínez, Pedro Marín, Fátima Martín-Sánchez, Elena Badillo, Francisca Fuentes-Hidalgo, María Teresa Yuste

Universidad de Murcia, Murcia, Spain.

E-mail: fatimams@um.es

Several studies have suggested that certain medications may contribute to the development or progression of cognitive impairment and dementia in elderly populations. However, the potential association between analgesics and dementia-related outcomes remains insufficiently characterized in real-world pharmacovigilance settings. A disproportionality analysis of individual case safety reports (ICSRs) was performed using VigiBase, the World Health Organization (WHO) global pharmacovigilance database. The study aimed to investigate potential associations between the analgesics most frequently used in clinical practice and the reporting of dementia or Alzheimer's disease-related events, as well as to explore individual cases for potential risk factors.

Reporting Odds Ratios (RORs) with 95% confidence intervals (95% CI) were calculated. Dementia-related adverse events were reported more frequently in women aged ≥75 years and originated predominantly from the Americas. In a relevant proportion of cases, drug withdrawal was associated with partial or complete attenuation of symptoms. These adverse events were classified as serious across all pharmacological groups evaluated. Latency periods were generally short for most therapeutic groups analyzed, except for amitriptyline, for which the latency period frequently exceeded one year.

Disproportionality analysis identified signals for the following therapeutic groups: psychoanaleptics (N06) (ROR = 2.04; 95% CI: 1.93–2.15) and antiepileptics (N03) (ROR = 2.04; 95% CI: 1.92–2.17). Among antiepileptics, pregabalin showed the highest disproportionality for dementia-related reporting. No pharmacovigilance signals were identified in the overall analysis of opioids (N02A), either in the general population or in sex- and age-stratified subgroup analyses. Similarly, no overall signal was detected for opioid combinations (N02AJ), although a specific signal was identified for the hydrocodone/ibuprofen combination and dementia (ROR = 3.01; 95% CI: 1.43–6.32). Within the non-steroidal anti-inflammatory drugs (NSAIDs) group (M01A), few signals were detected for the preferred terms analyzed. A slight disproportionality signal was observed for celecoxib and Alzheimer's disease (ROR = 1.78; 95% CI: 1.24–2.55), supported by a relevant number of reports (n = 30). No signals were identified for ibuprofen, diclofenac, or naproxen. Likewise, no pharmacovigilance signals were identified for other analgesics and antipyretics (N02B), either as a therapeutic group or for the individual drugs analyzed, including paracetamol, acetylsalicylic acid, and metamizole.

The pharmacovigilance signals identified for antiepileptics and psychoanaleptics should be interpreted with caution, as these drugs are frequently prescribed in patients with prodromal cognitive impairment or established dementia, potentially resulting in confounding by indication and protopathic bias. Although neuroinflammation has been implicated in the pathogenesis of Alzheimer's disease, the signal observed for celecoxib may also reflect protopathic bias and confounding factors associated with chronic pain, ageing, and comorbidity in exposed patients. Overall, most analgesics commonly prescribed in clinical practice demonstrated a relatively favourable pharmacovigilance profile regarding dementia-related adverse events.

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Teaching Innovation in Pharmacology

P25

Enhancing interdisciplinary interaction through collaborative practical activities among Pharmacology students from Medicine and Biomedical Engineering degrees

Laura Fernández-Sánchez[1], Mateo Ruiz-Conca[1], Mª Cristina García Cabanes[1], Pilar D'Ocón[2], Marisa Ferrándiz[2], María Dolores Ivorra[2], Luis Gandía[3], Estela Tébar-Garcerán[1], Marta Estalrich[1], Victoria Maneu[1]

[1]University of Alicante, Alicante, Spain
[2]University of Valencia, Valencia, Spain
[3]Autonomous University of Madrid, Madrid, Spain

E-mail: vmaneu@ua.es

Collaboration between healthcare professionals is key to ensuring effective clinical management and high-quality patient care. However, university programs often focus mainly on the specific competencies of each discipline, providing students with limited opportunities to interact with or learn from other healthcare fields. University training is therefore an ideal stage to encourage interprofessional learning and better understanding of the different roles within healthcare teams. Early exposure to other disciplines can help foster respect, communication, and collaboration among future professionals, ultimately benefiting patient care.

In this project, we implemented a joint activity involving third-year Pharmacology students from the Medicine and Biomedical Engineering degrees. The aim of this collaborative practical activity was to develop a mobile application to support healthcare professionals in selecting the most appropriate antidiabetic treatment for patients with diabetes. Each team consisted of two medical students and two biomedical engineering students. In the first joint session, students worked together for 2 hours to define the app structure, identify relevant clinical variables, and design the decision-making process. In the second session, biomedical engineering students were responsible for developing and programming the application.

A total of 120 students took part in the activity, distributed into three groups. Each session was supported by four instructors, which helped ensure smooth organisation and coordination throughout. In the anonymous post-activity surveys, students rated the experience as positive or very positive in 98.3% of responses. In addition, 95.0% reported that the activity helped them better understand the role of the other profession, and 95.8% felt it also contributed to a greater appreciation of their own knowledge. Moreover, 95.8% of the students stated they would consider engaging in interprofessional collaborations after having carried out the experience. The instructors considered the activity both highly instructive and a very positive experience.

We believe that joint experiences involving students from various degree programs are a valuable tool to foster interprofessional communication from early stages and will help to improve understanding and collaboration among future professionals.

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P26

Use of Quizizz® to Enhance Pharmacology Learning: Perceptions Among Medical Students

Vittoria Carrabs, Marta Marín, Lourdes Bosch

Department of Pharmacy, University CEU Cardenal Herrera, Valencia, Spain.

E-mail: vittoria.carrabs@uchceu.es

Introduction: The incorporation of gamification tools in higher education has increased in recent years due to their potential to improve student motivation, participation, and active learning. Among these tools, Quizizz® enables the integration of interactive quizzes with immediate feedback and competitive dynamics that may enhance student engagement in the classroom.

Material and methods: A cross-sectional observational study was conducted using a satisfaction questionnaire administered to medical students after the use of Quizizz® in teaching activities. The questionnaire included Likert-scale items (1–5) related to motivation, participation, ease of use, content comprehension, and overall perception of the tool. A descriptive analysis of the responses was performed.

Results: A total of 55 responses from medical students were analyzed. Overall, the use of Quizizz® received positive evaluations, with mean scores above 4 out of 5 for most assessed items. The highest-rated aspects were the fun and engaging nature of learning through Quizizz® (4.28, the ease of understanding how to use the platform (4.20), and the increase in active participation during teaching activities (4.17). Students also highlighted that Quizizz® makes learning more interesting (4.12) and that immediate feedback improves content comprehension (4.10). Most students additionally expressed interest in continuing to use Quizizz® in future courses.

Conclusions: The results suggest that Quizizz® is a well-valued gamification tool among medical students and that its use may contribute to improving motivation, active participation, and the overall learning experience in the classroom. The integration of interactive and dynamic methodologies could represent a useful strategy to enhance student engagement in Health Sciences education.

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In person teaching and learning methodology is essential in Pharmacy education

María Dolores Pérez-Carrión[1,2], Irene Rodriguez-Clemente[1,2], Inmaculada Posadas[1,2]

[1]*CIBER, Instituto de Salud Carlos III, Madrid, Spain*
[2]*Unidad Asociada Neurodeath, INAMOL, Universidad de Castilla-La Mancha, Albacete, Spain E-mail:*

inmaculada.posadas@uclm.es

The COVID-19 pandemic prompted a sudden shift in educational paradigms worldwide. This observational, cross-sectional study examined the perceptions of students and professors from the School of Pharmacy at the University of Castilla-La Mancha, regarding different teaching-learning methodologies implemented before, during and after the pandemic. A total of 157 participants (136 students and 21 faculty members) completed an anonymous questionnaire assessing satisfaction and perceived effectiveness of virtual teaching, limited face-to-face instruction, and traditional face-to-face teaching, using a 5-point Likert scale. The results revealed that both groups showed a clear preference for face-to-face modalities, particularly for practical-based activities. Fully in-person teaching received the highest satisfaction scores from students (4.04±0.73 to 4.23±0.85) and professors (4.57±0.51 to 4.76±0.43), followed by limited face-to-face instruction in small groups. While both groups recognized the benefits of virtual teaching in terms of flexibility, virtual teaching alone received the lowest rating, especially for practical components (students: 2.52±1.28; professors: 3.05±1.27) and competency-based assessment. Both groups agreed that exclusive virtual instruction negatively affected Pharmacy training (students: 3.17±1.46; professors: 4.00±1.26), although professors consistently reported slightly higher satisfaction scores than students across most items. In conclusion, these findings underscore the importance of integrating diverse teaching methodologies, highlighting face-to-face instruction as essential for optimizing learning outcomes in scientific disciplines such as Pharmacy.

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Implementation of a Breakout Box activity as a gamification strategy in the teaching of Pharmacology in the Degree of Optics and Optometry

Marta Estalrich-Soliveres, Estela Tébar-Garcerán, Lorena Vidal-Gil, Laura Fernández-Sánchez, Victoria Maneu, Mateo Ruiz-Conca

Universidad de Alicante, San Vicente del Raspeig, Spain

E-mail: marta.estalrich@ua.es

Gamification has emerged as an effective pedagogical strategy to improve motivation and learning in higher education. The Breakout Box format, inspired by escape rooms, uses padlocked containers whose combinatio codes are obtained by solving academic challenges, promoting active learning and teamwork. In a context where university student's absenteeism is rising and artificial intelligence use among students is widespread, methodologies that encourage engagement, reasoning and challenge over information retrieval are particularly relevant. We designed, implemented and evaluated a Breakout Box activity in a Pharmacology seminar session for students of the Degree in Optics and Optometry, and assessed its impact on student motivation, perceived learning and satisfaction. For this purpose, a Breakout Box consisting of two nested padlocked cases was prepared. The combination codes were obtained by solving six dichotomous (Yes/No) questionnaires covering general and ocular pharmacology topics previously taught (pharmacodynamics, pharmacokinetics, adverse reactions, anti-infective agents, anti-inflammatory drugs, and glaucoma treatment). The activity included a final challenge using a dissectible anatomical eye model. After the session, an anonymous voluntary satisfaction survey was administered, comprising 26 items on a 5-point Likert scale grouped into five constructs: motivation, perceived learning, satisfaction, comparison with traditional methodology, and teamwork. One attention-check item was included to ensure response validity. Seventeen students completed the survey (70.6% female; mean age 20 years). The majority (88.2%) had no prior experience with Breakout Box activities in higher education, and 64.7% reported using artificial intelligence regularly or always for study purposes. All responses passed the attention check. Mean construct scores (scale 1–5) were uniformly high: teamwork 4.84 ± 0.29, satisfaction 4.72 ± 0.37, comparison with traditional methodology 4.67 ± 0.37, motivation 4.53 ± 0.51, and perceived learning 4.25 ± 0.57. At the item level, three statements reached near-ceiling scores: clarity of instructions (4.94 ± 0.24), greater motivational effect compared to a traditional lecture (4.94 ± 0.24), and facilitation of group work (4.94 ± 0.24), each with 94% of responses at the maximum score. Active participation of all group members (4.82 ± 0.39) and the desire to use similar methodologies in other subjects (4.82 ± 0.39) were also rated very highly, the latter suggesting a strong transferability intent among students. Within the perceived learning construct, students most strongly agreed that the Yes/No format encouraged reflection rather than mere recall (4.59 ± 0.62), and that the activity helped consolidate ocular pharmacology content (4.29 ± 0.59). The lowest-rated item across the entire survey was content retention compared to self-study (4.00 ± 1.12), which also showed the greatest score variability, suggesting heterogeneity in how students perceived the long-term learning value of the activity. Interestingly, students who reported using AI regularly or always showed a higher mean perceived learning score (4.37 ± 0.47) compared to occasional users (4.04 ± 0.49), suggesting the activity may be particularly effective in engaging students who habitually rely on AI-assisted study. The Breakout Box activity was well received and positively rated across all dimensions evaluated. These results suggest that this methodology represents a viable and effective strategy to increase student motivation in Pharmacology teaching, particularly in contexts where active learning may help to enhance engagement. Its integration into university education merits further exploration through studies with larger sample sizes and comparative designs.

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A Pharmacology-themed escape room: implementation and students' perception

Ana M. Sahagun[1], E. Milena Vazquez[1], Beatriz Romero[1], José M. Rodriguez[1], M. Jesusa Martinez[1], Raquel Diez[1]

[1]*Pharmacology, IBIOMED, University of Leon, Leon, Spain*

E-mail: amsahp@unileon.es

In the last decade self-directed and lifelong learning has received increasingly attention as a way to achieve those attitudes and skills necessary to meet professional development in ever-changing healthcare environments. In this framework, the usage of virtual escape rooms in Higher Education settings may reinforce students' knowledge and learning motivation, as well as improve decision-making habilities and critical thinking (Piñero, 2020; Dittman et al., 2021). The study describes the usage of a virtual escape room in a practical session with students of the subject *Pharmacology applied to Nutrition* (Degree in Human Nutrition & Dietetics). The escape room was designed with Genially©, and included four clinical cases presented as puzzles and challenges related to food-drug interactions. They solved another case in the traditional way (PowerPoint presentation, pen and paper), and the five cases were finally reviewed and discussed as a group with the lecturer. A total of 25 students (64 % female) took part in the activity, working individually each case. The escape room took up about 1,15 h of the 2 hours established for the practical session. A satisfaction survey was provided them to find out their opinion on the activity. The survey was answered by 21 students (66.7 % female). For the vast majority of them (85.1 %), the number of cases worked on were appropriate or very appropriate, as were and their level of difficulty, the design of the cases and the instructions provided for solving them. Furthermore, they rated the escape room as appropriate or very appropriate for enabling them to learn in a fun way (85.7 %), and helped them to improve their skills in information retrieval and decision-making (76.2 %). It also increased their interest for the practical exercise (85.7 %), they felt it was a tool that promotes learning (95.2 %), and they pointed out that more activities like this should be organized (85.7 %). Their level of attention and interest was also higher with the escape room (71.4 %), they preferred it for carrying out the practical exercise (85.7 %), and expressed a high degree of satisfaction with the activity (90.5 %). The results obtained show that students prefer these activities over traditional practical sessions. Thus, appropriate use of educational escape rooms may have significant positive impacts on their engagement and learning.

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Empowering Teens Through Service-Learning: A University-Led Strategy for Substance Abuse Prevention

Cristina Arce-Recatalá, Rosa María Giner, María Amparo Blázquez, Nuria Cabedo

[1]*Universitat de València, Burjassot, Spain,*

E-mail: Cristina.arce@uv.es

Adolescent substance abuse is a major public health concern associated with physical, psychological, and social consequences. Educational interventions during adolescence are essential to promote healthy habits and prevent risky behaviors. Service-learning methodologies combine academic training with community engagement, allowing university students to apply their knowledge in real-life settings. In this project, Pharmacy students participated as active health educators for high school students. Through collaborative and interactive activities, the initiative aimed to increase awareness about addictive substances while strengthening communication and leadership skills among university students.
The project involved Pharmacy students in their third year and secondary school students in a classroom-based educational intervention. University students implemented flipped classroom and gamification methodologies using digital tools such as the Virtual Classroom platform, Google Forms®, and Wooclap to create interactive learning activities, complemented by a practical demonstration with tobacco leaves. In addition, the intervention was evaluated through two *ad hoc* five-point Likert scale questionnaires: a pre-activity survey assessing socio-demographic characteristics, risk perception, and consumption patterns, and a post-activity survey evaluating satisfaction and perceived usefulness of the activity. Data was analyzed to assess changes in knowledge, awareness, and attitudes toward substance abuse prevention

Results showed that caffeine and alcohol were the most commonly consumed substances among participants, whereas the use of cocaine, anxiolytics, and amphetamines was rarely reported. Tobacco and cannabis consumption remained low across the three participating educational groups. After the intervention, students reported positive changes in awareness and attitudes toward substance abuse prevention. The intervention improved adolescents' understanding of the risks associated with addictive substances and promoted healthier lifestyle habits. In fact, the post-activity evaluation revealed high levels of satisfaction, with most participants agreeing that the activity improved their learning, understanding, and awareness of healthy habits. Interactive methodologies, including practical activities and digital tools, were especially well valued, and students highlighted the usefulness, enjoyment, and recommendation of the service-learning experience.

This collaborative service-learning initiative successfully enhanced Pharmacy students' scientific knowledge, leadership, and communication skills through their active role as health educators. The close intergenerational interaction between university and high school students fostered a more engaging and effective learning environment, promoting meaningful discussions about healthy habits and substance abuse prevention.

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P31

Innovación docente en Atención Farmacéutica mediante metodologías activas orientadas a la práctica profesional

M. Carmen Montesinos[1,2], Isabel Andújar[1], M. Carmen Carceller[1,2], M. Carmen Recio[1,2], M. Luisa Ferrándiz[1,2], Isabel García-Arnandis[1,2]

[1]University of Valencia, Burjassot, Spain, [2]IDM, Valencia, Spain

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

Pharmaceutical Care (PC) currently requires a training model focused on the development of clinical, communication, and social competencies closely linked to professional practice. The aim of the study was to describe a teaching model based on the integration of active learning methodologies in a Clinical Pharmacy and Pharmaceutical Care course and to analyse its contribution to students' competency development. For this purpose, a descriptive study was conducted during the 2023–2024 academic year in the Clinical Pharmacy and Pharmaceutical Care course of the Pharmacy degree and the double degree in Pharmacy and Human Nutrition and Dietetics at the Universitat de València. The educational model integrated case-based learning (CBL), clinical simulation, service-learning (SL), and digital health communication through Instagram. Assessment included structured rubrics and anonymous 5-point Likert-scale satisfaction questionnaires.

The methodologies allowed the complementary development of competencies related to active dispensing, pharmaceutical indication, medication therapy follow-up, communication skills, and health promotion. Clinical simulation achieved a mean satisfaction score of 8.5/10, whereas in the SL activity, 57.14% of students assigned the highest score to the usefulness of the activity for improving communication and scientific dissemination skills, and the same percentage indicated that the experience fully met their expectations. Digital health communication reached more than 6,400 users, and 98.29% of students would recommend the activity to other classmates.

The structured integration of active learning methodologies may represent a useful educational strategy to align university training with the healthcare demands of community pharmacy practice and to support the development of key competencies required for PC.

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Online Faculty Training and Educational Innovation in Pharmacology: The REDFARMINN Experience

Cristina Arce, M. Pilar D'Ocon, M. Dolores Ivorra, M. Luisa Ferrándiz

Universitat de València, Burjassot, Spain

E-mail: Luisa.ferrandiz@uv.es

Current trends in Pharmacology education increasingly promote active learning methodologies and competency-based teaching approaches. In this context, inter-university collaboration represents an important strategy for promoting educational innovation and faculty development. Since 2015, the Inter-University Network for Innovation in Pharmacology Teaching, supported by teaching innovation projects at the University of Valencia, has facilitated collaboration among Pharmacology educators through its platform (www.uv.es/redfarminn). The network currently includes 229 professors from 45 universities across Spain, Portugal, Chile, Colombia, and Mexico.

The network develops activities aligned with its educational objectives, including annual scientific meetings, collaborative teaching innovation projects, educational resource sharing, and faculty training initiatives. In recent years, specialized online workshops have been incorporated to support teacher adaptation to emerging educational methodologies and digital teaching environments.

Annual meetings have become a central component of the network, promoting discussion on topics such as gamification, flipped classroom methodologies, collaborative assessment, and simulation-based learning in Pharmacology education. In addition, in recent years, several specialized workshops have been included: "How to get the most out of our online teaching through the Blackboard Collaborate platform" (2020), "Challenges of assessment in collaborative environments" (2021), "The value and usefulness of simulation methodologies in teaching Pharmacology" (2023). More recently, the network introduced training activities focused on the use of artificial intelligence (AI) as a teaching support tool in Pharmacology. During the current academic year, two introductory online workshops on AI applications in teaching were delivered through the University of Valencia virtual platform using Wooclap, with participation from 95 professors.

The sustained participation in these activities highlights the relevance of collaborative faculty development initiatives in Pharmacology education. The incorporation of AI-focused training workshops reflects emerging educational needs and supports the continuous adaptation of university teaching to evolving technological contexts. Future activities will include both introductory and advanced workshops on AI applications in teaching.

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Human Rights-Based Active Learning in Pharmacology Education: A UNESCO Chair-Supported Experience on Psoriatic Arthritis and Global Health

M. Carmen Carceller[1,2], Isabel García-Arnandis[1,2], Montserrat Robustillo-Villarino[3], Andrés Zúñiga-Vera[3], M. Carmen Montesinos[1,2]

[1]University of Valencia, Burjassot, Spain; [2]IDM, Valencia, Spain; [3]Hospital Universitario de la Plana, Castellón, Spain

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

Human rights, equity and global health are increasingly recognised as essential dimensions of pharmacology education and health sciences training [1,2]. Within this framework, an educational innovation focused on psoriatic arthritis (PsA) was developed in the course Foundations of Inflammation Pharmacology of the Master's Degree in Research and Rational Use of Medicines at the Universitat de València, as part of a UNESCO Chair project on psoriasis, psoriatic arthritis and global health.

The intervention involved twelve postgraduate students and combined active learning methodologies with a human-rights and global health perspective. Students first completed a pre-test assessing biomedical knowledge and awareness of inequities and stigma associated with PsA. Following a brief introductory lecture, students in pairs preparer oral presentations on targeted therapies and analysed Peru as a case study of barriers to diagnosis and access to biologics. In addition, they designed public-facing infographics and engaged with the patient perspective using materials provided by APAPSO Peru, a patient organisation for people living with psoriasis and psoriatic arthritis that advocates for awareness, patient support and equitable access to care. A post-test and satisfaction survey were subsequently administered.

Baseline results revealed heterogeneous prior knowledge, particularly regarding clinical manifestations and diagnostic concepts. Following the intervention, all students answered the post-test multiple-choice questions correctly, demonstrating consolidation of biomedical and pharmacological understanding. Open-ended responses evolved from limited reflections on inequity and stigma to more elaborate and contextually informed analyses. Student satisfaction was high, with participants highlighting that the Peru case study enhanced their awareness of global health inequities and barriers to biological therapies. Collaborative learning and integration of the patient-perspective were especially valued.

This experience demonstrates that integrating active learning, patient engagement, and a human-rights perspective into pharmacology education strengthens both scientific knowledge and awareness of healthcare inequities. Moreover, it promotes a proactive role of future professionals in advancing equity and access to medicines, in alignment with the Sustainable Development Goals (SDGs).

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Use of Artificial Intelligence in learning through the case method in Pharmacology (2nd year)

Luis Sendra Gisbert, Jesús Cosín-Roger, M. Pilar D'Ocon Navaza, M. Dolores Ivorra Insa, M. Carmen Recio, M. Carmen Terencio Silvestre, María José Herrero Cervera

Department of Pharmacology, Universitat de València (València, Spain)

E-mail: luis.sendra@uv.es

Background: The case method is an active learning strategy that involves presenting a real and specific situation, from which a problem is identified for students to solve, serving as a basis for reflection and learning. Students develop their knowledge by analysing and interpreting contextual factors, exploring possible solutions, comparing different ideas, and drawing conclusions that enable them to reach an appropriate response to the problem. In this project, this is applied to the resolution of clinical cases within the context of complex therapeutic scenarios, enabling the integration of content from an entire teaching unit in Pharmacology II of the Pharmacy degree and the Double Degree in Pharmacy and Nutrition. Furthermore, the project aims to compare students' outcomes with those generated by a generative artificial intelligence platform, which is capable of producing original responses based on existing data sources. Although natural language generation offers significant potential, it also presents limitations related to the accuracy and reliability of the output, which may include errors, particularly in tasks requiring logical reasoning. Generative AI is already widely accessible and commonly used by students. According to recent reports from the European Commission and CRUE, its integration into higher education is recommended, alongside training students to use it in an ethical and responsible manner.
Methods: during practical sessions (16 students/group, 4 hours), students work in subgroups to resolve 4 complex clinical pharmacology cases working in two stages: i) AI-assisted resolution; ii) review and correction of the responses provided by AI with the information retrieved from validated primary and secondary sources. Cases were 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; AI resolution of cases; information retrieval and critical review of AI answers; group analysis using both data sources and peer comparison of the information obtained, and the accuracy of different sources employed. A final collective discussion with instructors and a rubric-based evaluation to assess learning outcomes and students' perceptions of AI utility and limitations is also included. Pedagogical Strategies: Problem-Based Learning to encourage realistic contextualized knowledge construction and collaborative learning that enhances peer interaction.
Results: based on the results of the surveys conducted among 178 students from the two academic years during which we have implemented this activity, over 70% expressed improvement in their critical analysis ability. Around 60% of students believed they were more prepared to face real clinical situations. More than 75% found that AI improved their learning autonomy, but they also identified weaknesses in AI performance during complex clinical reasoning and remarked the high dependence on technology as its main disadvantage.
Conclusions: The case method is highly valued by students as it promotes meaningful learning and the development of practical skills. The integration of AI further strengthens critical professional judgement by allowing students to compare AI-generated outputs with validated academic sources, increasing their autonomy and understanding of both the strengths and Students' suggestion to make the activity more dynamic will be considered

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Active learning as a strategy to promote attendance and academic performance in pharmacology: experience in a biomedical Engineering degree

Mateo Ruiz-Conca, Marta Estalrich-Soliveres, Estela Tébar-Garcerán, Lorena Vidal-Gil, Laura Fernández-Sánchez, Victoria Maneu

Universidad de Alicante, San Vicente del Raspeig, Spain

E-mail: laura.fs@ua.es

Absenteeism in Spanish university classrooms has become a widespread concern in recent years, particularly in theory lectures. In this context, the integration of participatory activities into Pharmacology theory sessions was explored as a strategy to foster student engagement and attendance in a Biomedical Engineering degree programme. During the 2025-2026 academic year, interactive activities were designed and incorporated into Pharmacology theory classes. A total of eight activities were proposed. Three of them focused on pharmacokinetic aspects, including the evaluation of analytical software and the design and assessment of drug delivery devices. Another activity centered on the development of novel antimicrobial agents, while one activity involved the creation of a computer application related to drug interactions. The remaining three activities addressed practical challenges commonly encountered in a basic pharmacology research laboratory, including the design of devices to evaluate the effect of new therapies in mouse models of ocular disease. At the end of the course, the examination test of each student (n = 59) was collected. The correlation between the number of activities each student participated in and their examination grade was analysed using Pearson and Spearman correlation coefficients. A statistically significant positive correlation was observed between participation in classroom activities and the final examination grade (Pearson r = 0.27; p = 0.041; Spearman r = 0.25, p = 0.054). When students were grouped by level of participation, a clear dose-response trend emerged: those who attended 6 or 7 activities achieved the highest mean grade (8.22 ± 1.40), followed by students who participated in 4 or 5 activities (7.22 ± 1.50), those with no participation (6.86 ± 1.59), and finally those who attended only 1 to 3 activities (5.99 ± 1.67). The finding that students with no participation outperformed the low-participation group may reflect that students who attended sporadically could represent a less engaged subgroup, whereas those who did not attend at all may include highly autonomous learners who compensate through independent study. A perception survey administered at the end of the course (n = 8) revealed that students rated the activities positively in terms of their contribution to understanding the subject content (mean 4.5/5) and their professional applicability (mean 4.0/5). Activities that promoted creative problem-solving and applied pharmacological concepts to real biomedical engineering scenarios were most appreciated. These findings suggest that the active design of Pharmacology theory sessions may be an effective tool to increase student engagement and improve academic performance in biomedical engineering programmes, and contribute to the growing body of evidence supporting active learning strategies in health sciences education.

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P36

Academic Innovation in Pharmacology Education Through the Congreso Nacional de Proyectos Fin de Grado de Farmacia

Guillermo Cásedas[1], Estela Sangüesa[1], Nuria Berenguer[1], Elisa Langa[1], Loreto Sáez-Benito[1]

[1]*Departamento de Farmacia, Facultad de Ciencias de la Salud, Universidad San Jorge, Villanueva de Gállego, Zaragoza, Spain*

E-mail: gcasedas@usj.es

The organization of student conferences can be a valuable educational strategy to promote active learning, scientific communication, and the integration of pharmacological knowledge in undergraduate pharmacy education. In this context, the University San Jorge, together with TEVA, launched the "I Congreso Nacional de Proyectos Fin de Grado de Farmacia" in 2024, followed by a second edition in 2025, with a third edition scheduled for 2026. The initiative was designed as a national forum for students from Spanish pharmacy schools to present their final degree projects, exchange ideas, and engage with peers and faculty in a scientific environment.

Both editions were open to students from public and private Spanish universities and included oral and poster communications across thematic areas related to pharmacy, including pharmacology, pharmaceutical technology, biomedicine, patient-care pharmacy, public health, biochemistry, microbiology, chemistry, and environmental sciences. The conferences also incorporated keynote lectures, awards for the best presentations, and opportunities for interaction with the pharmaceutical industry, thereby reinforcing the connection between academic training and professional practice.

The event was made possible thanks to the collaboration of Spanish pharmacy faculties, with the active participation of professors and lecturers who contributed to both the organizational and scientific committees. Their involvement was essential for consolidating the conference as a national reference in pharmacy education.

From a teaching pharmacology perspective, these meetings helped students develop critical thinking, autonomy, and the application of pharmacological concepts to research-based projects. They also gave students to communicate scientific results in a competitive yet supportive environment, building skills that are highly relevant for future pharmacists and health professionals. The progressive consolidation of the event across three consecutive editions shows its potential as an educational model for active learning in pharmacology and related biomedical disciplines.

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Integrating AI-Based Continuous Assessment in Pharmacology: Student Perceptions of a NotebookLM-Supported Infographic Activity

Emma Barroso; Christian Griñán-Ferré; Manuel Vázquez-Carrera; Marcel.li Carbó; Marta Alegret; Mercè Pallàs

Pharmacology, Pharmacognosy and Therapeutics Unit, Faculty of Pharmacy and Food Sciences, University of Barcelona. ORFILA Teaching Innovation Group.

E-mail: pallas@ub.edu

The Pharmacology and Therapeutics course in the Pharmacy degree at the University of Barcelona incorporates continuous assessment activities aimed at promoting active learning and competency development. In the 2025–2026 academic year, a novel activity representing 10% of the final grade was introduced, consisting of the critical development of a scientific infographic on a topic related to the course using the AI-based tool NotebookLM. Students worked in small groups and were required to integrate at least eight high-quality scientific sources, critically review AI-generated outputs, and produce a final infographic aimed at a general audience, including pharmacological and patient-care perspectives.

To assess the educational impact of this activity, an anonymous survey (n≈100) was administered upon completion. Overall satisfaction was moderate (mean score: 3.19/4), with students positively rating the clarity of instructions, activity structure, and usefulness of the assessment rubric. Notably, students reported that the activity enhanced their ability to understand the limitations of AI-generated content and promoted a more critical attitude toward its use. Additionally, the activity was perceived as useful for developing skills related to literature search, identification of relevant information, and integration of theoretical knowledge into a practical context.

However, lower ratings were observed for items related to deeper learning outcomes, such as improvement in pharmacological knowledge, critical thinking, and synthesis skills. Qualitative feedback revealed recurrent concerns regarding the AI tool employed. Students reported significant limitations of NotebookLM, including lack of flexibility, slow performance, difficulties in iterative editing, and poor suitability for collaborative work. These factors often led to frustration and a perception that excessive time was spent managing the tool rather than engaging in meaningful learning. Some students also indicated that alternative methodologies, such as case-based learning or tutor-guided activities, might be more effective for fostering deeper understanding.

In conclusion, while the integration of AI tools in continuous assessment activities may enhance digital competence and foster critical awareness of AI, its educational effectiveness depends strongly on the usability and suitability of the selected tools. Future implementations should consider greater flexibility in tool choice and a stronger emphasis on pedagogical strategies that prioritize active student engagement over technological constraints.

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From Classroom to Community: An Educational Innovation Using Problem-Based Learning in Ocular Pharmacology

Patrice Marques[1], Luis Sendra[1], Jesús Cosín-Roger[1]

[1] *Department of Pharmacology, University of Valencia, Valencia, Spain*

E-mail: patrice.gomes@uv.es

Background: Active learning methodologies are increasingly used in health sciences education to enhance student engagement and develop professional competencies. Among these, Problem-Based Learning (PBL) promotes analytical reasoning, collaborative learning, and the integration of theoretical and practical knowledge through the exploration of real-world problems. In this context, a PBL-based activity was introduced into the Ocular Pathology and Pharmacology course (Degree in Optics and Optometry), with a particular emphasis on translating scientific knowledge into accessible information for non-specialist audiences.

Materials and Methods: Students (n=44) were presented with a problem focused on differentiating between bacterial and viral conjunctivitis. Working in groups, they were required to develop a science communication product in various formats (videos, presentations, posters, digital resources, or other creative proposals) designed to convey rigorous scientific information to non-specialist audiences across different age groups (from early childhood to late adolescence). The activity was carried out through supervised sessions and concluded with the presentation of the completed projects. Upon completion of the activity, students completed a satisfaction survey consisting of 15 items grouped into four dimensions: (1) overall evaluation of the activity, (2) content and methodology, (3) organization and evaluation, and (4) satisfaction and suggestions for improvement. Responses were collected using a 5-point Likert scale.

Results: Students rated the activity very positively, with an overall score of 4.68/5. Participants found the experience engaging and motivating, highlighted its relevance to their learning, and reported that it facilitated the understanding and practical application of key concepts of theoretical lessons. The activity was also perceived as effective in promoting critical thinking, problem-solving, teamwork, and peer collaboration, with teamwork receiving one of the highest ratings (4.88 ± 0.34). Organization, clarity of instructions, and feedback were particularly well valued (all ≥ 4.79/5). Overall, students expressed high levels of satisfaction and a strong willingness to participate in or recommend similar activities in future course editions. Median scores were ≥ 4.0 for all survey items.

Conclusions: The incorporation of PBL into the Ocular Pathology and Pharmacology course seems to represent an effective teaching strategy, highly valued by students. The activity facilitated the understanding of theoretical concepts, the practical application of knowledge, collaborative learning, and the development of science communication skills aimed at non-specialist audiences. The high levels of student satisfaction support the continued implementation of active learning methodologies in pharmacology education.

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Pharmacology of Ion Channels

P39

DECA-11 Restores Nav1.5 and Kir2.1 Channel Expression in the Membrane

Sara Pérez-Martín[1], Josu Rapún[1], Alba De La Iglesia[1], Teresa Crespo-García[1], Luis Loya[1], Gorka San José[2], Begoña López[2], Aránzazu González-Miqueo[2], Eva Delpón[1], Ricardo Caballero[1].

1. *Department of Pharmacology and Toxicology. School of Medicine. Universidad Complutense de Madrid. Instituto de Investigación Gregorio Marañón. CIBERCV, Instituto de Salud Carlos III, Madrid, Spain*
2. *Program of Cardiovascular Diseases, CIMA Universidad de Navarra and IdiSNA. CIBERCV, Instituto de Salud Carlos III, Pamplona, Spain*

E-mail: edelpon@ucm.es

Ventricular arrhythmias and ventricular fibrillation are the main cause of death in patients with heart failure (HF), who are estimated to be around 15 million in Europe. HF with reduced ejection fraction (HFrEF) induces an electrical remodeling characterized by downregulation of Nav1.5 and Kir2.1 channels (encoded by *SCN5A* and *KCNJ2* genes, respectively) at the cardiomyocyte membrane, reducing the Na^+ (I_{Na}) and inward rectifier K^+ (I_{K1}) current densities, thereby impairing ventricular excitability and delaying repolarization, factors that together constitute a highly arrhythmogenic substrate. We designed a gene therapy encoding an 11-mer peptide named DECA-11 (WO2025068623), capable of selectively increasing I_{Na} and I_{K1} in heterologous expression systems, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), and mouse ventricular cardiomyocytes that reduces VA risk in a mouse model of HF. In the present study, we aim to explore the mechanisms responsible of the effects of DECA-11. In spontaneously beating hiPSC-CMs, DECA-11 did not augment the late I_{Na} nor modify the amplitude and duration of intracellular Ca^{2+} transients or the contraction and relaxation speed at three frequencies (0.5, 1, and 1.8 Hz). Furthermore, DECA-11 did not modify the biophysical properties of Nav1.5 and Kir2.1 channels, as it had no effect on their conductance (γ), open probability (P_o) and kinetics characterized in single-channel experiments (cell-attached configuration of the patch-clamp technique). However, DECA-11 produced a pro-transcriptional effect on the promoter of the human *KCNJ2* gene (P=0.006) and increased *KCNJ2* mRNA expression levels (P=0.0003), while it did not modify the *SCN5A* gene transcription or mRNA expression levels. Flow cytometry experiments showed that DECA-11 augmented the number of fluorescent cells and their fluorescence intensity, which reflects an increase in the number of Nav1.5 and Kir2.1 channels at the cell membrane. Importantly, DECA-11 increased sarcolemmal and total expression of Nav1.5 and Kir2.1 channels ($P \leq 0.0001$) in ventricular samples of mice subjected to transverse aortic constriction. In conclusion, DECA-11 increases I_{Na} and I_{K1} by augmenting the sarcolemmal Nav1.5 and Kir2.1 channel expression without affecting their biophysical properties.

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Role of N-terminal domain of GLUN1 subunits ON NMDAR desensitization

Laura E. Maglio[1], Laura L. Hernández-Salas[2], Ricardo Gómez[2]

[1] *Departamento de Fisiología, Facultad de Medicina, Universidad Complutense de Madrid, Madrid, Spain*
[2] *Departamento de Farmacología y Toxicología, Facultad de Medicina, Universidad Complutense de Madrid, Madrid, Spain*

E-mail: ricardo_gomez@med.ucm.es

N-methyl-D-aspartate receptors (NMDAR) are glutamate-activated nonselective cation-permeable ion channels broadly distributed along the central nervous system, although recent studies demonstrated that they are also expressed in some types of tumours. These ion channels play a fundamental role in different brain functions, especially those related to learning and memory processes. One of their main function is modulating synaptic transmission and plasticity modulation, given the fact that their peculiar biophysical properties enable them to act as coincidence detectors of pre- and postsynaptic neuronal activity. After release of glutamate from the presynaptic terminal, NMDAR channel activation is translated into a transition from closed to open and, then, to desensitized state. Desensitization is defined as the decrement of a response (decrease in the current amplitude for NMDAR) in the continued presence of a stimulus (agonists binding for NMDAR), with recovery occurring after withdrawal of the stimulus (unbinding of agonists for NDMAR). Under physiological conditions, the rates into and out of the desensitized state can shape the synaptic response time course. In the present work, we aimed to properly elucidate the molecular determinants of NMDAR desensitization of the N-terminal domain (NTD). For this purpose, we used electrophysiological techniques (*patch-clamp*) combined with the use of different mutations on the GluN1 subunit of the NMDAR to elucidate the role of its NTD on NMDAR desensitization.
The results obtained unveil the role of different GluN1 NTD residues on NMDAR desensitization, pointing to the importance of this region in this physiological process and suggesting new drugable binding sites to modulate NMDAR desensitization process and, potentially, control synaptic transmission, synaptic plasticity, and, thus, brain functions such as learning and memory.

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Cardiovascular & Renal Pharmacology

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Proximal tubular FOSL1 deficiency aggravates streptozotocin-induced diabetes

Tania Sánchez-Díaz[1], Aránzazu Pintor Chocano[1,2], Alberto Ortiz[1,2,3], María Dolores Sánchez-Niño[1,2,3]

1 IIS-Fundación Jiménez Díaz, Madrid, Spain
2 RICORS2040, Madrid, Spain
3 Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain.

E-mail: tania.sdiaz@iis-fjd.es

Diabetes Mellitus is a major global health problem with an increasing worldwide prevalence, currently estimated to affect more than 10% of global population. Chronic hyperglycaemia promotes inflammation, oxidative stress and tissue injury, contributing to the development of kidney disease (1). FOSL1/FRA1 is a member of the AP-1 transcription factor family involved in cellular stress and inflammatory responses. Increased kidney expression of AP-1 transcription factor components has been reported in acute kidney injury (AKI) (2). However, its role in diabetes remains poorly understood.

In a mouse model of hyperglycaemia (streptozotocin-induced diabetes), kidney Fosl1 was overexpressed (mRNA and protein expression). Proximal tubular cell-specific Fosl1 knockout mice ($Fosl1^{\Delta tub}$) developed earlier, and more severe hyperglycaemia compared to wild-type diabetic animals. Moreover, $Fosl1^{\Delta tub}$ mice showed increased expression of inflammatory mediators and NADPH oxidase family members, while nephroprotective factors levels, such as Klotho and PGC1α, were lower than in wild-type diabetic animals. This was also associated with a trend toward more severe inflammatory cell infiltration in renal tissue.

Overall, these findings suggest a potential involvement of tubular Fosl1 in systemic metabolic regulation and in the renal response to experimental diabetes and support further studies to clarify its contribution to inflammatory and oxidative stress pathways during diabetic kidney injury.

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Proximal tubular Fosl2 deficiency induces and increases the severity of chronic kidney disease

Marta Ribagorda Bermejo[1], Arancha Pintor Chocano[1, 2], Alberto Ortiz[1, 2, 3], María Dolores Sánchez Niño[1, 2, 3]

1. *Instituto de Investigación Sanitaria de la Fundación Jiménez Díaz, Madrid, Spain.*
2. *RICORS2040, Madrid, Spain.*
3. *Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain.*

E-mail: marta.ribagorda@iis-fjd.es

Chronic kidney disease (CKD) affects more than 850 million people worldwide and is associated with high mortality, impaired quality of life, and an increased risk of acute kidney injury (AKI), which further accelerates disease progression. Despite its growing prevalence and clinical impact, effective treatments for CKD remain limited, with renal replacement therapy still being the only option for kidney failure. Therefore, understanding the molecular mechanisms underlying CKD is essential for the development of novel therapeutic strategies and early biomarkers. Increasing evidence suggests that AP-1 transcription factors could contribute to the inflammatory and fibrotic processes characteristic of CKD. In this study, we investigated the role of the AP-1 family member Fosl2 in CKD progression and its contribution to renal inflammation and fibrosis.

We first identified overexpression of Fosl2 in experimental models of AKI and CKD. Proximal tubule-specific Fosl2 deficient mice ($Fosl2^{PTKO}$) developed a spontaneous subclinical CKD, characterized by the acquisition of a secretory phenotype in proximal tubular cells, increased proinflammatory cytokines and profibrotic factors, and reduced nephroprotective molecules. Consistently, experimental induction of CKD by rich-adenine diet increased CKD severity in $Fosl2^{PTKO}$ mice compared to WT mice, promoting inflammation and extracellular matrix deposition, along with a reduction of nephroprotective factors.

Kidney transcriptomic analysis of $Fosl2^{PTKO}$ mice revealed downregulation of genes involved in transport and metabolism, together with upregulation of pathways related to hypoxia, immune response and collagen deposition. In CKD, $Fosl2^{PTKO}$ mice displayed enhanced inflammatory and immune responses, including overexpression of the cytokine IL-6. Importantly, IL-6 signaling blockade with specific antibodies attenuated kidney disease severity in $Fosl2^{PTKO}$ mice.

Overall, our results identify Fosl2 as a key regulator of renal homeostasis and suggest that Fosl2 expression in proximal tubular cells is required to preserve kidney integrity and limit CKD progression.

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Sex-dependent serotonergic modulation of perivascular CGRPergic neurotransmission involving 5-HT$_{2A}$ receptors

Anaïs Clara Terol Úbeda[a,b], **Mónica García Domingo**[a,b], **José Ángel García Pedraza**[a,b]

[a] *Universidad de Salamanca, 37007, Salamanca, Spain.*
[b] *Instituto de Investigación Biomédica de Salamanca (IBSAL). 37007, Salamanca, Spain.*

E-mail: aniter99@usal.es

Calcitonin gene-related peptide (CGRP) is a potent vasodilator agent released from sensory nerves, playing a key role in cardiovascular and neurovascular regulation [1] and in migraine pathophysiology, a disorder 3-4 times more prevalent in women than in men [2]. Previous studies in rats have shown that the serotonergic system modulates perivascular sensory CGRPergic neurotransmission through activation of 5-HT$_{1/7}$ receptors [3,4], with sex-dependent differences in both the 5-HT$_1$ receptor subtypes involved and the pre and/or postjunctional nature of 5-HT$_7$ receptors [4]. However, the role of 5-HT$_2$ receptors, which are known to participate in cardiovascular regulation and display marked sex-dependent differences [5], remains unknown. Therefore, the aim of this study was to investigate the sex differences in serotonergic regulation of perivascular sensory CGRPergic neurotransmission, with special focus on the role of 5-HT$_2$ receptors. Male and female Wistar rats were anaesthetised, pithed and prepared for electrical stimulation of perivascular sensory CGRPergic outflow [3,4]. Mean blood pressure (MBP) and heart rate were continuously monitored, and vasodilator responses were elicited by electrical stimulation of the entire spinal cord (0.1-5 Hz, 50 V) during infusion of serotonergic agonists or vehicle [3,4]. Basal MBP was lower in female (53±1 mmHg) than in male pithed rats (64±2 mmHg), whereas CGRP-mediated vasodilator responses were similar between sexes. In male rats, neither 5-HT (1 µg/kg/min) nor the non-selective 5-HT$_2$ agonist α-methyl-5-HT (5 µg/kg/min) modified the CGRPergic vasodilator responses. In contrast, in females, 5-HT (1 µg/kg/min), α-methyl-5-HT (5 µg/kg/min), and the selective 5-HT$_{2A}$ agonist TCB-2 (3 µg/kg/min) significantly inhibited CGRP release from sensory nerves, whereas BW723C86 and MK212 (selective 5-HT$_{2B}$ and 5-HT$_{2C}$ agonists, respectively; 20 µg/kg/min) did not modify the vasodilator responses obtained by electrical stimulation. These findings demonstrate sex-dependent differences in serotonergic modulation of perivascular sensory CGRPergic neurotransmission. In female rats, the inhibitory effect of 5-HT on CGRPergic vasodilator responses involves 5-HT$_{2A}$ receptor activation, whereas in males neither 5-HT nor 5-HT$_2$ receptor activation altered CGRPergic perivascular neurotransmission.

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Development of a miniaturized phenotypic assay for drug screening in polycystic kidney disease

Juan Abeigón-Moure[1], Mariña Durán-Rubí, Antón L. Martínez[1], José Brea[1] and María Isabel Loza[1]

[1]Innopharma Drug Screening and Pharmacogenomics Platform, BioFarma Research Group, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), Department of Pharmacology, Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela and IDIS (Health Research Institute of Santiago de Compostela), Santiago de Compostela, Spain.

E-mail: juan.abeigon@rai.usc.es

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the progressive formation of renal tubular epithelium-derived cysts and remains an area of unmet therapeutic need due to the limited availability of effective and safe disease-modifying treatments. While traditional approaches fail to capture the patient's biological reality and diversity, 3D culture systems offer more disease-relevant models than conventional 2D assays; however, their use in drug screening requires formats that are robust, scalable and compatible with high-throughput workflows.

The objective of this study was to develop a miniaturized 3D phenotypic assay using immortalized MDCK (Madin-Darby canine kidney) cells, with potential applicability in high-throughput screening (HTS). Culture conditions were optimized in a collagen matrix in 384-well plates, and forskolin-induced cyst formation was used as the phenotypic readout. The pharmacological responsiveness of the model was evaluated using rapamycin and birinapant as pharmacological modulators of cystogenesis.

Forskolin induced cyst formation at 5 and 10 µM compared with the DMSO control (both $p<0.0001$), confirming the ability of the model to generate a quantifiable cystogenic phenotype. In cultures undergoing forskolin-induced cystogenesis, co-treatment with rapamycin at 0.05 µM reduced the number of cysts compared with forskolin alone at 5 µM ($p<0.0001$) and 10 µM ($p<0.01$). Similarly, co-treatment with birinapant at 1 µM reduced cyst formation in comparison with forskolin alone at 5 µM ($p<0.0001$) and 10 µM ($p<0.05$).

In conclusion, we developed a miniaturized 3D phenotypic ADPKD model in 384-well plates that generates a reproducible and quantifiable cystogenic phenotype and detects pharmacological modulation by compounds with distinct mechanisms of action. This model provides a scalable platform for phenotypic drug screening and may support the identification and further characterization of candidate compounds for ADPKD.

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Digestive and Respiratory Pharmacology

P45

Emerging therapeutic role of fedratinib in ameliorating liver fibrosis associated with metabolic dysfunction-associated steatohepatitis

María-Jesús Sanz[1,2,3], Carmen de la Fuente-Gómez[1,2], Elena Domingo[1], Vera Francisco[1], Álvaro Gómez-Martín[1,2], Laura Martínez-Arenas[4,5], Ângela Carvalho-Gomes[4,5], Laura Piqueras[1,3,6], Marina Berenguer[2,4,5], Patrice Marques[2,4,5]

[1] INCLIVA Biomedical Research Institute, Valencia, Spain
[2] Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain
[3] CIBERDEM-Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, ISCIII, Madrid, Spain
[4] La Fe Health Research Institute (IIS La Fe), Valencia, Spain
[5] CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Madrid, Spain
[6]Department of Pharmacology, Faculty of Pharmacy, University of Valencia, Valencia, Spain

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

Background: Metabolic dysfunction-associated steatohepatitis (MASH) is a progressive liver disorder marked by excessive lipid accumulation, hepatocellular injury, and chronic inflammation, which can ultimately lead to fibrosis and advanced liver disease. Despite its increasing global prevalence, therapeutic options specifically targeting fibrotic progression remain limited. Fedratinib, a selective JAK2 inhibitor currently approved for myelofibrosis treatment, has recently shown promising antifibrotic activity in experimental models of pulmonary fibrosis. Therefore, we investigated the therapeutic potential of fedratinib in attenuating liver injury and fibrosis in an experimental model of MASH.

Material and Methods: Six-week-old male C57BL/6 mice were assigned to either a standard control diet or a choline-deficient, high-fat diet to induce MASH over 12 weeks. At 8 weeks, MASH-induced animals received subcutaneous administration of either vehicle or fedratinib (5 mg/kg/day) for 4 additional weeks via osmotic mini-pumps. Then, blood and liver samples were collected for comprehensive evaluation. Hepatic tissue was examined histopathologically using hematoxylin-eosin and picrosirius red staining to assess steatosis and fibrosis, respectively. In addition, hepatic α-SMA protein expression was determined by western blot analysis. Finally, liver immune cells were characterized by flow cytometry, while relevant biochemical markers and cytokines were measured in plasma using colorimetric assays and ELISA.

Results: Although fedratinib did not affect hepatic steatosis, it significantly attenuated liver fibrosis by 37.8% in MASH mice. Furthermore, fedratinib significantly decreased leukocyte infiltration in the liver, enhanced regulatory T-cell content, and promoted macrophage polarization toward an anti-inflammatory (M2-like) phenotype.

Conclusion: Our data support fedratinib as a potential therapeutic approach to reduce liver fibrosis in MASH, a disease with limited pharmacological options. However, further transcriptomic, proteomic, and histological studies are required to elucidate the underlying mechanisms and signaling pathways.

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Levothyroxine and Accommodative Disorders: A Disproportionality Analysis using VigiBase

Resurrección Riquelme-Nicolás, Francisco Lara-Lacárcel, Pedro Marín, Norberto López-Gil, María Teresa Yuste

Universidad de Murcia, Murcia, Spain.

E-mail: mariateresa.yuste1@um.es

The aim of this study was to investigate a potential association between levothyroxine (LT4) use, the standard treatment for hypothyroidism, and accommodative dysfunction using data from VigiBase, the WHO global pharmacovigilance database.

A retrospective pharmacovigilance study using a case–non-case disproportionality analysis was conducted in VigiBase, including the MedDRA Preferred Term (PT) "Accommodation disorder" (AD) and the High Level Term (HLT) "Refractive and accommodative disorders" (RAD) associated with LT4 and its combinations. Disproportionality was assessed using the reporting odds ratio (ROR) with 95% confidence intervals (CI). A signal was considered present when the lower limit of the 95% CI exceeded 1.0 and at least three cases were reported.

Although both were more frequent in women, RAD were more commonly reported in individuals aged 45–64 years, whereas AD predominated in those aged 18–44 years. Most cases of RAD originated from the Americas, whereas cases of AD were predominantly reported in Europe. For RAD, signals of disproportionality were detected in the overall database analysis associated with LT4 alone (307 cases; ROR 1.74; 95% CI: 1.55–1.95), combined with iodine (6 cases; ROR 7.88; 95% CI: 3.54–17.55), and with liothyronine (4 cases; ROR 3.37; 95% CI: 1.26–8.97). Subgroup disproportionality analysis by sex for LT4 alone, revealed signals for both males and females (males: 47 cases; ROR 1.59; 95% CI 1.19–2.12 and females: 242 cases; ROR 1.61; 95% CI 1.42–1.84) and by age group showed signals of disproportionality in all subgroups except patients aged 18–34: <18 years (8 cases; ROR 3.83; 95% CI 1.91–7.68), 35–44 years (34 cases; ROR 1.89; 95% CI 1.34–2.65), 45–64 years (100 cases; ROR 2.16; 95% CI 1.77–2.64) and ≥65 years (59 cases; ROR 1.79; 95% CI 1.38–2.32). No signal was detected for AD in the overall analysis; however, in the stratified analyses by sex and age, a signal of disproportionality was identified only in the 35-44 year age group (17 cases; ROR 1.92; 95% CI 1.18-3.10).

These findings suggest a possible association between LT4 and accommodative dysfunctions. Although causality cannot be established from spontaneous reports, clinicians should consider accommodative symptoms in patients receiving LT4.

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P47

Clinical profile and antidiabetic drug use in patients with diabetes mellitus attended in urban and rural pharmacies in the province of Valencia

Ana J. Dantas Fernandes[1], Isabel García Arnandis[1,2], Salvador Gutiérrez-Igual[1,3], Isabel Romero-Crespo[3], M. Carmen Montesinos [1,2]

[1]University of Valencia, Burjassot, Spain, [2]IDM, Valencia, Spain, [3]Muy Ilustre Colegio Oficial de Farmacéuticos de Valencia (MICOF), Valencia, Spain

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

Diabetes mellitus (DM) is one of the main chronic diseases affecting older adults and is commonly associated with comorbidities and polypharmacy. This study aimed to compare clinical characteristics and antidiabetic drug use between urban and rural community pharmacy patients with DM. A retrospective observational study was conducted using data collected through the Atenfarma® platform of the Muy Ilustre Colegio Oficial de Farmacéuticos de Valencia (MICOF). Patients with type 1 and type 2 DM attended in urban and rural community pharmacies from 42 municipalities from Valencia during 2024 were included. Data were analyzed using R statistical software. Demographic variables, type of DM, most frequent health problems, number of prescriptions and antidiabetic drug utilization according to ATC level 4 classification were evaluated.

A total of 185 patients were included: 118 attended in urban pharmacies and 67 in rural pharmacies. Type 2 DM was present in 84.7% of urban patients and 92.5% of rural patients. Mean age was over 80 years in all subgroups, and women predominated (59.3% in urban pharmacies and 67.2% in rural pharmacies). Patients attended in rural pharmacies showed a higher mean number of health problems (8.31 ± 2.47 vs 7.64 ± 2.36) and prescribed medications (11.61 ± 3.68 vs 10.70 ± 3.28) than urban patients. The most commonly used antidiabetic therapeutic groups in urban pharmacies were SGLT2 inhibitors, followed by oral hypoglycemic combinations, DPP-4 inhibitors, and biguanides (27.7%, 22.8%, 16.8%, and 14.1%, respectively). In rural pharmacies, oral hypoglycemic combinations were the most frequently used group, followed by SGLT2 inhibitors and DPP-4 inhibitors (26.7%, 19.2%, and 15.8%, respectively). Insulin use was more frequent in rural pharmacies, particularly long-acting insulin analogues (8.3% vs 6.5%). Regarding the antidiabetic therapeutic regimen, monotherapy and dual therapy were more common in urban pharmacies (53.8% vs 47.0% and 37.0% vs 31.8%, respectively). In contrast, rural pharmacies showed higher use of triple (13.6% vs 7.6%) and quadruple regimens (7.6% vs 1.7%).

Patients with DM attended in community pharmacies showed a high burden of comorbidities and polypharmacy. Those in rural areas showed greater medication use and more complex antidiabetic regimens, including increased insulin use. These findings suggest structural differences in healthcare access, clinical management, and patient profiles between urban and rural settings.

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P48

Investigating the role of pleiotrophin in lung adenocarcinoma growth and angiogenesis *in vivo*

Pinelopi Kastana, Konstantinos Papadimatos, Sophia Gkotsopoulou, Olga Michali, Katerina Karavasili, Ioanna Ilia, Andriana Plevriti, Konstantinos Mikelis, Evangelia Papadimitriou

Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Greece

E-mail: epapad@upatras.gr

Background: Lung adenocarcinoma (LUAD) is the most common subtype of non-small cell lung cancer, strongly linked to smoking and accounting for almost half of non-small cell lung cancer cases. Pleiotrophin (PTN) is a growth factor that regulates cancer growth and angiogenesis, acting through various receptors. It has been shown previously that serum PTN levels are elevated in patients with LUAD, negatively affect treatment response, and might serve as a biomarker for both prognosis and diagnosis. Indeed, lower PTN expression is associated with better survival of LUAD patients, smokers and non-smokers.

Methods: To elucidate the role of PTN in LUAD growth and angiogenesis in vivo, we used C57BL/6J mice that do not express PTN (*Ptn*$^{-/-}$) and induced carcinogenesis by administering urethane parenterally, which leads to LUAD development.

Results: The number of tumors developed in the lungs was lower in *Ptn*$^{-/-}$ than in *Ptn*$^{+/+}$ mice, but tumor diameter and volume, and consequently, the tumor burden were similar between the groups. Using the same mice, we also studied another in vivo LUAD model by subcutaneously injecting the murine lung carcinoma cell line LL3. Tumor growth was significantly lower in the *Ptn*$^{-/-}$ compared with the *Ptn*$^{+/+}$ mice, suggesting that PTN expression in the host plays a role in LUAD growth. In vitro, PTN does not seem to affect LUAD cell proliferation, but significantly enhances their migration and clonogenic growth. Angiogenesis was enhanced in the lungs of *Ptn*$^{-/-}$ mice but was not detected in tumors from the urethane model. Similarly, angiogenesis was enhanced in the LL3 xenografts grown in *Ptn*$^{-/-}$ mice compared with those in *Ptn*$^{+/+}$ mice. In vitro, although endothelial cells derived from *Ptn*$^{-/-}$ lungs have slightly but statistically significantly decreased proliferation and motility compared with those derived from wild-type mice, their response to hypoxia and $VEGFA_{165}$ was significantly higher, favoring the notion that PTN acts as a brake for VEGFA actions on endothelial cells. To identify target genes associated with the differential behavior of *Ptn*$^{+/+}$ and *Ptn*$^{-/-}$ endothelial cells, total mRNA was isolated for RNA sequencing. The number of differentially expressed genes was high: 474 genes were upregulated, and 256 were downregulated. GO analysis showed that PTN is involved in functions related to extracellular matrix, cell adhesion, cytoskeleton regulation, cell migration/motility, protein localization to membrane, and angiogenesis, among others. Mechanistically, VEGFR2 localization on the cell membrane was higher in *Ptn*$^{-/-}$ compared with *Ptn*$^{+/+}$ endothelial cells, although there was no significant difference in the total VEGFR2 mRNA or protein levels.

Conclusions: Collectively, our data suggest that PTN positively regulates LUAD development in vivo, despite its negative effect on angiogenesis in vivo and the effects of hypoxia and VEGFA in vitro. Further studies are warranted to identify strategies to effectively exploit the PTN pathway as a target to suppress LUAD growth.

Pharmacology of Senescence and Aging

P49

A 10-year retrospective study on the treatment of canine cancer in a Veterinary Teaching Hospital

Beatriz Romero[1], E. Milena Vázquez[1], José M. Rodríguez[1], Julen Susperregui[2], Raquel Díez[1]

[1]*Pharmacology. IBIOMED. University of León, Leon, Spain*
[2]*Applied Mathematics. University of Leon, Leon, Spain*

E-mail: bromg@unileon.es

The relationship between owners and pets, frequently regarded as part of the family, has led people to dedicate greater care and financial resources to their animals, including medical treatments for cancer (1). The Veterinary Cancer Society reports that one out of four dogs will develop cancer at some point during their lives (2). Regarding treatment possibilities, although guidelines have been established in companion animals (3), no information is available on their use in field conditions. Therefore, the aim of this study was to characterize the population affected, the types of tumours diagnosed and the modalities of treatment followed.

A retrospective and descriptive study was carried out among dogs with cancer diagnosis attending at the Veterinary Teaching Hospital of the University of León between 2015 and 2024. Data collection, processing and storage was carried out in accordance with Spanish regulations. Cytotoxic agents were classified according to ATC/ATCvet index (4,5), and adverse events with VCOG-CTCAE v2 (6). Information was processed and analysed with Microsoft Excel 2019 and SPSS Statistics v.26.

A total of 107 dogs were diagnosed with cancer. Many of them were females (65.4%), purebred (73.8%), senior (59.8%), and large-giant sized (43.9%). Tumors were most frequently detected in Golden Retriever and Boxer breeds (6.5% each). Most tumors were malignant (86.9%), being epithelium the predominant tissue origin (42.1%). The most frequent histotype was carcinoma (36.4%), and the most affected system was mammary gland (29.9%) followed by skin/mucosa (25.2%). Regarding treatments, 44.0% received surgery, 38.3% chemotherapy, and 8.4% both modalities. The probability of being treated with surgery was 3.8 times higher in female (OR=3.8; 95% CI: 1.2–11.9; p=0.020), and 11.3 times higher when chemotherapy was not employed as treatment modality (OR=11.3; 95% CI: 2.9–43.6; p<0.001). Euthanasia was carried out in 26.2% of patients. A total of 60 different chemotherapeutic treatments were prescribed and they included 10 different drugs, being toceranib the most prescribed (35.0%), followed by cyclophosphamide, vincristine and doxorubicin (13.3% each one). Only 2 were veterinary drugs (administered orally) and 8 were used off-label (3 oral and 5 intravenous). Adverse events caused by these agents were recorded in 19 animals, with neutropenia (grade 1-5) and vomiting (grade 1-2) as the most frequent.

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P50

Effect of new carvone derivatives on glioblastoma viability

[4]Chamero, A, [2]Naranjo, J., [3]Nuñez, I., [2]De la Cruz-Martínez, F., [2]Lara-Sanchez, A., [4]Ceña, V., [4]Perez-Carrion, M.D., [4]Posadas, I.

[1]Facultad de Medicina, Albacete, Spain, Universidad de Castilla-La Mancha; [2]Departamento de Química Inorgánica, Orgánica y Bioquímica-Centro de Innovación en Química Avanzada (ORFEO-CINQA), Facultad de Ciencias y Tecnologías Químicas and Instituto Regional de Investigación Científica Aplicada-IRICA, 13071, Universidad de Castilla-La Mancha, Ciudad Real, Spain;3Facultad de Ciencias Ambientales y Bioquímica, and INAMOL, Universidad de Castilla-La Mancha, Spain; [4]CIBER, Instituto de Salud Carlos III, Madrid, Spain; Unidad Asociada Neurodeath, INAMOL, Universidad de Castilla-La Mancha, Albacete, Spain.

E-mail: inmaculada.posadas@uclm.es

Glioblastoma multiforme (GBM) is the most common and aggressive of the primary brain tumours developed in adults. Despite the relatively low incidence, 3.19 per 100,000 people per annum, the median survival of GBM patients is only approximately 12-18 months, and merely 10% of them currently survive for more than 5 years. Currently, the standard treatment is based on surgical resection followed by radiotherapy plus concomitant chemotherapy with temozolomide (TMZ), daily. However, complete surgical excision is highly improbable due to invasion of surrounding brain. As a result, glioblastoma patients have a poor prognosis, with a median survival of 14 months from diagnosis (1). Therefore, new therapeutic approaches are needed to treat this kind of tumour.

Carvone derivatives are biodegradable compounds that has recently demonstrated in vitro antitumoral properties against breast and lung cancer (2).

In the present work, we investigated the antitumoral effect of a new family of carvone derivatives (CR01-CR04) on human glioblastoma T98g cells, and their structure-activity relationship. To this end, we determined the effect of these derivatives on cell proliferation measured by BrdU assay, and cell viability by measuring the percentage of LDH released to culture medium. In addition, redox status and intrinsic apoptotic pathway was analysed by measuring glutathione (GSH) content spectrophotometrically; reactive oxygen species (ROS) production and caspases activity by fluorometry.

Our preliminary results suggest that these new derivatives promote glioblastoma cell death in a concentration- and time-dependent manner, being CR04 the most potent with an IC_{50} of 9.5 µM. Moreover, CR04 increases ROS production, promotes GSH depletion and activates the intrinsic apoptotic pathway.

In conclusion, our findings support this new carvone derivative as a lead compound for the development of novel antitumour drugs for glioblastoma treatment.

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Statins Enhance the Antitumor Activity of KRAS Inhibitors in KRAS-Mutant Lung and Pancreatic Cancer Models

Rodrigo López-Muñoz, Alberto Maestre, Francisco Díaz-Lazcano, Carina Chipón

Universidad Austral de Chile, Valdivia, Chile.

E-mail: rodrigo.lopez@uach.cl

KRAS mutations drive approximately one-third of human cancers and are particularly prevalent in non-small cell lung cancer (NSCLC), where codon 12 variants predominate. Although mutant KRAS inhibitors such as adagrasib and zoldonrasib have demonstrated promising clinical activity, therapeutic responses remain incomplete and resistance frequently emerges. KRAS signaling depends on membrane localization through farnesylation, a process sustained by the mevalonate pathway. Therefore, inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) may represent a strategy to enhance the efficacy of KRAS-targeted therapies.

Publicly available CRISPR screening datasets from the DepMap platform were analyzed using Python-based bioinformatics workflows to evaluate HMGCR dependency across cancer cell lines. NSCLC cell lines harboring $KRAS^{G12C}$ mutations and lung and pancreatic cancer cell lines carrying $KRAS^{G12D}$ mutations were treated with statins alone or in combination with KRAS inhibitors. Cell viability was assessed by MTT reduction assays, protein expression and signaling pathway activity were analyzed by Western blotting, and drug interactions were evaluated using Combenefit software.

DepMap analyses identified HMGCR as an essential gene for the viability of multiple lung and pancreatic cancer cell lines. Among the statins tested (simvastatin, atorvastatin, rosuvastatin, and pravastatin), simvastatin consistently showed the strongest combinatorial effect with the KRAS inhibitors adagrasib and zoldonrasib, whereas pravastatin was the least effective compound across all models. In NSCLC cells, simvastatin reduced MAPK pathway activity, as evidenced by decreased ERK phosphorylation, while simultaneously increasing total RAS protein levels, suggesting the accumulation of non-functional KRAS unable to properly localize to the plasma membrane. In KRAS^G12D^ models, simvastatin enhanced the inhibitory effects of zoldonrasib on both ERK phosphorylation and cell viability. Furthermore, inhibition of sterol regulatory element-binding protein 2 (SREBP2), a key transcriptional regulator of HMGCR expression, using fatostatin also produced synergistic effects when combined with adagrasib.

These findings indicate that pharmacological inhibition of the mevalonate pathway through statins, particularly simvastatin, enhances the antitumor activity of mutant KRAS inhibitors. Targeting HMGCR or its upstream regulator SREBP2 may represent a promising therapeutic strategy to improve the efficacy of emerging KRAS-targeted therapies in lung and pancreatic cancers.

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P52

Role of alamandine in the prevention of inflammation and senescence in human endothelial cells and age-related endothelial dysfunction

Diego Berlanas Vicente[1,2]; Ana Cuadrado Gómez[1,2]; Raquel Romero González[1]; Alicia Villacampa[1,2]; Carmen María Parra Moreno [1,2]; Francisco López Sánchez[3]; José Luis Bartha[1,2,3]; Robson Santos [4]; Guillermo Díaz Araya[5]; Carlos F. Sánchez Ferrer[1,2]; Concepción Peiró[1,2]

1.-Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain; 2. Instituto de investigación Hospital Universitario La Paz (IdiPAZ), Madrid, Spain; 3. Gynecology and Obstetrics Service, La Paz University Hospital, Madrid, Spain; 4. Federal University of Minas Gerais, Brazil.; 5. Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile.

Endothelial dysfunction and cardiovascular disorders are largely driven by premature vascular ageing, vascular cell senescence, and inflammation. In this project, we investigated the effects of alamandine as a senotherapeutic drug. Alamandine is a novel heptapeptide of the protective branch of the renin-angiotensin system (RAS), a key peptide hormonal system involved in the maintenance cardiovascular homeostasis. This branch of the RAS constitutes a counter-regulatory axis formed by different peptides and receptors that activate protective signalling pathways mediating anti-inflammatory and antifibrotic effects, counteracting the potentially deleterious effects of classical axis. We investigated whether alamandine could prevent the pro-inflammatory, pro-senescence, and endothelial dysfunction response triggered by a harmful stimulus for the vasculature, such as the pro-inflammatory cytokine interleukin-1β (IL-1β).

We used human umbilical vein endothelial cells (HUVEC) isolated, cultured, and treated with IL-1β (2.5 ng/ml), as an *in vitro* model of pro-senescent stress, alone or in combination with alamandine (100 nM). Cellular senescence and inflammation were assessed by β-galactosidase (β-gal) activity, Western blotting and qPCR. In addition, both 3-month-old C57BL/6J mice treated for 7 days with *in vivo* infusion of IL-1β ± alamandine using a mini-pump and 24-month-old animals were used to study age-related endothelial dysfunction. Vascular reactivity in mouse mesenteric microvessels was measured using an isometric wire myograph.

In HUVECs, alamandine prevented the priming and activation phases of the NLRP3 inflammasome, an inflammatory protein complex of the innate immune system related to cellular senescence, by reducing 1) the levels of NF-κB, IL-1β and NLRP3 protein expression, as markers of the priming phase, and 2) ASC specks formation and the levels of active caspase-1 and cleaved IL-1β protein expression, as markers of the activation phase. Additionally, alamandine decreased the number of β-gal+ cells, the levels of the cell cycle arrest effector p16 and the onset of the senescence-associated secretory phenotype (SASP) by inhibiting the transcription of inflammatory mediators like CCL2, IL-6, and IL-8. The partial loss of alamandine effects in the presence of D-Pro^7-Angiotensin-(1-7) (1 μM), an antagonist of the Mas-related G protein-coupled receptor member D (MrgD), confirmed the involvement of MrgD in mediating the actions of alamandine. Alamandine also demonstrated the ability to activate the cytoprotective Nrf2-heme oxygenase pathway. Regarding vascular function, alamandine attenuated endothelial dysfunction in isolated microvessels from 24-month-old male and female C57BL/6J mice, an effect mediated by the MrgD receptor. In an *in vivo* model, alamandine protected against the impaired endothelium-dependent relaxation induced by IL-1β when co-infused *in vivo* with IL-1β in 3-month-old male C57BL/6J mice.

These results indicate that alamandine may represent a novel senotherapeutic compound with anti-senescence, anti-inflammatory and vasoprotective properties capable of slowing the development of age-related cardiovascular diseases.

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Direct anti-inflammatory and anti-senescent effects of metformin on human peripheral blood mononuclear cells stimulated with IL-1β

Carmen M. Parra-Moreno[1,2], Alicia Villacampa[1,2], Ana Cuadrado-Gómez[1,2], Diego Berlanas[1,2], Ana Ortega Pita[2,3], Alberto M. Borobia[1,2,3], Concepción Peiró[1,2], Carlos F. Sánchez-Ferrer[1,2]

1. *Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain*
2. *Institute for Health Research (IdiPAZ), Madrid, Spain*
3. *Central Unit for Clinical Research and Clinical Trials (UCICEC-HULP), La Paz University Hospital, Madrid, Spain*

E-mail: carmenm.parra@uam.es

Diabetes is a disease associated with low-grade chronic inflammation and accelerated cellular senescence, leading to premature vascular ageing and inmunosenescence. Metformin is an AMPK activator drug used as first-line pharmacological for type 2 diabetes mellitus. In recent years metformin has been claimed to provide anti-inflammatory and anti-aging benefits beyond its metabolic properties. In this study we explored this potential protective effect of metformin in human peripheral blood mononuclear cells (PBMCs) stimulated with the proinflammatory cytokine IL-1β which is known to favor vascular damage and progeric actions.

PBMCs were obtained from the peripheral blood of six healthy control male and female subjects, with an age ranging from 25 to 43 years old and a body mass index ranging from 18,50 to 28,07. The cells were isolated by Ficoll density gradient centrifugation and cultured in RPMI 1640 medium containing 11 mmol/l glucose. Cells were stimulated for 18-24 hours with IL-1β (10 ng/ml) with or without metformin (10 µmol/l). The expression of senescence markers (p21 and p53 as growth arrest effectors proteins), pro-inflammatory markers (NLRP3, IL-1β, IL-6, IL-8 and TNF-α and CCL2 as component of the NLRP3 inflammasome and/or the senescence-associated secretory phenotype (SASP) components) and antioxidant and cytoprotective markers (klotho and the enzymes catalase and heme-oxygenase 1) were assessed by RT-qPCR. We observed that PBMNCs stimulation with IL-1β induced a pro-inflammatory and pro-senescent profil, while antioxidant and cytoprotective markers were enhanced probably as a compensatory mechanism against IL-1 β-mediated injury. Importantly, metformin could prevent the increase in senescence and pro-inflammatory markers induced by the cytokine. In conclusion, in PBMCs from young healthy controls challengend with a pro-inflamatory and progeric mediator such as IL-1β, metformin exhibits cytoprotective and anti-immunosenescence effects in addition to its metabolic actions which supports this drug as a candidate for senotherapeutics.

Keywords: Metformin, PBMCs, inflammation, senescence, antioxidant.

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Cationic phosphorus dendrimers as potential siRNA carriers for cancer therapy

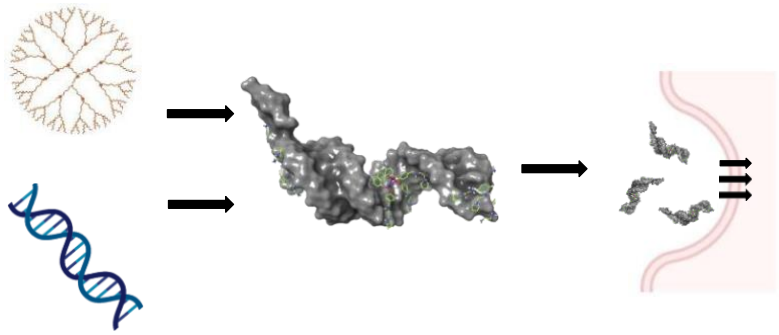
Angel Buendía[1,2], Irene Rodríguez-Clemente[1,2], Andrii Karpus[3], Krzystof Sztandera[1,2] Elzbieta Regulska[4], Jerome Bignon[3], Anne-Marie Caminade[3], Carlos Romero-Nieto[4], Anke Steinmet[5], Serge Mignani[6], Jean-Pierre Majoral[3], and Valentín Ceña[1,2]

[1]Unidad Asociada Neurodeath, INAMOL, University of Castilla-La Mancha, Spain; [2]CIBER Instituto de Salud Carlos III, Spain; [3]Laboratoire de Chimie de Coordination, CNRS, Toulouse 31077, France;[4]Department of Inorganic, Organic and Biochemistry, University of Castilla-La Mancha, Spain; [5]Sanofi R&D, CMC Synthetics/BTDV/EB, CRV, France; [6]CQM_Centro de Química da Madeira, Universidade da Madeira, Portugal

E-mail: angelbuendiab23@gmail.com

Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor in adults, with poor survival rates [1]. Small interfering RNA (siRNA) has potential for treating GBM by silencing genes involved in tumor growth [2], but delivering it efficiently into cells remains a challenge. We developed a series of cationic phosphorus dendrimers with 6, 12, or 24 pyrrolidinium or piperidinium surface groups to facilitate siRNA delivery. These dendrimers were able to bind siRNA, with the pyrrolidinium-based versions showing the strongest interactions. However, their ability to protect siRNA from RNase degradation varied.

Molecular modeling suggested that, in addition to charge, factors such as flexibility and binding mode influenced the dendrimers' properties. Despite their capacity to form complexes with siRNA, these dendrimers did not achieve sufficient siRNA delivery into GBM cells for effective transfection. This highlights the need to further refine dendrimer design to enhance siRNA delivery and improve therapeutic outcomes for GBM.



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P55

Cytokine Signatures in Active Ulcerative Colitis: TNFα/IL-6 Upregulation and Exploratory Association with Smoking Status

Mariña Durán-Rubí[1,2], Cristina Calviño-Suárez[2,3], Juan Abeigón-Moure[1,2], José Brea[1,2], David Moreira-Álvarez[1], Manuel Barreiro-de Acosta[2,3], María Isabel Loza[1,2], Antón L. Martínez[1,2].

1. *BioFarma research group. CiMUS. University of Santiago de Compostela, Santiago de Compostela, Spain*
2. *Instituto de Investigacións Sanitarias de Santiago de Compostela (IDIS), Santiago de Compostela, Spain*
3. *Department of Gastroenterology and Hepatology, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain*

E-mail: marina.duran@rai.usc.es

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by dysregulated immune activation. Several current therapies target cytokine-mediated inflammatory pathways, including those involving TNFα, IL-6, and IL-12/23. However, treatment response remains highly heterogeneous, underscoring the need for better molecular profiling.

Fifty-seven adult patients diagnosed with active ulcerative colitis (Mayo Endoscopic Score >0) undergoing routine colonoscopy were prospectively enrolled at the Digestive Department of the Complejo Hospitalario Universitario de Santiago de Compostela (CHUS). Biopsies from inflamed and non-inflamed colonic mucosa were obtained and processed by chemical and mechanical digestion. Mucosal levels of TNFα, IL-6, IL-12, and IL-23 were quantified using a Luminex-based immunoassay. Associations with demographic and clinical variables were explored using non-parametric statistical analyses.

TNFα and IL-6 were significantly elevated in inflamed mucosa ($p<0.0001$), whereas IL-12 and IL-23 showed no significant differences. TNFα and IL-6 were positively correlated ($r=0.67$, $p<0.0001$), as were IL-12 and IL-23 ($r=0.53$, $p<0.0001$). In the exploratory analysis according to smoking status, TNFα levels were higher in non-smokers than in smokers and former smokers (median 1.732 vs 1.289, $p=0.022$, Mann-Whitney) whereas IL-6, IL-12, and IL-23 showed no significant differences. No significant associations were observed between cytokine levels and age, sex, disease extent, or treatment group.

These results contribute to a better understanding of mucosal inflammatory mechanisms underlying UC and suggest that cytokine profiling, particularly assessment of TNFα levels, may provide useful biological information for future studies aimed at identifying anti-TNF response biomarkers and supporting more personalized therapeutic strategies in UC.

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Neuropharmacology

P56

Psilocybin Induces Antidepressant- and Hedonic-like Responses in a Validated Preclinical Model of Premenstrual Dysphoric Disorder

Jordi Jornet-Plaza[1,2], Francesca Sansó-Elle[1,2], M. Julia García-Fuster[1,2,3]

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

E-mail: j.jornet@uib.es

Premenstrual dysphoric disorder (PMDD) is a debilitating neuroendocrine condition affecting approximately 3-10% of women of reproductive age. The disorder is characterized by emotional and physical symptoms, including anxiety, irritability, depressive-like states, and somatic discomfort, which substantially impair daily functioning and quality of life. Despite its clinical relevance, currently available treatments often show limited efficacy and may produce adverse effects, highlighting the need for new therapeutic approaches. The present study aimed to validate a preclinical PMDD model in female Sprague-Dawley rats that replicates key behavioral and neuroendocrine alterations associated with the disorder, in which to evaluate the potential therapeutic effects of psilocybin. For model validation, ovariectomized rats received daily injections of progesterone (500 µg, 1 ml/kg) for 5 or 10 days vs. vehicle (control group). Rats were then exposed to a forced withdrawal period to mimic the abrupt progesterone decline associated with PMDD. Sequential behavioral phenotyping during withdrawal included the marble burying, forced swim and sucrose preference tests, performed 2, 4, and 6-7 days post-treatment respectively. The main results revealed a significant reduction in sucrose preference in rats treated with 10 days of progesterone (-37 ± 12%, $^{**}p = 0.0076$ vs. control rats), suggesting impaired hedonic-like responses. In a second procedure, a new cohort of ovariectomized rats was exposed to the 10-day PMDD model and treated with psilocybin (1 mg/kg, i.p.) or vehicle for 7 consecutive days (1 dose/day) during withdrawal while behavioral responses were scored as previously described. The main results proved that psilocybin ameliorated the behavioral impact of PMDD; it exerted antidepressant-like responses as measured in the forced swim test (i.e., decreased immobility: -13 ± 4s, $^{**}p = 0.005$; increased climbing: +12 ± 4s, $^{*}p = 0.011$) while also improved hedonic-like behavior (i.e., increased sucrose preference: +16 ± 5%, $^{**}p = 0.01$) when compared to vehicle-treated rats. In conclusion, we validated a translationally relevant animal model of PMDD suitable for testing novel therapeutic strategies. More importantly, we proved, for the first time, the potential preclinical use of psilocybin for PMDD, with demonstrated antidepressant- and hedonic-like responses, proving its efficacy in yet another affective-like disorder. Current studies are focused on elucidating the molecular mechanisms underlying the observed effects.

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P57

MKP-1 emerges as a novel therapeutic target for neurodegeneration

Nerea Peña Montero[1], Ariadna Pérez Pérez[1], Silvia Santamaría[1], Silvia Murillo[2], Isabel Varela[2], Elsa Cortés[1], Manuela G. López[1]

[1]*NeuroProtectionLab. Departamento de Farmacología. Instituto Teófilo Hernando. Facultad de Medicina. Universidad Autónoma de Madrid. Madrid.*
[2]*Grupo de Neurobiología de la audición. Instituto de investigaciones Biomédicas Sols-Morreale. CSIC.*

E-mail: nerea.penna@uam.es

The family of dual specificity phosphatases (DUSPs) is responsible for the dephosphorylation and inactivation of MAPKs and plays a role in neuronal survival, learning, and memory. Previous studies have demonstrated that DUSP1, also known as MKP-1, is involved in several neurodegenerative diseases such as Huntington's disease, in which lower levels of MKP-1 have been detected in postmortem patient samples. Likewise, in murine models of Alzheimer's disease, upregulation of MPK-1 has been shown to improve cognitive deficits and reduce Aβ amyloid plaques. Furthermore, transcriptomic profiling of patients with Parkinson's disease has revealed lower MKP-1 levels compared with healthy individuals. Taken together, these findings position MKP-1 as a promising novel therapeutic target for neurodegenerative diseases.

To evaluate the involvement of MKP-1 in neurodegenerative processes, we used male and female wild-type (WT) and MKP-1 knock-out (KO) mice at different ages. Phenotypic characterization was performed using a battery of behavioral tests, while inflammatory and oxidative stress markers in brain samples were analyzed by qPCR, western blot, and immunofluorescence.

Our results demonstrate that MKP-1 KO mice display cognitive impairment in the novel object recognition test (NOR) with a more pronounced effect in females. This cognitive deficit correlates with reduced long-term potentiation. In social interaction assessments at 6, 9 and 12 months of age, MKP-1 KO mice show significantly reduced interaction with juvenile mice, an effect mainly driven by males. Furthermore, the marble-burying test revealed anxiety-like behavior in MKP-1 KO mice at 6 and 9 months of age, with an earlier onset in females, which exhibited this phenotype at 3 months of age. Biochemical analyses showed increased expression of genes and proteins related to oxidative stress responses and inflammatory responses in MKP-1 KO animals. Structurally, MKP-1 KO mice exhibited reduced thickness of the cerebral cortex, dentate gyrus, and the CA1 and CA3 hippocampal layers.

These results allow us to conclude that MKP-1 may represent a novel therapeutic target for the development of drugs aimed at slowing neurodegeneration.

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P58

Small-Molecule GLP-1R Positive Allosteric Modulators and Agonists as Novel Therapeutic Strategies for Neuroprotection and Neuroinflammation

Ariadna Pérez Pérez[1], Nerea Peña Montero[1], Ignacio Férnandez Ángel[1], Sonia Godoy Pérez[2], Marina Amores Borge[1], Lucía Viqueira Díaz-Alejo[1], Marta Mirón Alcalá[1], Silvia Santamaría García-Minguillán[1], Yaiza Rodríguez García[1], Rafael León[2], Sergio Senar[3], Manuela G. López[1]

[1]*NeuroprotectionLab, Universidad Autónoma de Madrid, Madrid, Spain.*
[2]*Instituto de Química Médica, CSIC, Madrid, Spain.*
[3]*DrTarget, Machine Learning Applied to Drug Discovery, Alcalá de Henares, Madrid, Spain.*

E-mail: ariadna.perez@uam.es

Glucagon-like peptide-1 receptor (GLP-1R) signaling has emerged as a promising therapeutic target in neuroinflammatory and neurodegenerative disorders, in addition to obesity and diabetes. While peptide-based GLP-1R agonists have demonstrated neuroprotective effects, the development of small-molecule agonists and positive allosteric modulators (PAMs) may offer improved pharmacological properties and central nervous system accessibility.

In this study, we investigated the anti-inflammatory and neuroprotective potential of novel small-molecule GLP-1R agonists and PAMs identified through an algorithm-based screening strategy developed by Sergio Senar. The main objective was to evaluate whether pharmacological modulation of GLP-1R could disrupt the neurotoxic microglia–astrocyte–neuron axis associated with neuroinflammation.

Murine immortalized IMG microglial, IMA astrocytic, and HT22 neuronal cell lines were used to model inflammatory and neurodegenerative conditions. Microglial activation was induced with lipopolysaccharide (LPS), and conditioned media from activated microglia were transferred to astrocytes to induce a neurotoxic phenotype. Subsequently, astrocyte-conditioned media were applied to neuronal cultures to evaluate neuronal phenotype. In parallel, direct neuroprotective effects of GLP-1R modulators were assessed in neuronal cells exposed to rotenone or okadaic acid. Inflammatory and neurodegenerative markers, including TNF-α, IL-1β, IL-6, nitric oxide production, ROS generation, NF-κB signaling, and cAMP-related pathways, were analyzed using MTT assay, Griess reaction, qPCR, ELISA, and cAMP quantification assays.

Treatment with selected GLP-1R agonists and PAMs reduced inflammatory activation in microglia and prevented the acquisition of a neurotoxic astrocytic phenotype. Direct neuroprotective effects against several stimuli are also being tested.

Preliminary results suggest that effects are mediated, at least in part, through GLP-1R-dependent mechanisms. Ongoing studies using organotypic cultures aim to further validate the translational potential of these compounds, while GLP-1R dependency will be further evaluated through receptor silencing using RNA interference. In parallel, molecular docking and molecular dynamics simulations are being performed on the compounds showing the most promising biological activity to further characterize their interaction with GLP-1R and support structure–activity relationship analyses.

Taken together, these findings support small-molecule GLP-1R modulation as a promising therapeutic strategy targeting neuroinflammation and neurodegeneration.

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P59

Insights into the MOR-D1R heteromer interface: implications for heteromer-selective ligand design

Natalia Llopart[1], Aràntzazu Alonso-Carrasco[1], Itziar Muneta-Arrate[2], Estefania Moreno[1], Leonardo Pardo[3], Miriam Stoeber[2], Verònica Casadó-Anguera[1], Vicent Casadó[1]

[1]Universitat de Barcelona, Institut de Biomedicina de la Universitat de Barcelona (IBUB), and Nanomedicine and Molecular Neuropharmacology Research Group, Barcelona, Spain. [2]Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland. [3]Laboratory of Computational Medicine, Biostatistics Unit, Faculty of Medicine, Autonomous University of Barcelona, Bellaterra, Spain.

E-mail: nllopart@ub.du

Chronic pain in Europe is a major health issue, compounded by the opioid crisis due to the widespread use of opioids for managing severe pain[1]. While opioids alleviate pain by binding to the mu opioid receptor (MOR), a G protein-coupled receptor (GPCR), they also have adverse effects, such as tolerance and addiction. GPCRs exist not only as monomers but also as dimers, tetramers, and higher-order complexes, known as heteromers, which have new pharmacological properties compared with monomeric receptors.[2] MOR can form higher-order complexes, of particular interest is the MOR-dopamine D_1 receptor (D_1R) heteromer[3], potentially contributing to opioid adverse effects like the restlessness associated with opioid withdrawal[4] and restless legs syndrome (RLS)[5]. Recent unpublished findings from our laboratory support the existence of a MOR-D_1R heterotetramer with a characteristic pharmacological fingerprint and defined transmembrane interface. Here, we aimed to further characterize its interaction interface by building a computational model to identify key amino acids involved in MOR-D_1R interaction. Molecular dynamics simulations identified candidate residues with bulky side chains predicted to stabilize the heteromeric interface. MOR ($D179^{4.55}$, $Y229^{5.33}$, $W230^{5.34}$, $L233^{5.37}$, and H225) and D_1R ($R133^{4.55}$, $I157^{4.59}$, $L161^{4.63}$, and W163) residues were mutated to alanine by site-di-rected mutagenesis to evaluate their contribution to MOR-D_1R heteromerization and validate the proposed structural model. Immunocytochemistry confirmed proper plasma membrane ex-pression of all mutants. Bioluminescence resonance energy transfer (BRET) saturation assays revealed that only the MOR-H225A mutant completely abolished MOR-D_1R heteromerization, identifying it as a critical residue within the heteromeric interface. Functional cAMP assays showed that, unlike MOR-WT, the MOR-H225A mutant selectively lost cross-antagonism over D_1R signaling while preserving cross-talk. Together, these results validate the proposed heter-omeric interface and demonstrate that preservation of the receptor-receptor interface is required for the characteristic heteromeric fingerprint. These results further refine the structural organi-zation of the MOR-D_1R complex and provide a basis for the future design of heteromer-selec-tive ligands, including heterobivalent compounds.

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P60

Structural and functional characterization of striatal MOR–D$_1$R heterotetramers underlying opioid–dopamine antagonistic interactions

Verònica Casadó-Anguera[1], Aràntzazu Alonso-Carrasco[1], Natalia Llopart[1], Cèlia Rodríguez-Casas, Estefanía Moreno[1], Leonardo Pardo[2], Vicent Casadó[1]

[1]*Universitat de Barcelona, Institut de Biomedicina de la Universitat de Barcelona (IBUB), and Nanomedicine and Molecular Neuropharmacology Research Group, Barcelona.*
[2]*Laboratory of Computational Medicine, Biostatistics Unit, Faculty of Medicine, Autonomous University of Barcelona, Bellaterra.*

E-mail: veronica.casado@ub.edu

Striatal μ-opioid receptors (MORs) and dopamine D$_1$ receptors (D$_1$Rs) are co-expressed in striosomal projection neurons implicated in opioid reward. Previous studies demonstrated increased sensitivity of striosomal D$_1$R signaling during restlessness associated to opioid withdrawal and in experimental models of restless legs syndrome (RLS), together with reduced MOR expression, suggesting the existence of a functional antagonistic interaction between MOR and D$_1$R signaling[1-3]. Although MOR-D$_1$R heteromers have been previously proposed[4], their structural and functional properties remain unclear. Here, we characterized MOR-D$_1$R complexes *in vitro* and in native striatal tissue.

Bimolecular luminescence complementation (BiLC) assays combined with transmembrane (TM) interfering peptides demonstrated that MOR-D$_1$R heteromerization depends on TM4 and TM5 domains of both receptors. MOR co-expression reorganized the D$_1$R homodimerization interface from TM4/TM5 toward TM5/TM6, supporting higher-order receptor assemblies (MOR-D$_1$R-D$_1$R). Radioligand binding assays showed negative cooperativity of endomorphine-1, consistent with MOR homodimerization in native tissue, while D$_1$R activation selectively altered MOR agonist binding, supporting allosteric communication within a MOR-MOR-D$_1$R-D$_1$R heterotetramer. *In situ* proximity ligation assays (PLA) in mouse striatal slices confirmed MOR-D$_1$R complexes *ex vivo,* and a TM4-interfering peptide selectively reduced PLA signal, confirming the specificity and native preservation of the interface. Functional studies revealed bidirectional cAMP interactions between MOR and D$_1$R. MOR agonists inhibited D$_1$R-mediated cAMP accumulation, whereas MOR antagonists suppressed D$_1$R signaling through cross-antagonism. Heteromer-interfering TM peptides selectively abolished cross-antagonism, whereas crosstalk required simultaneous disruption by two peptides, suggesting distinct allosteric mechanisms. Together, these findings support a structurally organized MOR-D$_1$R heterotetramer whose integrity is required for heteromer-specific modulation of D$_1$R signaling. These receptor complexes may therefore mediate opioid modulation of striatal D$_1$R signaling in physiological and pathological conditions, and may represent potential therapeutic targets in opioid withdrawal and RLS.

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Dose- and sex-dependent modulation of social behavior by psilocybin, LSD and MDMA in adult mice

Pedro Bergas-Cladera[1,2], Rubén García-Cabrerizo[1,2,3]

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

E-mail: r.garcia-cabrerizo@uib.es

Social interactions are a key component of mental health and are frequently impaired in stress-related and psychiatric disorders [1]. Psychedelic and entactogenic compounds have gained interest for their capacity to modulate socio-emotional processing, yet whether different compounds produce comparable prosocial profiles across sex, dose and time remains poorly understood [2]. In the present study, we compared the dose-response effects of psilocybin, lysergic acid diethylamide (LSD) and 3,4-methylenedioxymethamphetamine (MDMA) on social behavior in adult C57BL/6 mice (82 males and 83 females). Each compound was tested in an independent experiment with its own vehicle control. Mice received vehicle or two doses of psilocybin (0.3 or 1 mg/kg), LSD (30 or 100 µg/kg) or MDMA (5 or 15 mg/kg) by oral gavage for seven consecutive days. Direct social interaction was assessed using the reciprocal social interaction test with a novel age- and sex-matched conspecific after acute administration (30 min post-administration), after repeated administration (1 day after the last administration) and at long-term time points (7 and 21 days after repeated treatment). Statistical analyses were performed separately for each compound using two-way ANOVAs with sex and treatment as independent variables. At the acute time point, psilocybin and LSD increased direct social interaction in a dose- and sex-dependent manner, whereas MDMA produced the opposite behavioral profile, reducing social interaction. Psilocybin showed significant treatment $F_{2,45}=5.672$, $p=0.0064$, sex $F_{1,45}=9.559$, $p=0.0034$ and interaction effects $F_{2,45}=3.226$, $p=0.0491$. Similarly, LSD showed significant treatment $F_{2,54}=8.601$, $p=0.0006$, sex $F_{1,54}=50.06$, $p<0.0001$ and interaction effects $F_{2,54}=4.055$, $p=0.0229$. In contrast, MDMA significantly reduced direct social interaction, as supported by significant treatment $F_{2,43}=5.774$, $p=0.0060$ and sex effects $F_{1,43}=9.842$, $p=0.0031$, without a significant interaction. After repeated treatment, psilocybin showed robust treatment $F_{2,50}=18.01$, $p<0.0001$ and sex effects $F_{1,50}=18.02$, $p<0.0001$, while LSD showed significant treatment $F_{2,54}=3.261$, $p=0.0460$, sex $F_{1,54}=40.32$, $p<0.0001$ and interaction effects $F_{2,54}=4.306$, $p=0.0184$. MDMA showed no significant prosocial effect 24 h post-treatment. At 7 days post-treatment, psilocybin and LSD still maintained significant treatment effects (psilocybin: $F_{2,50}=6.293$, $p=0.0036$; LSD: $F_{2,53}=5.027$, $p=0.0100$, whereas MDMA showed no long-lasting modulation. By 21 days post-treatment, no significant treatment effects were detected for any compound. Overall, these findings reveal compound-specific effects on social behaviour, with psilocybin and LSD enhancing social interaction across selected time points, while MDMA showed an acute reduction in social interaction without evidence of sustained prosocial effects.

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Reduced class II HDAC activity in prefrontal cortex of schizophrenia and bipolar disorder subjects: possible inhibition by antipsychotic drugs

Sergio Rabadán-Merino [1], Zuriñe Eguizabal [1], Inés Bonis [1], Jon Ander Santas-Martín [1], Alfredo Ramos-Miguel [1,3,4], Benito Morentin [2], Luis F. Callado [1,3,4], J. Javier Meana [1,3,4], Guadalupe Rivero [1,3,4]

[1] University of the Basque Country, EHU, Leioa; [2] Basque Institute of Legal Medicine, Bilbao; [3] Centro de investigación Biomédica en Red de Salud Mental (CIBERSAM), Leioa and [4] Biobizkaia Health Research Institute, Leioa, Spain.

E-mail: guadalupe.rivero@ehu.eus

Schizophrenia and bipolar disorder share etiological factors and show overlapping symptomatology[1]. Treatment of schizophrenia subjects is based on antipsychotic drugs, which are also first-line drugs in bipolar disorder due to their antimanic and mood-stabilizing properties. Susceptibility for schizophrenia and bipolar disorder is determined by interactions between genes and environment, possibly via epigenetics. Histone deacetylases (HDAC) are key epigenetic enzymes and have been postulated as targets of antipsychotic and mood stabilizing drugs[2]. Own studies have shown that class I HDAC activity is reduced in dorsolateral prefrontal cortex (DLPFC) of schizophrenia subjects treated with antipsychotics[3] but not in bipolar disorder subjects. However, information on class II HDAC expression and kinetic characterization of class II HDAC in these disorders is scarce[4,5]. Thus, the aim of this study was to evaluate class II HDAC activity in the DLPFC of schizophrenia and bipolar disorder subjects.

To this end, DLPFC samples of two cohorts formed by schizophrenia (n=19) and bipolar disorder cases (n=18) and sex-, age- and postmortem delay-matched control subjects were used. Case subjects were classified in antipsychotic-treated or antipsychotic-free subgroups according to blood toxicology at the time of death. Nucleosolic class II HDAC activity on fluorogenic Boc-Lys(TFA)-AMC substrate was resolved via Michaelis-Menten non-linear regression. Kinetic parameters of maximum velocity (V_{max}) and Michaelis constant (K_M) were obtained. Data fitting to a global or to 2 separate curves in cases and controls was statistically compared by extra sum-of-squares *F* test. Complementary experiments explored possible differences in the protein density of class II HDAC enzymes HDAC4 and HDAC5.

Class II HDAC activity was not different from respective controls neither in schizophrenia nor in bipolar disorder cohorts. Nevertheless, antipsychotic-treated schizophrenia subjects showed a slight trend towards a lower activity compared to respective controls ($F_{2,80}$ = 1.51, p = 0.226) due to differences in V_{max} (-26%). This tendency reached statistical significance in antipsychotic-treated subjects of the bipolar disorder cohort ($F_{2,79}$ = 17.71, p<0.0001; Δ in V_{max} = - 68%). HDAC4 and HDAC5 protein density remained unaltered in all comparisons.

Past and present results on class I and II HDAC activity suggest that the mechanism of action of antipsychotic drugs may involve selectively reducing HDAC activity in brains of subjects with either schizophrenia or bipolar disorder. The implications of the epigenetic regulation by antipsychotic drugs in these disorders deserves further study.

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P63

The Parkinson´s disease E193K LRRK2 mutation alters gap junction structure and activity

Alex Peña Terrón[1,2], Jaime Rubio Sanz[1,2], Valentín Ceña[1,2], Inmaculada Posadas[1,2] and María Dolores Pérez-Carrión[1,2]

[1]*CIBER, Instituto de Salud Carlos III, Madrid, Spain*
[2]*Unidad Asociada Neurodeath, INAMOL, Universidad de Castilla-La Mancha, Albacete, Spain*

E-mail: mariad.perez@uclm.es

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor symptoms including tremor, rigidity, and postural instability. Among the genetic factors associated with PD, mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene represent one of the most common causes of familial and sporadic forms of the disease. LRRK2 is a multidomain protein composed of seven functional domains, including the N-terminal Armadillo (ARM) domain. The E193K variant, located within the ARM domain, has been associated with altered mitochondrial dynamics and defective vesicular trafficking[1]; however, its potential involvement in Gap Junctions (GJs) regulation remains unclear. GJs, formed by connexin (CX) proteins, are essential for intercellular communication and neuronal homeostasis.[2]. In this study, we investigated the effect of the E193K LRRK2 variant on GJs organization and function using complementary approaches including western blotting, flow cytometry and cell viability assays. Our results revealed significant alterations in GJs organization in fibroblasts derived from an E193K LRRK2 carrier compared with healthy control cells. In addition, the E193K variant reduced hemichannels activity and increased cellular sensitivity to 1-methyl-4-phenylpyridinium (MPP+) treatment. Together, these findings demonstrate that the E193K LRRK2 variant critically affects GJs organization and function, supporting its potential contribution to PD-related cellular dysfunction.

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The roles of glucocorticoids in the accumulation and secretion of dopamine by PC12 cells

Noemí Socas-Pérez [1], Santiago Yanes [1], Sergio Pérez-Herrera[1], Natalia Domínguez [2], José David Machado[1], Marcial Camacho, Ricardo Borges[1].

[1] Unidad de Farmacología. Facultad de Ciencias de la Salud - Medicina. Universidad de La Laguna.
[2]Departamento de Bioquímica, Microbiología, Biología Celular y Genética. Facultad de Ciencias. Universidad de La Laguna. E-38200 - La Laguna. Tenerife. Spain

E-mail: rborges@ull.du.es

Biogenic amines, such as dopamine and norepinephrine, are stored in large dense-core vesicles (LDCVs), where they are retained through interactions with ATP and intravesicular matrix proteins. In addition to the well-known role of vesicular monoamine transporters (VMATs) in amine transport, the efficient accumulation of these amines depends on the coordinated action of accessory components, such as the vesicular nucleotide transporter (VNUT) and chromogranin A (CgA).

Dopamine concentration inside secretory vesicles and the efficiency of its release can be significantly enhanced by modulating the molecular machinery responsible for amine storage. In this study, we investigate how glucocorticoids influence the aminergic phenotype in PC12 cells by upregulating key enzymes involved in catecholamine biosynthesis—specifically tyrosine hydroxylase—while also increasing the expression of VNUT and CgA.

To assess the functional impact of these changes, we employed Ultrahigh-performance liquid chromatography (UHPLC) to quantify intracelluar dopamine levels, along with on-line secretion and single-cell amperometry to monitor stimulus-evoked secretion dynamics. Our findings suggest that corticosteroid treatment promotes a more efficient and stable storage of dopamine within secretory vesicles, potentially through enhanced matrix formation and ATP-dependent loading.

By combining corticosteroid treatment with strategies that optimize ATP availability and VNUT function, we enhance vesicular dopamine content and boost stimulus-evoked secretion, as confirmed by HPLC and amperometry. These results highlight the pivotal role of glucocorticoids in modulating vesicular dopamine storage and secretion efficiency, through upregulation of both enzymatic machinery and vesicular transporters.

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Role of Thrombospondin-1 in the Protection of the Blood–Brain Barrier Following Traumatic Brain Injury

Céline Decouty-Perez [1], Inés Valencia[1], María Álvarez Rubal[1], María del Pilar Madrigal[2], Lobete M[3], Salvador Martínez[2], M Dolores Martin-de-Saavedra[3], Ana Belén Lopez-Rodríguez[1] and Javier Egea[1]

[1]*Laboratorio de Neuroinflamación Molecular y Plasticidad Neuronal, Unidad de Investigación, Hospital Universitario Santa Cristina. Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, España.*
[2]*Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas, Universidad Miguel Hernández de Elche, Elche, Spain.*
[3]*Sheddome and Disease Lab, Departmento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid (Spain)*

E-mail: javier.egea@inv.uam.es

Traumatic brain injury (TBI) is the second most common cause of acquired brain damage in adults. Approximately 420,000 people in Spain are estimated to live with this type of injury, and around 100,000 new cases occur annually. TBI is caused by an external force, and its consequences can have a wide-ranging impact on the quality of life of affected individuals. In previous studies from our group, we demonstrated that the protein thrombospondin-1 (TSP-1) plays a key role in maintaining the blood–brain barrier (BBB) following TBI in our closed-head traumatic brain injury animal model. However, data obtained from patient serum samples showed a marked discrepancy, as reduced serum levels of this protein appeared to correlate with a better prognosis six months after injury (Decouty-Pérez C, 2024). Therefore, we decided to investigate the proteolysis of TSP-1, focusing on the potential protective effect of its N-terminal fragment.

In *in vitro* studies using cerebral microvascular endothelial cells, we observed an increase in eNOS phosphorylation following treatment with this fragment. Furthermore, *in vitro* vessel formation experiments revealed that the fragment induced a significant increase in vascular formation. These findings were also confirmed in our TBI animal model, where animals treated with the N-terminal fragment exhibited reduced BBB disruption. In addition, due to the extensive vascular damage observed after TBI in TSP-1-deficient animals, we used Light Sheet microscopy to study the vasculature of these animals under basal conditions. Moreover, proteomics experiments are currently being conducted to identify the receptors or interacting proteins involved in the mechanism of this TSP-1 fragment. Taken together, our findings suggest that this protein is regulated through proteolysis, and further investigation of this regulatory mechanism is warranted.

Decouty-Pérez C, Valencia I, Alvarez-Rubal M, et al. Uncovering the Role of Thrombospodin-1 and Occludin as Potential Prognostic and Diagnostic Biomarkers in Traumatic Brain Injury. Int J Mol Sci. 2026;27(2):571. Published 2026 Jan 6. doi:10.3390/ijms27020571

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Sexually dimorphic behavioural and physiological effects of a chronic corticosterone-induced mouse model of stress

Ane Larrea[1], Cristina Bruzos-Cidón[2], María Torrecilla[1].

[1]Department of Pharmacology. University of the Basque Country, Leioa, Spain. [2]Department of Nursing. University of the Basque Country, Leioa, Spain.

E-mail: cristina.bruzos@ehu.eus

Chronic stress and dysregulation of glucocorticoid signalling are strongly associated with major depressive disorder (1). Accordingly, corticosterone (CORT) administration represents a widely used and translationally relevant model of chronic stress, as it reliably induces depression-like phenotypes in rodents (2). However, sex-dependent differences in its behavioural and metabolic effects remain incompletely understood. The aim of this study was to evaluate behavioural, metabolic and physiological effects of chronic corticosterone intake in male and female mice.

CORT (0,1 mg/ml) was dissolved in tap water to a final ethanol concentration of 1%at After 4 weeks of CORT treatment, adult mice (male and female) were subjected to a behavioural battery in the following 4 days: sucrose preference test (SPT), nesting test, open field test (OFT), elevated plus maze (EPM), food intake assessment and tail suspension test (TST). Body weight, coat condition and organ weights were also recorded. Control group received vehicle (1% ethanol solution). Data were analysed using two-way ANOVA followed by Sidak's post hoc test.

Model induction was confirmed by behavioural and physiological alterations consistent with chronic stress consequences. A significant sex × treatment interaction was found for body weight ($F_{(1,53)}$ =15.28, p=0.0003). Corticosterone increased body weight in females (p=0.0244) but not in males, and treated females showed higher values than treated males (p=0.0024). Food intake decreased in both sexes (females: p<0.0001; males: p=0.0148), with a significant interaction ($F_{(1,47)}$ =5.958, p=0.0185). Despite this, females showed increased white adipose tissue (p<0.0001), whereas males did not. Corticosterone reduced adrenal weight in females (p=0.0095) and spleen weight in both sexes (p<0.001), while brain weight decreased only in males (p=0.0020). Behaviourally, corticosterone increased immobility in the TST and reduced nesting behaviour selectively in males (p<0.001), indicating greater behavioural vulnerability. In the SPT, corticosterone reduced sucrose preference in males compared to controls (males: 67.52 ± 4.7 vs 90.89 ± 1.6, p<0.0001), indicating anhedonia, whereas females showed no changes. Coat condition was impaired in both sexes, with no differences in OFT or EPM. Overall, chronic corticosterone treatment induces both shared and sex-specific effects. Although both males and females exhibit reduced food intake and decreased spleen weight, males appear more vulnerable to depression-like behaviours, as evidenced by increased despair and anhedonia. In contrast, females show predominantly metabolic alterations, including increased body weight, enhanced visceral white adiposity, and reduced adrenal gland weight. Beyond their descriptive value, these findings suggest that this chronic corticosterone exposure may provide a useful framework to investigate the sex-specific mechanisms underlying divergent behavioural and physiological responses to chronic stress.

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Development of *In Situ* Gelling Systems for Neuropharmacological Applications: Impact of Cyclodextrins on Drug Release and Structural Integrity

Fernando Yáñez-Gómez[1,2,3], María Antonia Vallés-Sansó[1], M. Julia García-Fuster[1,2,3]

[1]IUNICS, University of the Balearic Islands Palma, Spain.; [2]Health Research Institute of Balearic Islands (IdISBa), Palma, Spain; [3]Department of Medicine, University of the Balearic Islands Palma, Spain.

E-mail: f.yanez@uib.es

Background: The use of controlled drug-release systems could be a good tool for optimizing *in vivo* treatments [1] by ensuring stable administration and minimizing experimental animal stress caused by repeated handling. In this context, this study aimed to develop and optimize *in-situ* gelling hydrogels for the controlled release of several drugs with a recent proven antidepressant-like efficacy (i.e., ketamine, cannabidiol [2]) vs. the antidepressant goal-standard, fluoxetine.
Materials and Methods: Thermosensitive hydrogels were formulated using Pluronic F127 (26–29% w/v) as the main polymeric matrix, and combined with different cyclodextrins (α-, β, hydroxypropyl-β-, and γ-cyclodextrin) to optimize drug encapsulation and modulate release kinetics. Cyclodextrins were incorporated at different molar ratios relative to drug loading in order to evaluate their influence on hydrogel stability, drug incorporation, and release behavior. Hydrogel formulations were characterized by differential scanning calorimetry and oscillatory rheology to evaluate thermal transitions, gelation behavior, and viscoelastic properties. Additionally, injectability and structural stability were qualitatively assessed throughout the experimental period. *In vitro* release studies were performed at 37 °C and released drug concentrations were quantified by UV–Vis spectrophotometry.
Results: Hydrogels containing 26-29% Pluronic F127 exhibited optimal thermo-responsive behavior, showing rapid sol–gel transition near physiological temperature together with stable viscoelastic properties. Rheological analyses confirmed the formation of structurally stable gel networks with predominant elastic behavior after gelation. Furthermore, the addition of cyclodextrins simplified the formulation, resulting in better mechanical stability and more predictable release profiles. Drug release behavior was strongly dependent on the type of cyclodextrin employed. Ketamine-loaded hydrogels containing γ-cyclodextrin exhibited sustained and controlled release profiles. In contrast, CBD formulations failed to achieve satisfactory encapsulation or controlled release under the evaluated conditions. Finally, fluoxetine formulations incorporating hydroxypropyl-β-cyclodextrin showed favorable incorporation and release kinetics. Despite these differences, all optimized systems maintained structural stability and mechanical integrity for up to 7 days at 37 °C.
Conclusions: These findings support 26–29% Pluronic F127-based thermosensitive hydrogels as sustained delivery platforms for neuropharmacological compounds. Optimal stability and release behavior were achieved by incorporating cyclodextrins, which allowed for the modulation of drug release kinetics based on the physicochemical properties of each compound through molar adjustments. While cannabidiol formulations require further optimization, the systems maintained structural and mechanical integrity for 7 days at physiological temperature, facilitating prolonged administration with reduced handling. Future *in vivo* studies will evaluate their pharmacokinetic behavior and therapeutic applicability in animal models.

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Cell Painting morphological profiling of drugs acting on Central Nervous System through on SH-SY5Y cell line differentiated to neurons

Rodrigo Guerra, María M. Asensio, María J. Varela, José M. Brea, Antón L. Martinez, María I. Loza

Universidade de Santiago de Compostela, Santiago de Compostela, Spain

E-mail: rodrigo.guerra.hiniesto@usc.es

Phenotypic models constitute a highly useful strategy for studying multifactorial pathologies, such as neuropsychiatric disorders, given the elevated complexity of the pathological mechanisms involved in their development and the difficulty of identifying individual therapeutic targets on which to act effectively. In this framework, we put forward the hypothesis that morphological profiling of in vitro neuronal cultures following their exposure to different drugs active on the Central Nervous System (CNS) could lay the groundwork for a translational strategy aimed at identifying new compounds with therapeutic potential for these pathologies.

The aim of this work was to adapt the JUMP CP protocol (1) and the image analyses to the SH-SY5Y cell line differentiated into neurons, and to validate this approach by testing clinically used drugs active in CNS disorders, including antipsychotics, antidepressants, and anticonvulsants.

To this end, a seeding and differentiation methodology for the SH-SY5Y cell line compatible with performing the CP assay in a 384-well plate was developed, and the fixation, staining, and washing conditions of the protocol were optimized. Analysis pipelines in the CellProfiler software were likewise adapted to incorporate and quantify parameters such as neurite formation and branching, together with other morphological variables enabling cell profiling. Finally, a small set of molecules active against different CNS pathologies was evaluated using this methodology, which allowed the establishment of compound-specific phenotypic profiles.
Taken together, we present here the development of a new morphological profiling protocol with high translational value for the identification of potential new drugs intended for the treatment of neuropsychiatric disorders.

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STMN2 loss induces cholesterol dysregulation in amyotrophic lateral sclerosis

Claudia Fernández-Cuétara[1], Gemma de la Fuente[2], Silvia Romero-Murillo[3], Joaquín Fernández-Irigoyen[3], Enrique Santamaría[3], Pilar Negredo[2], Elena Tortosa[1]

[1] *Autonomous University of Madrid. Madrid, Spain*
[2] *Autonomous University of Madrid. Madrid, Spain*
[3] *Navarrabiomed, Hospital Universitario de Navarra (HUN), Universidad Pública de Navarra (UPNA), IdiSNA. Pamplona, Spain*

E-mail: elena.tortosa@uam.es

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of motor neurons, leading to muscle weakness, atrophy and ultimately respiratory failure. Despite advances in defining its genetic and molecular basis, effective therapies remain limited. Cytoplasmic mislocalization and aggregation of TDP-43 is a defining pathological feature in most ALS cases and drives disease through loss of its nuclear RNA regulatory functions. Among its key downstream targets is stathmin-2 (STMN2), a neuron-specific regulator of microtubule dynamics required for axonal growth and maintenance. Although STMN2 depletion is emerging as a central driver of ALS pathology and a promising therapeutic target in this disease, the downstream molecular pathways leading to neurodegeneration remain poorly understood. In this study, we investigated the functional and molecular consequences of STMN2 deficiency using a constitutive STMN2 knockout mouse model. We performed an extensive behavioral characterization revealing significant alterations in motor performance, consistent with the motor deficits observed in ALS. To further explore the molecular mechanisms underlying this phenotype, we conducted an unbiased proteomic profiling of spinal cord tissue. STMN2 loss induced broad alterations in pathways associated with neuronal structure and trafficking. Notably, cholesterol homeostasis emerged as a significantly dysregulated pathway. STMN2-deficient mice exhibited downregulation of the cholesterol transporter ABCA1, accompanied by upregulation of the cholesterol-metabolizing enzyme CYP46A1 and reduced circulating levels of 24S-hydroxycholesterol. In parallel, neuronal analyses revealed intracellular accumulation of perfringolysin O, consistent with altered cholesterol distribution and turnover. Together, our findings identify cholesterol dysregulation as a previously unrecognized consequence of STMN2 deficiency and suggest that cholesterol homeostasis pathway may represent a novel pharmacological targets and translational biomarker in ALS.

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Development of an in vitro phenotypic model of cognitive deficit

María Magdalena Asensio[1], María I. Loza[1], José M. Brea[1], Antón L. Martínez[1], Rodrigo Guerra[1], María Arrabal[1], Neila Cosme[1]

[1]Innopharma Drug Screening and Pharmacogenomics Platform, BioFarma Research Group. Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), Deparment of Pharmacology, Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela- Santiago de Compostela (Spain).

E-mail:mariamagdalena.asensio.lopez@usc.es

Cognitive impairment is a prevalent and highly disabling symptom across several neurological and neuropsychiatric disorders, including Alzheimer's disease, Parkinson's disease, schizophrenia, and depression. Unlike other symptoms that are better characterized and more effectively treated, current pharmacological interventions show limited efficacy in improving cognitive dysfunction. This limitation underscores the need for predictive models capable of supporting the development of new therapeutic strategies.

The aim of the present work is to extend and validate a previously developed translational *in vitro* phenotypic pharmacological model of schizophrenia-associated cognitive impairment[1] using the SH-SY5Y cell line differentiated into dopaminergic neurons. To this end, a co-culture model was established between the neuronal SH-SY5Y cell line and the oligodendroglial MO3.13 cell line. Differentiation of the co-culture system was assessed in a 2D format using different induction protocols. SH-SY5Y cells were first treated with retinoic acid (RA, 10 µM) for five days to induce neuronal differentiation. On day 7, MO3.13 cells were added, and a seven-day co-culture was maintained in the presence of Glucagon-Like Peptide-1 (50 or 100 nM). An alternative protocol using phorbol 12-myristate 13-acetate [2] (PMA,100 nM) was also tested. Comparison of these conditions identified the combined RA + PMA protocol as the most effective for promoting 2D co-culture differentiation.

Cellular differentiation was examined through morphological analysis and fluorescence microscopy, confirming the expression of Myelin Basic Protein expression in mature oligodendrocytes and β-tubulin in neurons. Functional assays were also conducted to evaluate neuronal excitability by comparing SH-SY5Y monocultures with co-cultures, using fluorescence lifetime measurements obtained with an FDSS 7000EX reader (Hamamatsu Photonics).

The system is now being adapted to a three-dimensional co-culture format to more accurately reproduce the cellular and tissue interactions present in vivo. This model will serve as a platform for screening of chemical compound libraries aimed at improving neuronal activity through electrophysiological readouts.

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Chemotherapy-Induced Neurobehavioral Dysfunction: Temporal Profile After FOLFOX in Rats

Ana Bagues, Claudia Blázquez, Blanca Silván-Ros, Lara Merchán-Sánchez, Cristina Ogando, Yolanda López-Tofiño, José A. Uranga, Raquel Abalo

Universidad Rey Juan Carlos, Alcorcón, Spain

E-mail: raquel.abalo@urjc.es

FOLFOX chemotherapy is widely used for the treatment of colorectal cancer but is frequently associated with adverse effects, including neurobehavioral alterations. Preclinical models provide a valuable framework to investigate the temporal progression of these effects under controlled conditions. This study aimed to evaluate the impact of FOLFOX on behavioral parameters at different time points in a rat model. Young male Wistar rats (250–300 g) received a single FOLFOX cycle previously shown to reduce tumor growth in a colorectal cancer liver metastasis model[1], consisting of oxaliplatin (6.1 mg/kg, i.p.) on day 1 and 5-fluorouracil (71.4 mg/kg, i.p.) with leucovorin (14.3 mg/kg, i.p.) on days 1 and 2. Control animals received the corresponding vehicles. Behavioral assessments were conducted 1, 2, and 6 weeks post-treatment. Two cohorts were studied. In cohort 1, exploratory behavior, anhedonia, and anxiety were evaluated using the hole-board test, splash test, and elevated plus maze (EPM), respectively. In cohort 2, nociceptive thresholds were assessed using the von Frey test (mechanical allodynia), plantar test (thermal hyperalgesia), and PAM test (mechanical muscle hyperalgesia). Locomotor activity was measured with an actimeter, and visceral sensitivity was evaluated using phasic colorectal distension. FOLFOX-treated rats showed reduced locomotor activity and exploratory behavior at week 2, reflected by decreased total distance travelled, rearing, and hole exploration. A shift in spatial exploration was observed at weeks 2 and 6, with reduced activity in the center and increased activity in the periphery of the hole-board arena. Anhedonia was not detected at any time point, as grooming latency and duration in the splash test remained unchanged. Anxiety-like behavior was evidenced by a decreased number of open arm entries in the EPM at weeks 1 and 2, while reduced time spent in open arms was only observed at week 6. Signs of somatic neuropathy were detected, including mechanical allodynia at weeks 2 and 3 and muscle hyperalgesia at weeks 1 and 2 post-treatment, whereas thermal hyperalgesia was not observed. Visceral sensitivity was also altered: at week 1, the number of abdominal contractions increased while their duration decreased at the highest intracolonic pressure (80 mmHg); at week 2, contraction duration remained reduced without changes in frequency; by week 3, contraction frequency and duration normalized, but the percentage of time spent contracting increased at 80 mmHg. Overall, these findings suggest that FOLFOX induces transient alterations in exploratory behavior, anxiety, somatic neuropathy, and visceral sensitivity in male rats, partially mimicking clinically relevant side effects observed in cancer patients.

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Neuroprotection by Imidazoline 2 ligands via proteolytic and mitochondrial modulation in 5xFAD mice

Marta Ribalta-Vilella[1], Teresa Taboada-Jara[1], Christian Griñán-Ferré[1,4], Rafael Franco[3,4], Gemma Navarro[2,4], Mercè Pallàs[1,4*]

[1]*Department of Pharmacology, Toxicology and Medicinal Chemistry,*
[2]*Department of Biochemistry and Physiology, School of Pharmacy and Food Sciences,*
[3]*Department of Biochemistry and Molecular Biomedicine, Faculty of Biology, Institute of Theoretical and Computational Chemistry (IQTCUB), Institute of Neurosciences (UBNeuro).University of Barcelona, Barcelona, Spain*
[4]*Network Center for Biomedical Research in Neurodegenerative Diseases, CiberNed, Spanish National Health Institute Carlos III, Madrid, Spain.*

E-mail: pallas@ub.edu

Background: Given the multifactorial nature of Alzheimer's disease (AD), therapeutic strategies capable of modulating multiple pathological pathways simultaneously are increasingly favoured. Imidazoline I2 receptor (2IR) ligands have emerged as promising candidates due to their pleiotropic neuroprotective effects.
Material and methods: Primary microglial cultures challenged with LPS were used to evaluate ROS production, calcium entry, and neuroinflammatory cytokine release following treatment with the I2IR ligands 2-BFIand idazoxan. In parallel, brains from 5XFAD mice chronically treated with both compounds were analyzed. Calpain activation was assessed by quantifying spectrin breakdown, and neuronal morphology was examined through Golgi staining.
Results: Treatment with I2IR ligands significantly reduced reactive oxygen species (ROS) production in LPS-stimulated microglia and decreased calcium entry, an effect that paralleled changes in neuroinflammatory cytokine levels. The reduction in calcium entry observed *in vitro* corresponded *in vivo* to a marked decrease in calpain activation in 5XFAD brains, evidenced by reduced spectrin degradation. Since calpain promotes the generation of p25, a co-activator of CDK5, these findings suggest interference with the pathway leading to tau hyperphosphorylation, a hallmark of AD. Golgi staining revealed that 2-BFI and idazoxan preserved dendritic arborization and neuronal network organization in the hippocampus of 5XFAD mice. Because cytoskeletal integrity is essential for mitochondrial trafficking, these results represent an indirect indicator of improved mitochondrial health.
Conclusions: I2IR ligands modulate multiple pathological mechanisms relevant to AD, including oxidative stress, neuroinflammation, calcium homeostasis, calpain activation, and neuronal structural integrity. These results support the idea that I2IR ligands enhance neuronal resilience to neurodegeneration, potentially through indirect modulation of mitochondrial function and preservation of neuronal architecture.

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Pharmacology of Inflammation

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P2X7 Receptor Blockade as a Strategy to Mitigate Inflammation and cell death in Murine Models of Retinal Degeneration

Marta Estalrich-Soliveres[1], Mateo Ruiz-Conca[1,2], Estela Tébar-Garcerán[1], Eva Tudurí[1,2], Lorena Vidal-Gil[1], Natalia Martínez-Gil[1,2], Victoria Maneu[1], Laura Fernández-Sánchez[1,2]

[1] *University of Alicante, Alicante, Spain*
[2] *Alicante Institute for Health and Biomedical Research (ISABIAL), Alicante, Spain.*

E-mail: laura.fs@ua.es

Retinal degenerative diseases are a group of pathologies characterized by neuronal cell death, chronic glial activation, and neuroinflammation, ultimately leading to visual loss and blindness. A common pathogenic mechanism shared across these conditions is the activation of the purinergic receptor P2X7 (P2X7R), which plays a key role in the induction of neuroinflammation, retinal gliosis, and neuronal degeneration. ATP released by damaged cells activates P2X7R, promoting microglial activation and the release of pro-inflammatory cytokines, thereby contributing to the propagation of gliosis and retinal degeneration. In addition, overactivation of P2X7R in neurons may induce excessive Ca^{2+} influx through its pore, contributing to excitotoxicity-induced cell death. The aim of this study was to evaluate the therapeutic potential of P2X7R blockade, using biological agents, to slow down the progression of retinal degeneration. We used two mouse models with different origin and clinical progression: the rd10 mouse, a model of retinitis pigmentosa, characterized by with a rapid progression; and an ischemia-reperfusion (I/R) model whose severity can be experimentally modulated, induced in C57BL6/J mice. I/R injury contributes significantly to the pathophysiology of retinal neurodegenerative diseases such as glaucoma and diabetic retinopathy, inducing oxidative stress, massive release of ATP, metabolic dysfunction, and an exacerbated inflammatory response that compromises neural and vascular integrity. Adeno-associated viral vectors (AAV8) encoding selective antibodies against P2X7R were administered intraperitoneally to C57BL6/J mice one month prior to ischemia induction. In the rd10 model, specific nanobodies against P2X7R were administered in the same route. The effect of the treatment on retinal function was evaluated using electroretinography. *In vivo* structural analysis was performed using optical coherence tomography (OCT) and OCT angiography (OCTA), quantifying morphospatial parameters of the different retinal vascular plexuses by using AngioTool software. No significant changes in electroretinography, OCT, or OCTA were observed in rd10 mice compared with the control group. In eyes subjected to I/R, the results showed a decrease in retinal function, which was more pronounced in treated animals. These findings suggest that preventive blockade of P2X7R may exacerbate the tissue response to I/R injury. Since inflammation plays a protective role in early stages, premature blockade might be counterproductive. Therefore, therapeutic success may depend on identifying an appropriate temporal window for intervention according to the progression of retinal damage.

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Impact of bariatric surgery on the immunophenotype profile of patients with morbid obesity: a flow cytometry study

Lylia Lakehal [1,2], Michele Malaguarnera [1,2], Blanca Alabadí [2,3], José T. Real [1,2,3], Andrea Cabrera-Pastor[1,2]

[1]*Universitat de València, Valencia, Spain*
[2]*Fundación de Investigación del Hospital Clínico Universitario de Valencia, Valencia, Spain*
[3]*Hospital Clínico Universitario de Valencia, Valencia, Spain*

E-mail: andrea.cabrera@uv.es

Morbid obesity is associated with chronic low-grade inflammation and alterations in peripheral immune cell profiles. This study evaluated the longitudinal impact of bariatric surgery on circulating leukocyte populations.
The study included 21 patients with morbid obesity, predominantly women (17 women and 4 men), aged 25–65 years, with a mean baseline BMI of 44.5 ± 5.0 kg/m². Peripheral whole blood samples were collected before and after bariatric surgery and analyzed by multiparametric flow cytometry. A fluorochrome-conjugated antibody panel targeting CD45, CD16, CD62L, CD3, CD4, CD8, CD19 and CD14 was used to identify total leukocytes, neutrophil activation phenotypes, lymphocyte subsets and monocyte subpopulations. Data were expressed as relative frequencies and absolute counts (cells/µL).
Results showed that bariatric surgery was associated with selective remodeling of circulating immune cell populations. Total $CD45^+$ leukocytes increased in absolute counts without major changes in relative frequency. $CD45^+CD16^+$ neutrophils increased in absolute counts, whereas their relative frequency remained statistically unchanged despite heterogeneous individual responses. However, detailed neutrophil phenotyping revealed a marked reduction in activated $CD16^{high}/CD62L^{dim}$ neutrophils, accompanied by changes in immature and mature resting subsets. This pattern suggests a qualitative reshaping of the neutrophil profile toward a less activated phenotype after surgery, rather than a uniform proportional change in total neutrophils. Changes were also observed within the lymphocyte profile, including increased absolute $CD3^+$ T-cell counts and proportional enrichment of $CD4^+$ helper T cells, while CD8+ T cells and $CD19^+$ B cells remained largely unchanged. Monocyte profiling showed an expansion of total circulating monocytes together with redistribution of monocyte subsets, characterized by reduced relative representation of intermediate/pro-inflammatory monocytes and increased absolute counts of classical and non-classical monocyte populations.
Overall, these findings indicate that bariatric surgery induces a selective remodeling of peripheral immune cell profiles in morbid obesity, characterized by reduced activated neutrophils, redistribution of neutrophil phenotypes, modulation of T-cell subsets and changes in monocyte populations. These findings suggest that surgical weight loss is accompanied by measurable changes in systemic immune cell dynamics.

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Functional effects of Osteoarthritis-Derived Synovial Fluid Extracellular Vesicles on Joint Cells

Vittoria Carrabs[1], Álvaro Compañ[2], María Luisa Ferrándiz[2,3], Antonio Silvestre[4], Ignacio Muñoz[5], María Carmen Carceller[2,3], María Isabel Guillén [1,2]

[1]Department of Pharmacy, University CEU Cardenal Herrera, Valencia, Spain.
[2]Interuniversity Research Institute for Molecular Recognition and Technological Development (IDM), Universitat Politècnica de València, Universitat de València, Burjassot, Valencia, Spain.
[3]Department of Pharmacology, University of Valencia, Burjassot, Valencia, Spain.
[4]Service of Orthopedic Surgery and Traumatology, University Clinical Hospital,Valencia, Spain.
[5]Hospital Ribera IMSKE, Valencia, Spain.

E-mail: vittoria.carrabs@uchceu.es

Background: Osteoarthritis (OA) is a degenerative joint disorder associated with aging, characterized by persistent inflammation and progressive extracellular matrix degradation[1]. Extracellular vesicles (EVs) present in synovial fluid (SF) are increasingly recognized as key mediators of intercellular communication and may contribute to OA pathophysiology[2].

Materials and methods: Synovial fluid, cartilage, and synovial membrane samples were obtained from 68 patients with knee OA undergoing arthroplasty. EVs isolated from SF (SF-EVs) were purified by size exclusion chromatography and subsequently characterized. Primary synoviocytes and chondrocytes were stimulated with IL-1β in the presence or absence of SF-EVs, and inflammatory and catabolic mediators were evaluated.

Results: SF-EVs (mean size ~261 nm; concentration ~7×10^8 particles/mL) expressed characteristic EV markers. In IL-1β-stimulated synoviocytes, SF-EVs reduced cell proliferation, IL-6, and MMP-3 levels, while increasing hyaluronic acid (HA) production. In chondrocytes, SF-EVs decreased IL-6 and MMP-13 expression and enhanced HA and IL-1Ra release, with sustained effects over time. These findings indicate that SF-EVs attenuate inflammatory and catabolic responses while promoting protective mediators involved in joint homeostasis. Despite being derived from an inflammatory OA environment, SF-EVs displayed predominantly protective effects, suggesting the presence of adaptive regulatory mechanisms within the osteoarthritic joint.

Conclusion: The present study demonstrates that EVs derived from osteoarthritic synovial fluid exert anti-inflammatory and chondroprotective effects on synoviocytes and chondrocytes under inflammatory conditions. By reducing pro-inflammatory and catabolic mediators while enhancing protective factors associated with tissue repair and lubrication, SF-EVs may contribute to maintaining joint integrity. These findings reinforce the context-dependent role of EVs in OA and support their potential as therapeutic targets for modulating inflammation and limiting disease progression.

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Osteoarthritic Synovial Fluid Induces Transcriptomic Reprogramming and Immunometabolic Adaptation in Healthy CD14+ Monocytes

Marta Marín Vázquez[1], Nazaret Prieto Aranda[1], Luís Álvarez Fernández[1], Antonio Silvestre Muñoz[2], Vicente Mirabet Lis[3], Vittoria Carrabs[1], Paloma Guillem-Llobat[1]

1. *Facultad de Ciencias de la Salud, Universidad Cardenal Herrera-CEU, CEU Universities. Valencia. España.*
2. *Hospital Clinic Universitari, Valencia. España*
3. *Banco de Células y Tejidos. Centro de Transfusión de la Comunidad Valenciana. Valencia. España*

E-mail: nazaret.prietoaaranda@alumnos.uchceu.es

Osteoarthritis (OA) is characterized by chronic low-grade inflammation and a complex synovial microenvironment capable of modulating innate immune responses [(1)]. The aim of this study was to characterize the transcriptomic response of healthy peripheral blood CD14+ monocytes exposed to osteoarthritic synovial fluid (SF).

Monocytes (CD14+) were isolated from peripheral blood samples of healthy donors obtained from the Valencian Community Blood Transfusion Center by using magnetic bead isolation. Monocytes were cultured in the absence or presence of OA SF (20%, v/v), and RNA sequencing (RNA-seq) was performed in three independent paired experiments. Sequencing quality was high, with >97% alignment to the human reference genome and Q30 values above 96%. Exploratory analyses included principal component analysis (PCA), volcano plot visualization, and identification of consistently regulated genes across all experiments.

PCA showed clear transcriptomic separation between control and OA SF-treated monocytes while maintaining paired clustering between experiments. Differential expression analysis identified a robust OA SF-induced transcriptional signature characterized by upregulation of genes associated with innate immune activation, chemokine signaling, extracellular matrix remodeling, macrophage differentiation, and immunometabolic adaptation, including ALOX5AP, CXCL5, SERPINE1, SPOCK1, CLEC5A, MRC1, and ANGPTL4. In contrast,

genes involved in chemotactic and immune-regulatory pathways, such as IDO1, CCR7, CCL19, CCL18, CCL23, SIGLEC1, and CCL15, were downregulated. Moreover, the absence of relevant cartilage-associated gene expression (COL2A1, ACAN, SOX9) suggested minimal tissue contamination and supported that the observed profile predominantly reflects monocyte responses to the osteoarthritic synovial microenvironment.

Overall, OA synovial fluid induced a reproducible transcriptomic reprogramming of healthy CD14+ monocytes characterized by inflammatory activation, extracellular matrix-related remodeling, macrophage-associated differentiation, and immunometabolic adaptation. These findings provide further insight into innate immune mechanisms involved in OA pathophysiology.

Osteoarthritis; Synovial fluid; CD14+ monocytes; RNA-seq; Transcriptomics; Inflammation; Immunometabolism; Extracellular matrix remodeling; Macrophage differentiation; Chemokine signaling.

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Immunomodulatory effects of diosmetin and diosmin on pro-inflammatory cytokines in stimulated human PBMCs

Inés Sánchez; Iván Hernández; Cristina Zaragozá; Lucinda Villaescusa; Francisco Zaragozá

Universidad de Alcalá

E-mail: ines.sanchezl@uah.es

Background: Diosmin and its aglycone diosmetin are well-studied citrus flavonoids with anti-inflammatory and immunomodulatory properties. Both compounds suppress pro-inflammatory cytokines such as TNFα, IL-6 and IL-1β through inhibition of the NF-κB and MAPK signalling pathways in preclinical models. However, their direct ex vivo effects on human peripheral blood mononuclear cells (PBMCs) stimulated with polyclonal activators, and specifically their capacity to modulate TNFα, IFNγ and IL-18, have not been systematically characterised in healthy volunteers.

Material and methods: PBMCs were isolated by density-gradient centrifugation (Ficoll-Paque) from heparinised venous blood of three healthy adult volunteers (mean age 55±5 years), after obtaining informed consent. Cells were resuspended in RPMI-1640 supplemented with 10% FBS at 37°C/5% CO^2. Prior to stimulation, PBMCs were pre-incubated for 1 h with diosmetin or diosmin (both at 0.02, 0.05, 0.1, 0.5, 1 and 2 mM). Cytokine production was induced by PMA (50 ng/mL) plus ionomycin (1 µg/mL) for 6 h. Levels of TNFα, IFNγ and IL-18 in the obtained supernatants were simultaneously quantified by multiplex immunoassay (Luminex® xMAP®). The percentage inhibition was calculated relative to the stimulated, untreated control.

Results: Diosmetin showed its greatest inhibitory capacity in all three volunteers at a concentration of 0.05 mM for the interleukins TNFα and IFNγ, with a mean inhibition of more than 50%; however, to achieve the same effect on IL-18 a concentration of 1 mM was required. At these same concentrations of diosmin, a slight inhibition of TNFα was observed, along with an activation of between 20% and 50% of IL-18 and IFNγ.

Conclusions: These results in human PBMCs confirm that diosmetin exerts a more potent inhibitory effect on the pro-inflammatory cytokines TNFα, IFNγ and IL-18 than its glycosylated form, diosmin, in line with published preclinical evidence. The greater activity of diosmetin is consistent with the well-known in vivo metabolic conversion of diosmin to diosmetin, which could explain the clinical efficacy of the glycoside. These findings reinforce the therapeutic potential of diosmetin as an immunomodulatory agent in inflammatory conditions in which T lymphocyte and macrophage activation plays a central role.

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Up-regulation of CCL25–CCR9 chemokine axis in diabetic patients with morbid obesity

Álvaro Gómez-Martin[1,2], María Abad[2], Patrice Marques[1,2,4], Cristina Arce[2,5], Elena Domingo[2], Norberto Cassinello[6], María Lapeña[5], Blanca Alabadí[2], José T. Real[2,3], María Jesús Sanz[1,2,3], Laura Piqueras[2,3,5]

[1]Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain
[2] INCLIVA Biomedical Research Institute, Valencia, Spain
[3]CIBERDEM: Diabetes and Associated Metabolic Diseases Networking Biomedical Research- ISCIII, Madrid, Spain
[4]CIBEREHD-Spanish Biomedical Research Centre in Hepatic and Digestive Diseases, ISCIII, Madrid, Spain
[5]Department of Pharmacology, Faculty of Pharmacy, University of Valencia, Valencia, Spain
[6]Digestive Surgery Unit, Hospital Clinic of Valencia, Valencia, Spain

E-mail: Laura.piqueras@uv.es

Background: Obesity is a multifactorial and chronic metabolic disorder that represents a major and growing burden on global public health systems. It is widely recognized that obesity is closely associated with a state of persistent low-grade systemic inflammation, which plays a critical role in the development of insulin resistance and the subsequent onset of type 2 diabetes mellitus. In this context, the present study aims to investigate circulating levels of the chemokine CCL25, as well as the expression of CCL25 and its receptor CCR9 in both visceral and subcutaneous adipose tissue depots in patients with morbid obesity. Furthermore, the study explores the potential association between the CCL25/CCR9 axis and the presence of diabetes in this population.

Methods: Plasma concentrations of CCL25 were quantified using enzyme-linked immunosorbent assay (ELISA). Adipose tissue samples, including subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VCAT), were obtained from patients with morbid obesity undergoing bariatric surgery. Gene and protein expression levels of CCL25 and CCR9 were assessed by quantitative real-time polymerase chain reaction (RT-PCR), Western blot analysis, and immunohistochemistry.

Results: Circulating levels of CCL25 were significantly elevated in patients with morbid obesity compared to non-obese control subjects ($p < 0.05$). At the tissue level, both CCL25 and CCR9 expression were markedly higher in visceral adipose tissue compared to subcutaneous adipose tissue ($p < 0.05$). Notably, this upregulation was more pronounced in patients with morbid obesity who also had diabetes, as compared to non-diabetic obese individuals. Immunohistochemical analyses further revealed that CCL25 is predominantly localized in endothelial cells and infiltrating macrophages within visceral fat, suggesting its involvement in vascular inflammation and immune cell recruitment.

Conclusions: Taken together, these findings provide preliminary evidence that the CCL25/CCR9 signaling axis may play a significant role in the inflammatory mechanisms linking obesity and diabetes, particularly within visceral adipose tissue.

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Critical changes in pro-survival and pro-death glial markers during the early stages of retinal degeneration in the rd10 mouse

Estela Tébar-Garcerán[1], Mateo Ruiz-Conca[1,2], Marta Estalrich-Soliveres[1], Lucía Tornero[1], Victoria Maneu[1,2], Laura Fernández-Sánchez[1,2]

[1] *University of Alicante, Alicante, Spain*
[2] *Alicante Institute for Health and Biomedical Research (ISABIAL), Alicante, Spain.*

E-mail: laura.fs@ua.es

Retinal glial cells are pivotal regulators of the initiation and dissemination of gliosis in retinal degenerative diseases. Classically, microglia are considered the primary initiators of inflammation and the main contributors to its propagation. However, Müller cells and astrocytes may also play a significant role during the early stages of degeneration. Our main purpose was to analyze the contribution of microglia, Müller cells and astrocytes to the initial stages of retinal degeneration by examining changes in their cellular phenotypes.

Using the rd10 mouse model of retinitis pigmentosa and C57BL/6J (control) mice. We examined the expression of the pro-death complement component C3 and S100 calcium-binding protein A10 (also known as annexin II light chain or p11), a marker associated with pro-survival phenotypes in brain astrocytes, Müller cells, retinal astrocytes and CD11b-immunopositive cells at postnatal day (P) 17, P18, P22, P26, P40 by flow cytometry and immunohistochemistry, corresponding to representative timepoints from initial to advanced degeneration.

We observed a higher expression of complement component C3 in rd10 mice retinas compared to C57BL/6J mice at all the ages analyzed from P18. In contrast, rd10 mice exhibit a pronounced decline in S100A10 in astrocytes and Müller cells occurring from P17-P18, whereas C57BL/6J mice maintain a uniform and sustained expression of S100A10 at all ages examined. In all the three main glial cell types in rd10 retina, we observed transient glial subpopulations showing a higher expression of C3 at the first stages of retinal degeneration (from P18 to P22 in microglia and activated Müller cells, and at P18 in astrocytes).

Microglia, Müller cells and astrocytes populations show changes in their phenotype from the very first stages of retinal degeneration. Subpopulations showing increased expression of complement component C3 together with the decreased S100A10 expression could counteract the balance towards pro-death phenotypes in the early stages of retinal degeneration, so pointing to a possible role in triggering the degenerative process. Critical changes in expression levels of C3 and S100A10 were observed at P17-P18, pointing to this age as a potential time window for therapeutic intervention. A deeper understanding of glial subpopulations with different phenotypes, and how they drive disease progression, may help to identify new therapeutic targets and determine the most effective timing for a successful treatment to slow down the degenerative process.

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