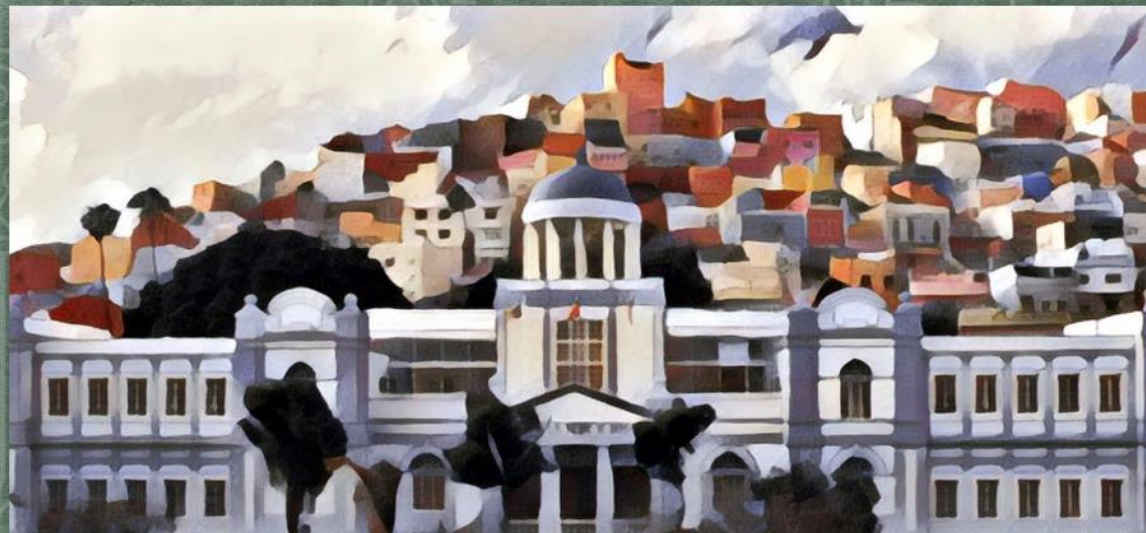


39TH

SPANISH SOCIETY OF PHARMACOLOGY MEETING

● BOOK OF ABSTRACTS

● 3 - 5 JULY 2019 ● LAS PALMAS DE GRAN CANARIA



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1. Welcome

On behalf of the Spanish Society of Pharmacology, we heartily welcome pharmacologists, physicians and clinicians, scientists and researchers, health professionals, patients and representatives of the pharmaceutical industry to this 39th Spanish Society of Pharmacology meeting, Las Palmas, 2019.

Pharmacology is an interdisciplinary science which has a close relationship to almost all the areas of medical and pharmaceutical sciences. Its final aim is, of course, to cure and prevent diseases by drugs, thereby maintaining the well-being of the human community, but it is also the innermost pleasure of a pharmacologist to reveal the secrets of life with the aid of miraculous drugs.

It is the first time that our city hosts this event and that the team participates in its organization; it is a strong incentive to get all the participants to take a pleasant memory of their stay in Las Palmas.

The Spanish organization committee and also the scientific committee have done a fantastic work preparing our congress and I would like to thank them for their energy, competence and professionalism during the organization process. For sure, the success I anticipate to this congress will certainly be the result of the effective work carried out.

We will focus in the following thematic areas

- Cardiovascular
- Clinic Pharmacology
- Cancer
- Drug Safety and Toxicology
- Endocrinology
- Gastrointestinal/Respiratory Pharmacology
- Pharmacology of Pain and Inflammation
- Receptors
- Drug design
- Teaching
- Pharmacogenetics

In this congress we will count on the inestimable collaboration of the German, Chilean and Dutch pharmacological societies

The success of a congress lies, no doubt, in the quality of its scientific contents. However, in our personal experience, the congresses in which it is developed an attractive complementary social activity survive in memory. The city of Las Palmas, undoubtedly one of the most beautiful in Spain, gives us a warm and friendly atmosphere and offers historical, cultural and gastronomic attractions that we will know how to take as people with concerns beyond our specialty.

We look forward to welcoming you to Las Palmas to attend the 39th Spanish Society of Pharmacology meeting during the first week of July 2019.

Juan Francisco Loro Ferrer
Chair of the Organizing Committee

2. Committees

Honorary Committee

Antonio Morales Méndez
President of Cabildo de Gran Canaria

Rafael Robaina Romero
Rector of Universidad de Las Palmas de Gran Canaria

Organizing Committee

Juan Francisco Loro Ferrer (President)

José Manuel Brea Floriani

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Bonifacio Díaz Chico

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Mattijs Blankesteijn

Ricardo Caballero

Amadeu Gavalda

Francisco Estévez Rosas

Thomas Wieland

Jorge Fuentealba Arcos

Juan Jose García Vieitez

Francisco Ciruela

Juan Francisco Loro Ferrer

3. Program

Wednesday – July 3rd, 2019

Sala Baldaquino

08:00h	Technical Secretariat opens for Registration
09:30h - 11:30h	<p>Session 1: New in vivo tools in Pharmacology: CRISPR/Cas and conditional mice Moderators: M^a Ángeles Moro and Lluís Montoliu Speakers: - "New Animal Models with CRISPR Tools". Lluís Montoliu. CNB. Madrid, Spain - "Conditional Mouse Models, Strategies and Applications". Sagrario Ortega. CNIO. Madrid, Spain - "Investigation of Rodent Adult Neurogenesis in Homeostasis and Disease: an example of the use of Conditional Mice in Neuropharmacology". M^a Ángeles Moro. Universidad Complutense. Madrid, Spain</p>
11:30h - 12:00h	Coffee Break
12:00h - 12:30h	Opening Ceremony
12:30h - 13:30h	<p>Opening Lecture - "Stop the Mast Cells and the Eosinophils by any means!" Francesca Levi-Schaffer. IUPHAR, Jerusalem, Israel</p>
13:30h - 15:00h	Lunch
15:00h - 16:00h	<p>Plenary Lecture - "Protease-Activated Receptors (PARs) in Inflammation and Pain: What should we target?" Natalie Vergnolle. INSERM. Toulouse, France.</p>
16:00h - 16:30h	Coffee Break and Poster Session 1
16:30h - 18:30h	<p>Session 2: New targets in cardiac and cardiovascular disorders Moderators: Ricardo Caballero and Thomas Wieland Speakers - "Targeting Histidine Phosphorylation by Nucleoside Diphosphate Kinases in Cardiovascular Disease". Thomas Wieland. Heidelberg University. Mannheim, Germany - "Interventions in WNT Signaling Stimulate Cardiac Regeneration". Mattijs Blankesteijn. Maastricht University. Maastricht, Netherland - "Recent Advances in the Pharmacological Treatment of Cardiovascular Diseases". Ricardo Caballero. Universidad Complutense. Madrid, Spain</p> <p>Oral communications Abstracts Title: - "Systemic Inflammation is Reduced in Primary Hypercholesterolemia Patients after an Oral Fat Load Administration". Aida Collado. Universidad de Valencia. Valencia, Spain - "Probiotics Prevent Hypertension in a Murine Model of Systemic Lupus Erythematosus Induced by TLR7 Activation" Néstor De La Visitación. Universidad de Granada. Granada, Spain</p>
20:30h	Welcome Reception

Sala Fogón

Session 3:**Parallel Roundtable. Education in Pharmacology****Moderators:** Michael Spedding and Concha Peiró**Speakers:**

- "*IUPHAR, Education and Collaborations World-Wide*". **Michael Spedding**. IUPHAR, Edimburgh, UK

- "*The Doctoral Programme in Pharmacology, University of Chile*". **Guillermo Díaz**. Universidad de Chile. Santiago, Chile

16:30h - 18:30h

- "*Doctoral Studies in Spain: Structure and Management*". **Jose Miguel Doña**. ULPGC. Las Palmas de Gran Canaria, Spain

Oral communications**Abstracts Title:**

- "*Combination of Innovative Teaching Techniques in Pharmacology Seminar Classes*".

M^a Carmen Montesinos. Universidad de Valencia. Valencia, Spain

- "*Information about Drugs and Related Topics in the Spanish General, Economic and Professional Press*". **Gonzalo Casino**. Universitat Pompeu Fabra. Barcelona, Spain

Thursday – July 4th, 2019

Sala Baldaquino

08:00h	Technical Secretariat opening time
09:00h - 11:00h	<p>Session 4: Open innovation in drug discovery Moderator: María Jesús Sanz. Speakers: - "Health Care and Impact of Migraine from the Patients Perspective". Isabel Colomina. AEMICE. Madrid, Spain - "Migraine Pharmacology Evolution". María del Buensuceso Fernández del Pozo. Consejo General de Colegios Oficiales de Farmacéuticos. Madrid, Spain - "Physiopathology and epidemiology of Migraine". Ayoze González. Hospitales San Roque. Las Palmas de Gran Canaria, Spain Sponsored by NOVARTIS</p>
11:00h - 11:30h	Coffee Break and Poster Session 2
11:30h - 13:30h	<p>Session 6: New advances in the Pharmacology of Respiratory System. Moderators: Angel Cogolludo and Andreas Ludwig Speakers: - "Targeting K⁺ Channels in Pulmonary Hypertension". Ángel Cogolludo. Universidad Complutense. Madrid, Spain - "ADAM Family Proteases: Friends or Foes in Pulmonary Inflammation?" Andreas Ludwig. RWTH Aachen University. Aachen, Germany</p> <p>Oral communications Abstracts Title: - "Role of il-11 in Pulmonary Fibrosis Associated to Pulmonary Hypertension". Inés Roger. CIBERES. Madrid, Spain - "The Role of MUC1 in a Bleomycin Induced Pulmonary Fibrosis Mouse Model". Paula Montero Magalló. INCLIVA. Valencia, Spain</p>
13:30h - 15:00h	Lunch
15:00h - 16:00h	<p>Plenary Lecture <i>"Somatic Mutations and Clonal Hematopoiesis as Drivers of Inflammation and Cardiovascular Disease"</i>. José Javier Fuster. CNIC. Madrid, Spain</p>
16:00h - 16:30h	Coffee Break and Poster Session 3
16:30h - 18:30h	<p>Session 8: Free Radicals in traslational Pharmacology. Moderators: María José Alcaraz and Pietro Ghezzi Speakers: - "Relevance of Nrf2 and Heme Oxygenase-1 in Articular Disease". María José Alcaraz. Universidad de Valencia. Valencia, Spain - "Free Radicals in Translational Pharmacology: NOS, NOX & sGC as a Network Pharmacology-Based Approach for Brain Ischemia". Ana Casas. Maastricht University. Maastricht, Netherlands - "The Oxidative Stress Theory of Disease". Pietro Ghezzi. Brighton & Sussex Medical School. Sussex, UK</p> <p>Oral communications Abstracts Title: - "Evaluation of Nrf2 Activators for the Treatment of Chronic Obstructive Pulmonary Disease (COPD)". Cristina Estornut. Universidad de Valencia. Valencia, Spain - "Adipose Tissue Mesenchymal Stem Cell-Derived Extracellular Vesicles as a Biological Therapy in Osteoarthritic Cells". Miguel Tofiño-Vian. Universidad de Valencia. Valencia, Spain</p>

Sala Fogón	
09:00h - 11:00h	<p>Session 5: Young Scientist Forum</p> <p>Part 1 <u>Moderators:</u> Cristina Arce, Marta Cimadevila and Aida Collado <u>Speaker:</u> - "<i>Creativity in Science</i>". Michael Spedding. IUPHAR, Edimburg, UK</p> <p>Part 2 <u>Moderators:</u> Jesús Cosín, Patrice Marques, Alejandra Romero and Álvaro San Hipólito <u>Speakers:</u> - "<i>How to become a good University Professor</i>". María Luisa Ferrándiz. Universidad de Valencia, Valencia, Spain - "<i>Research after PhD</i>". Nuria Godessart. Almirall, Barcelona, Spain - "<i>From Science to Industry</i>". Amadeu Gavaldá. Almirall, Barcelona, Spain - "<i>How to bring Science to Society: from School to Parliament</i>". Eduardo Oliver. CNIC, Madrid, Spain Sponsored by Almirall</p>
11:30h - 13:30h	<p>Session 7: New advances in Neuropharmacology</p> <p><u>Moderators:</u> Ricardo Borges and Ramón Sotomayor <u>Speakers:</u> - "<i>Inside a Secretory Vesicle. Novel Targets for Modulating the Exocytosis of Neurotransmitters</i>". Ricardo Borges Jurado. ULL. Tenerife, Spain - "<i>Pamam Dendrimers Effects on Neuronal Functionality in vitro</i>". José Guzmán González. Universidad de Concepción. Concepción, Chile - "<i>Programming of Dopaminergic Neurons by Early Exposure to Sex Hormones: is a Vulnerability Factor for Drug Addiction?</i>". Ramón Sotomayor Zárata. Universidad de Valparaíso. Valparaíso, Chile - "<i>Supplementation with Melatonin Impedes Cognitive Decline in tau-related Alzheimer Models, by Restoring the Autophagic Flux, once the Pathology is Initiated</i>". Izaskun Buendía Abaitua. UAM. Madrid, Spain</p>
16:30h - 18:30h	<p>Session 9: Poster Presentation (8 selected posters)</p> <p><u>Moderators:</u> Mabel Loza and Juan Manuel Duarte <u>Abstracts Titles:</u> - "<i>CCL11/CCR3 Axis is Involved Atherosclerosis Development</i>". Elena Domingo. Universidad de Valencia. Valencia, Spain - "<i>The Adipokine Visfatin Induces Endothelial Dysfunction through TLR-4 and NLRP3 Inflammasome Activation</i>". Álvaro San Hipólito-Luengo. UAM. Madrid, Spain - "<i>Characterization and Evaluation of Hemp Protein Hydrolysates on Neuroprotection</i>". Sergio Montserrat De La Paz. Universidad de Sevilla. Sevilla, Spain - "<i>Obesity-Induced Hypogonadism: Pathophysiological Roles of a Novel Hypothalamic miRNA/Kisspeptin Pathway and Potential Therapeutic Implications</i>". M^a Soledad Avendaño. Universidad de Córdoba. Córdoba, Spain - "<i>In vitro Characterization of a TRPV1 Soft Antagonist</i>". Magdalena Nikolaeva Koleva. IDiBE. Elche, Spain - "<i>Quantification of Functional Bias of Serotonin 5-HT_{2A} Receptor Agonists and Validation of Structural Hypotheses on Functional Selectivity at 5-HT_{2A} Receptor</i>". Andrea García Silva. Universidad de Santiago de Compostela. Santiago de Compostela, Spain - "<i>MUC1-CT as an IPF Potential Druggable Target</i>". Beatriz Ballester. Universidad de Valencia. Valencia, Spain - "<i>Mechanisms of Resistance to Egfr-Targeted Therapy Induced by Nicotine in Human Lung Cancer</i>". María Extremera Mazuela. UAM. Madrid, Spain</p>

Friday – July 5th, 2019

Sala Baldaquino

08:00h	Technical Secretariat opening time
09:00h - 11:00h	<p>Session 10: Aging from Mechanisms to Pharmacological Perspectives Moderators: José Viña and Jorge Fuentealba Speakers: - "Aging from Mechanisms to Pharmacological Perspectives". José Viña. Universidad de Valencia. Valencia, Spain - "P2XR Overexpression Contribute to Beta Amyloid Toxicity: New Pharmacological Target to AD". Jorge Fuentealba Arcos. Universidad de Concepción. Concepción, Chile - "Cardiac Fibroblast Role on Inflammatory Process: Interaction with Immune Cells". Guillermo Díaz. Universidad de Chile. Santiago, Chile</p> <p>Oral communications Abstracts Title: - "Targeting Soluble Epoxide Hydrolase to Improve Neurodegenerative Traits in SAMP8 Mouse". Mercè Pallàs. Universidad de Barcelona. Barcelona, Spain - "The Angiotensin-(1-7)/Mas Receptor Axis Attenuates Human Endothelial Cells Senescence through the Activation of Klotho and Nrf2". Alejandra Romero Martínez. UAM. Madrid, Spain</p>
11:00h - 11:30h	Coffee Break
11:30h - 12:30h	<p>Session 12: Oral Communications: Pain and Inflammation Moderators: Francesca Levi-Schaffer and Eva Delpón Abstracts Title: - "Amitriptyline, a New Therapeutic Tool to Block Innate Immune Responses in Osteoarthritis Patients". Eloi Franco Trepas. Instituto IDIS. Madrid, Spain - "Methadone is an analgesic with Low Addictive Side Effects due to its Weak Potency on Opioid-Galanin Receptor Heteromers". Verónica Casadó. Universidad de Barcelona. Barcelona, Spain - "Identification and Validation of two small Molecules Targeting the IL-17 Inflammatory Pathway". Elia Álvarez Coiradas. Universidad de Santiago de Compostela. Santiago de Compostela, Spain</p>
12:30h - 13:30h	<p>Closing Lecture - "Resolution Pharmacology". Mauro Perretti. Queen Mary University of London. London, UK Sponsored by EPHAR</p>
13:30h - 14:00h	Closing Ceremony
15:30h - 17:30h	SEF Assembly

Sala Fogón

09:00h - 11:00h	<p>Session 11: New Advances in the Pharmacology of Cancer <u>Moderators:</u> Francisco Estévez Sarmiento and Patricia Rijo <u>Speakers:</u> - "<i>Abietane Diterpenoids from Plectranthus spp. as Lead Protein Kinase C Modulators for Cancer Therapy</i>". Patricia Rijo. Universidade Lusófona. Lisboa, Portugal - "<i>Sesquiterpene Lactones as Potential Agents against Cancer</i>". Francisco Estévez Sarmiento. ULPGC. Las Palmas de Gran Canaria, Spain - "<i>Novel Biomarkers and Therapeutic Targets in Clear Cell Renal Cell Carcinoma (ccRCC)</i>" Anna Meseguer Navarro. VHIR. Barcelona, Spain</p> <p>Oral communications <u>Abstracts Title:</u> - "<i>CM-728 as a Potential and Novel Compound for Metastatic Clear Cell Renal Cell Carcinoma (ccRCC) Treatment</i>". Jazmine Arévalo. Universitat Autònoma de Barcelona. Barcelona, Spain - "<i>Preliminary Screening for Selective Anticancer Activity of Andalusian Plants</i>". Víctor Jiménez González. Universidad de Sevilla. Sevilla, Spain</p>
11:30h - 12:30h	<p>Session 13: Oral Communications: Neuro/Psychopharmacology and Receptors <u>Moderators:</u> Paco Ciruela and Mattijs Blankesteyn <u>Abstracts Title:</u> - "<i>Protective Effect of Phosphorus Dendrimers in a Murine Model of Multiple Sclerosis</i>". Valentín Ceña. Universidad de Castilla-La Mancha. Castilla-La Mancha, Spain - "<i>NLRP3 Inflammasome Inhibition Protects the Neurovascular Unit, Reduces Infarct Volume and Inflammation in Cerebral Ischemia</i>". Alejandra Palomino Antolín. IIS Princesa. Madrid, Spain - "<i>Cannabidiol Antidepressant-Like Effects in Rats: Decreased Sensitivity in Adolescent versus Adult Rats</i>". M^a Julia García Fuster. Universitat de les Illes Balears. Palma de Mallorca, Spain - "<i>Essential Role of C148-C227 Disulfide Bridge in Human 5-HT_{2A} Receptor Functionality and Trafficking</i>". Marta Cimadevila. University of Santiago de Compostela. Santiago de Compostela, Spain</p>

4. ABSTRACTS

4.1. Plenary Sessions

Stop the mast cells and the eosinophils by any means!

Francesca Levi-Schaffer

The Hebrew University of Jerusalem, Israel

The pivotal effector cells of allergic inflammation are the mast cells and the eosinophils. Mast cells, as activated by IgE mechanisms via allergens, are the recognized *primum movens* while eosinophils infiltration and persistence in the inflamed tissue with the mast cells are the accepted features of the late stage and of the chronic outcome of allergy.

During the years we have defined a pro-inflammatory cross-talk between these two cells that we have named the Allergic Effector Unit (AEU). We found that mast cell/eosinophil interactions result in increased eosinophils chemotaxis, survival, degranulation, cytokine production and in mast cell survival, IgE-dependent and independent degranulation and cytokine production. These effects are mediated by both released mediators (soluble interactions) and by receptor/ligands binding (physical interactions). Prominent players of the activating "physical" AEU are the two activating receptors (ARs)/ligands CD48 and 2B4. Nevertheless, we have also described the presence and functional activity of two inhibitory receptors (IRs), i.e. CD300a and Siglec-7, on mast cells and on eosinophils that can indicate a possible anti-inflammatory or even pro-resolution activity within the AEU and globally as mediated by mast cells and by eosinophils.

The goal of our research is to define potential new targets for immunopharmacological intervention in allergic diseases by blocking ARs, i.e. CD48, or by activating IRs, i.e. CD300a and Siglec-7. We indeed found that CD48 is significantly upregulated on human and murine asthma on mast cells and eosinophils and in the presence of *S. aureus*, the prominent bacteria infecting atopic tissues. We have therefore studied CD48 modulation *in vitro* and *in vivo* and the outcome of its blockade and found that CD48 is a key player in allergic diseases. Similarly, we have found that CD300a and Siglec-7 are expressed by eosinophils and mast cells of allergic patients and described their role in downregulating these cell functions.

Thus, our strategy is to treat allergy by inhibiting activation and/or by activating inhibition of mast cells, eosinophils and the AEU. Translationally this strategy will have to take into account the allergic patient endotype.

Protease-Activated Receptors (PARs) in inflammation and pain: what should we target?

Nathalie Vergnolle
INSERM U1220, France

In addition to their role in protein degradation and digestion, proteases can send specific signals to cells, by activating in particular a family of receptors: the Protease-Activated Receptors (PARs). Four members of this family have been described: PAR-1 to -4. These are 7-transmembrane domain G protein-coupled receptors that are expressed on almost all cell types, where they can control important physiological and pathophysiological processes. In particular, members of this receptor family have been implicated as “emergency signals” in inflammation and pain processes.

Known proteases such as thrombin or trypsin cleave PARs at established sites on the extracellular domain of the N-terminal domain of the receptors to induce specific intracellular signals. However, a growing number of other proteases have been identified and cleave PARs at divergent sites, activating distinct signaling pathways. With this concept of biased agonism, the pharmacology of these receptors has revealed to be more complicated than anticipated. A better understanding of the nature and specificity of the proteases present in physiological and pathophysiological conditions is therefore urgently needed. This lecture will focus on presenting the pharmacology of PARs, their involvement in inflammation and pain mechanisms, and will reflect on how we should consider possible therapeutic interventions targeting PARs or other members of the proteolytic balance.

Somatic Mutations and Clonal Hematopoiesis as Drivers of Inflammation and Cardiovascular Disease

José Javier Fuster

Centro Nacional de Investigaciones Cardiovasculares, Spain

Recent exome sequencing studies in humans have shown that normal aging is unavoidably associated with the accumulation of somatic mutations in the hematopoietic system, which in some cases provide a competitive growth advantage to the mutant cell and allow its clonal expansion. Remarkably, this somatic mutation-driven clonal hematopoiesis has been associated with increased mortality due to both cancer and non-cancerous conditions, most frequently cardiovascular disease. This talk will summarize the results of several epidemiological and experimental studies supporting the possibility that clonal hematopoiesis represents a new and unexpected risk factor and driver of atherosclerotic cardiovascular disease and, potentially, many other age-related inflammatory conditions. Knowledge gained in these studies could eventually lead to the design of personalized therapies or preventive care strategies for individuals carrying specific somatic mutations in blood cells.

Resolution Pharmacology

Mauro Perretti

Queen Mary University of London, UK

Inflammation is a defensive response we mount against pathogens and injurious agents. While a properly regulated inflammatory response is integral to physiology and well-being, a loss of regulation can lead to fibrosis and other chronic diseases. Over the last two decades, we have proposed that important cues to correct persistent inflammation which typifies virtually all chronic pathologies may derive by studying how physiological inflammation resolves. As such we and others have identified specific pro-resolving mediators and receptors that are engaged for an efficient resolution. In this context, specific G-protein coupled receptors signal on macrophages and neutrophils to evoke pro-resolving and tissue-protective effects. More recently, the importance to signal resolution in stromal cells is emerging as necessary for an efficient resolution. Altogether, we propose that this wealth of scientific knowledge makes time ready to harness the biology of resolution for therapeutic innovation. The development of novel strategies to inform new treatments for chronic inflammatory diseases will establish a new branch of pharmacology we term 'Resolution Pharmacology'. The flexibility of the biology of resolution can provide opportunities for monotherapies or poly-pharma approaches for better clinical management of complex diseases.

4.2. Symposiums

Session 1: New in vivo Tools in Pharmacology: CRISPR/Cas and conditional mice

New Animal Models with CRISPR Tools

Lluís Montoliu

CNB-CSIC and CIBERER-ISCIII, Spain

Genome editing has boosted our capacity to produce numerous animal models, for investigating in vivo the cause of altered gene expression and its resulting pathological consequences. Now, with the use of CRISPR genome editing tools, a large variety of genome-edited mice, zebrafish and other useful animal models, can be created easily and rapidly, and at a fraction of the cost that used to be limiting for the generation of transgenic and knockout mice using classical techniques. Single base pair insertions, deletions, substitutions, larger deletions can readily be made with CRISPR reagents directly on mouse embryos, without requiring the use of embryonic stem cells. Reproducing patient-specific mutations in the homologous mouse gene counterparts is now feasible and it is perhaps one of the most powerful benefits of genome editing techniques for the production of in vivo models. Other more challenging projects, including knockin developments, are still difficult but doable with this new technology. In this presentation, I will review the current status of the CRISPR revolution in what it concerns for the generation of new animal models for biomedical research. I will illustrate my talk with numerous examples of mouse models of rare diseases, such as albinism, the genetic condition we have been studying in the lab for many years.

Conditional Mouse Models, Strategies and Applications

Sagrario Ortega

Centro Nacional de Investigaciones Oncológicas, Spain

The laboratory mouse is widely considered the model of choice to study gene function and regulation and the best suited for modeling human disease. The genetic modification of the mouse germ line, achieved for the first time in the last quarter of the 20th century when animal transgenesis was first established, has substantially progressed along the last 20-30 years. The development of gene-targeting technologies based in the use of mouse embryonic stem cells and more recently the new tools for genome editing, especially the CRISP/Cas system, have facilitated the targeted and precise modification of the mouse genome. The introduction of virtually any kind of genetic modification including gene deletions, point mutations, tag or reporter insertions, gene replacement and even chromosomal translocations in the mouse genome is currently relatively simple. However, constitutive genetic modification, in all the cells of the animal and at all stages of development, has many limitations including, in many cases, embryonic lethality that prevents the use of this strategy for modelling disease or studying gene function in the adult. Conditional models, in which the genetic modifications are controlled temporally and spatially have overcome these and other limitations.

Two of the most widely used strategies for conditional genetic manipulation in mammals are exported from prokaryotic organisms. The tetracycline inducible system, used by E.coli strains carrying the Tn10 transposon to control tetracycline resistance, was initially adapted by Mosen and Bujard to control gene expression in eukaryotes. The system, based in the use of a tetracycline responsive promoter to drive gene transcription, has evolved and has been optimized so that we have many tools today to use it in mouse models for inducing or repressing expression of any given gene. While the tetracycline system controls gene transcription and, therefore, is an epigenetic modulator, another conditional system, based in the use of the Cre recombinase of the E. coli bacteriophage P1, induces site-specific recombination by binding and recombining two target DNA sequences of 34 nucleotides called loxP sites. The loxP sites are not present in the mammalian genome but can be introduced by gene targeting or gene editing to create "primed" alleles, called floxed alleles, that behave as wild type ones but are susceptible of Cre-mediated recombination to be converted to knockout (loss of function) or knockin (precise mutations) alleles. Cre may also catalyze "in trans" the targeted integration of a loxP containing transgene into a pre-defined position in the genome where a loxP site has been previously introduced or even precise chromosomal translocations. The Flp recombinase from *S. cerevisiae* works in a similar way to Cre, recombining two flt target sites whose structure is similar to loxP sites but have different sequence, so that both systems can be used in the same mouse simultaneously. Fusions of these recombinases with the ligand-binding domain of the estrogen receptor (ER), previously modified (ERT2) to reduce its affinity for the endogenous 17 β estradiol and specifically bind to 4-OH tamoxifen (4OHT) allows temporal control of Cre or Flp activity. These fusions are retained in the cytoplasm of the cells, bound to the HSP90 chaperon protein and are released and internalized in the nucleus upon 4OHT administration.

Several examples of the use of these systems for studying gene function, tracing cell fate during development, modelling cancer and identifying new potential targets for cancer therapy in the mouse will be presented.

Two of the most widely used strategies for conditional genetic manipulation in mammals are exported from prokaryotic organisms. The tetracycline inducible system, used by E.coli strains carrying the Tn10 transposon to control tetracycline resistance, was initially adapted by Mosen and Bujard to control gene expression in eukaryotes. The system, based in the use of a tetracycline responsive promoter to drive gene transcription, has evolved and has been optimized so that we have many tools today to use it in mouse models for inducing or repressing expression of any given gene. While the tetracycline system controls gene transcription and, therefore, is an epigenetic modulator, another conditional system, based in the use of the Cre recombinase of the E. coli bacteriophage P1, induces site-specific recombination by binding and recombining two target DNA sequences of 34 nucleotides called loxP sites. The loxP sites are not present in the mammalian genome but can be introduced by gene targeting or gene editing to create “primed” alleles, called floxed alleles, that behave as wild type ones but are susceptible of Cre-mediated recombination to be converted to knockout (loss of function) or knockin (precise mutations) alleles. Cre may also catalyze “in trans” the targeted integration of a loxP containing transgene into a pre-defined position in the genome where a loxP site has been previously introduced or even precise chromosomal translocations. The Flp recombinase from *S. cerevisiae* works in a similar way to Cre, recombining two flt target sites whose structure is similar to loxP sites but have different sequence, so that both systems can be used in the same mouse simultaneously. Fusions of these recombinases with the ligand-binding domain of the estrogen receptor (ER), previously modified (ERT2) to reduce its affinity for the endogenous 17 β estradiol and specifically bind to 4-OH tamoxifen (4OHT) allows temporal control of Cre or Flp activity. These fusions are retained in the cytoplasm of the cells, bound to the HSP90 chaperon protein and are released and internalized in the nucleus upon 4OHT administration.

Several examples of the use of these systems for studying gene function, tracing cell fate during development, modelling cancer and identifying new potential targets for cancer therapy in the mouse will be presented.

Investigation of Rodent Adult Neurogenesis in Homeostasis and Disease: An example of the use of Conditional Mice in Neuropharmacology

María Ángeles Moro

Universidad Complutense de Madrid, Spain

The conditional gene knockout method is a very ingenious strategy based on the elimination of a specific gene in a certain cell type. It is of great utility for the study of the function of genes in selected cell types. It is also valuable in the study of the relevance of cellular processes which depend upon the gene which is conditionally knocked-out. In this presentation, we will review how conditional knock-out mice can provide essential information in the study of the function of specific receptors in adult neurogenesis in the healthy brain, and also how these mice can be applied to explore the role of this process in the injured brain.

Session 2: New targets in cardiac and cardiovascular disorders

Targeting Histidine Phosphorylation by Nucleoside Diphosphate Kinases in Cardiovascular Disorders

Thomas Wieland
Heidelberg University, Germany

Nucleoside diphosphate kinases (NDPKs) are important housekeeping enzymes for nucleotide triphosphate homeostasis. Early reports during the 1990s already indicated a close association of NDPKs with heterotrimeric G proteins to replenish GTP required for their activation. We meanwhile identified oligomers of the NDPK isoform B and C to form a complex with heterotrimeric G proteins. Apparently, NDPK C is essential for a complex formation with the G protein $G\beta\gamma$ dimer. As proven by knockdown experiments in zebrafish embryos the interaction formation of NDPK B and C with $G\beta\gamma$ is required for the contractility of the heart. Intriguingly, besides its classical enzymatic activity, its ability to function as protein histidine kinase also contributes to its specific interaction with $G\beta\gamma$. Within the complex a specific histidine residue is phosphorylated in $G\beta$. If this His is mutated to Ala, the isoprenaline-induced cAMP formation and single cell contractility in cardiomyocytes is significantly impaired. Interestingly, NDPK C is upregulated in human heart failure and is preferentially found in complexes with the inhibitory G protein of the adenylyl cyclase, G_i . In contrast in non-failing hearts, NDPK is preferentially bound to the stimulatory G protein G_s . Therefore, NDPK C likely contributes to well-known chronic inhibition of cAMP formation in failing heart muscle.

A second protein which is regulated in its activation by histidine phosphorylation by NDPKs, is the intermediated conductance potassium channel SK4, which is expressed in proliferating but not in mature vascular smooth muscle cells. We could show that the phosphorylation of the channel by NDPK B is required for vascular smooth muscle cell proliferation and neointima formation after vessel injury occurring for example during re-stenosis or atherosclerosis.

Therefore small molecule inhibitors targeting specifically the histidine phosphorylation by NDPKs might be relevant for new therapeutic strategies in cardiovascular disease. Out of a library of several hundred potential candidates, we identified 3 substances, of which has a preference for NDPK C. This substance is able to inhibit the essential histidine autophosphorylation of NDPK C in living cells and like the knockdown of NDPK C, attenuates the isoprenaline-induced cAMP formation and PKA activation in cardiac myocytes. It is therefore an interesting candidate to be structurally optimized and further tested the potential use of NDPK inhibitors as novel therapeutic option.

Recent Advances in the Pharmacological Treatment of Cardiovascular Disease

Ricardo Caballero

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Cardiovascular disease (CVD) is a group of disorders of heart and blood vessels that includes among others: hypertension, coronary artery disease, heart failure, cerebrovascular disease, and peripheral vascular disease. CVD is the leading cause of death in the world: more people die annually from CVD than from any other cause. During the last decades there were huge advances in the pharmacological treatment of some of these diseases. Nowadays, drugs for the treatment of hypertension or coronary artery disease are very efficacious, safe, and cheap. Furthermore, in recent years new anticoagulants, hypolipidemic and antidiabetic drugs, or drugs for the treatment of some forms of heart failure have been developed and successfully used in many patients. However, pharmacology of CVD is not devoid of remarkable failures and mistakes at all stages of drug development. In this regard, there are some examples of drugs with serious concerns about their efficacy and/or safety. Furthermore, even after extensive basic and clinical research in the field the pharmacological treatment of some severe forms of CVD such as acute heart failure or heart failure with preserved ejection fraction is clearly suboptimal. To circumvent all these challenges, the use of novel strategies and tools is necessary in order to improve the development of new drugs for treating CVD patients.

Interventions in WNT Signaling Stimulate Cardiac Regeneration

Mattijs Blankesteyn
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Activation in WNT signaling has been identified in many cardiovascular diseases, but its potential implications for therapeutic intervention are only beginning to be understood. Several studies from different research groups have shown, however, that pharmacological inhibition of WNT signaling after myocardial infarction reduces scar size and improves cardiac function. A plausible explanation for this is the induction of newly formed cardiomyocytes in the border zone of the infarct. Given the importance of WNT signaling in the control cell differentiation and stem cell biology it is tempting to propose WNT inhibition as a mediator of cardiac regeneration.

Session 3: Parallel Roundtable. Education in Pharmacology**IUPHAR, Education and Collaborations World-Wide**

Michael Spedding
IUPHAR, UK

The International Union of Basic and Clinical Pharmacology (IUPHAR) represents ~37,000 pharmacologists world-wide and is affiliated to WHO for Health Care. The nomenclature committee (current chair Steve Alexander) has structured modern pharmacology and IUPHAR has published 125 publications with an H-Index of >80. NC-IUPHAR organises two meetings/year. Over 90 subcommittees of about 700 expert scientists back our publicly available databases, accessed freely by scientists in 160 countries:

- The IUPHAR/BPS guidetopharmacology.org, with help from the BPS and Wellcome Trust
- The guidetoimmunopharmacology.org (see Francesca Levi-Schaffer's presentation)
- The IUPHAR/MMV guide to malaria pharmacology.org, financed by the Medicines for Malaria Initiative.

We have also set up, helped by ASPET, CNPHARS, JPS, and HPS, the pharmacology education project website, which is an ongoing project, freely available to all. The IOSP project run by David Lewis has given multiple training courses on animal use and ethics in China, Africa and India. Courses in clinical pharmacology, and on pharmacovigilance have been run in China, India and Africa. We have signed an agreement with Karolinska Institute to progress academic drug discovery and clinical pharmacology, obtaining two seed grants. We have organised 8 meetings worldwide on advances in natural products, with an emphasis on how technological advances can engage evidence-based medicine. The next IUPHAR World Congress on natural products is to be held in Hyderabad December 4th-7th, 2019, hosted by IPS. An enthusiastic young investigator's committee is now picking up the IUPHAR baton!

The Doctoral Programme in Pharmacology

Guillermo Díaz
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The Doctoral Programme in Pharmacology was established in the year 2002, with the participation of the Faculties of Chemical & Pharmaceutical Sciences, Medicine, Sciences and the Institute of Nutrition & Food Technology (INTA). From 2003, had received a national accreditation by the National Accreditation Institute (CNA-Chile).

Main features of the Doctoral Programme in Pharmacology

The programme entails a face-to-face, full dedication schedule. Candidates hold undergraduate degrees in chemistry, biochemistry, biology and pharmaceutical sciences, including medicine, dentistry, veterinary medicine and biotechnology. Since 2009, the candidate postulates by an on-line application system (<https://postulacionpostgrado.uchile.cl>). Successful applicants can apply to a CONICYT Scholarship or to any other National or International Granting Institutions, including support by the Faculty of Chemical & Pharmaceutical Sciences, University of Chile. Formal activities are based on two mandatory courses: Advanced Molecular Pharmacology, and Experimental Pharmacology, which focus on molecular modelling, genomics, pharmacological chemistry and clinical pharmacology. It is mandatory that the doctoral thesis work, must generate at least one article published by an international ISI-indexed Journal, where the candidate as a first author. All graduates from the Program are inserted in Research and Academic Institutions, both in the country or abroad. Several graduates proceed to postdoctoral positions in internationally recognised institutions, either locally, or in Europe and North America. The insertion rate is 100% for the total graduate cohorts.

Academic core

At present, the academic core is composed of 34 faculty members with the highest national and international prestige, selected among associate and full professors from the University of Chile. Based on the expertise of academic Core, six lines of research have been established, which warrant proper competitiveness, i.e. Cardiovascular, Cancer, CNS, Immunology, Antimicrobial and Chemotherapy, and Drug Development. Core have collectively authored a total of about 700 ISI articles in the past last 10 years. The Core has also files and obtained 16 patents reflecting an outstanding record.

Achievements

From 2002, more than 90 PhDs in Pharmacology have graduated from the Program. In the past few years, an average of 10 candidates per year have been accepted in the Program, and at present there are 47 candidates carrying out their thesis work. The demand for admission has also increased substantially, in particular by students from other Latin American countries (i.e. Colombia, Venezuela, Costa Rica, Ecuador, Cuba, Peru), thus presenting the Program as attractive choice in the region. The Doctoral Programme in Pharmacology has substantially prioritized international networking, including the University of Regensburg, Germany, for the development of epigenetics; and the University of Mississippi, USA, for Clinical Pharmacology. There are several agreements in progress with the Universidad Autónoma de Madrid, Spain, focusing on system pharmacology, and UNICESUMAR, Paraná, Brazil, focusing on veterinary.

Doctoral studies in Spain. Structure and management

Jose Miguel Doña Rodríguez
Universidad de Las Palmas de Gran Canaria, Spain

The aim of this communication is to provide an overview of the structure and management of doctoral studies in Spain today, following the process of adaptation to the European Higher Education Area. Presently Doctoral studies regulations in Spain promotes to fit these studies to the Salzburg's Principles which can be summarized as mainly research-focused, time-limited and controlled, under collegial supervision and highly interdisciplinary, collaborative and international. Doctoral Programs do not require to be structured in ECTS. The essential activity will be the research. Training is not regulated in any way except in the expected learning outcomes and skills. During the development of the doctorate, the doctoral candidate should develop a series of formative activities adequate to reach the expected learning outcomes and skills. Of course, Doctoral studies should finish with the elaboration and the defense of a doctoral thesis that incorporates original results of research^{1,2}.

During doctoral studies, the candidates should develop different formative activities:

- **Transversal training:** Teamwork, preparation of presentations, improvement of oral and written communication skills, knowledge of the principles of ethics and integrity in research, management of scientific projects, management of intellectual and industrial property, prevention of occupational risks, collaborations in teaching activities, etc.
- **Specific training in the scope of the Doctoral Program:** Attendance and participation in advanced seminars, congresses, courses, oral presentations, article writing, professional practices, etc.

Doctoral studies at the University of Las Palmas de Gran Canaria are organised by the Doctoral School. We have 13 doctoral programs, three of them in the field of health sciences. These three programs develop, among other lines of research:

- Pharmacology applied to experimental, clinical and epidemiological studies, pharmacosurveillance, pharmacoconomics, quality of life and use of medicines.
- Molecular mechanisms that control the immune response, infection and inflammation.

¹ Royal Decree 99/2011, of 28 January, regulating official doctoral studies.

² Regulation of doctoral studies of the University of Las Palmas de Gran Canaria.

Session 4: Open innovation in drug discovery

Health Care and Impact of Migraine from Patients Perspective

Isabel Colomina
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Migraine affects more than 4 million people in Spain, of which 80% are women between 20 and 40 years old. Historically migraine has been associated with a simple headache, but it is not, migraine is manifested by recurrent episodes of moderate or severe headache, throbbing and accompanied by nausea, vomiting, sensitivity to light, sounds or the smells.

What we need the patients and how we want to be treated must be part of the active listening of health professionals. The pharmacological innovation that is coming, restricted to a group of patients, we are sure will improve our quality of life, but in the meantime and for those who cannot benefit from it, we must take into account the impact that this disease has on our lives, in all dimensions: personal, family, work, social or educational.

Migraine Pharmacology Evolution

María del Buensuceso Fernández del Pozo
AEMICE, Spain

Migraine is a highly disabling disease, with recurrent episodic crises, that could progress in some cases to chronicity, having a significant economic and social cost. Its multifactorial etiology and genetic influence makes difficult the comprehension of its pathophysiology, which is not entirely clarified yet. Until the 40s the only alternative for treating migraine was the symptomatic treatment with non-specific analgesics for this condition, linking antiemetics if necessary, and with which many patients did not find relief. Ergotic drugs were a first step towards more specific drugs, but their efficacy remain limited and their adverse effects and contraindications are considerable. The introduction in the 90's of the triptans (5-HT_{1B/1D} agonists), which become the gold standard for the treatment of acute crises, relegates the ergotic drugs to the background. However, many patients doesn't respond yet to treatment. More recently, the first 5-HT_{1F} receptor agonist, lasmiditan, has been authorized, which opens the family of the ditans and allows treating patients with risk or diagnosis of cardiovascular pathology, in which triptans are contraindicated. FDA has also authorized another small molecule, ubrogepant, CGRP receptor antagonist. Even so, almost half of patients fail to reduce the frequency or intensity of seizures. In these cases of chronic migraine, preventive treatment is indicated. Beta-blockers, antiepileptics and antidepressants have been used to prevent migraine with limited effectiveness. In recent years, advances in the knowledge of the pathophysiology of migraine have led to the recent authorization by the EMA and the FDA of monoclonal antibodies against CGRP (galcanezumab, fremanezumab), and its receptor (erenumab), to which other products, such as eptinezumab and the gepants rimegepant and atogepant, in advanced stages of research can be shortly included.

Other targets of interest are the pituitary adenylate cyclase activating polypeptide (PACAP), with a monoclonal antibody in preclinical assays, and its PAC₁ receptor, in relation to which a monoclonal antibody already exists in phase II clinical trials that may give rise to a new family of antimigranous. Transient receptor potential (TRP) channels, linked to the release of neuropeptides, constitute another hypothetical target for the control of migraine that is currently under investigation, as well as acid-sensing ion channels (ASICs), implicated in pain perception and who are inhibited by AINE, or NOD-like receptor protein 3 (NLRP3) inflammasome related to neuroinflammation.

Physiopathology and epidemiology of Migraine

Ayoze González

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Migraine is a common disorder, with a prevalence of 10-12%. It is more frequent in women and is an important cause of morbidity and loss of daily function. Currently, the main pathophysiological mechanism of migraine includes interactions of the cerebral cortex, brainstem and trigeminovascular system. This spread cortical depression causes sensitization and hyperexcitation of the trigeminovascular system, which leads to inflammatory pathways and to pain aetiology. Various neuropeptides, such as the P substance or the peptide related to the calcitonin gene (GCRP), regulate the trigeminovascular system. It has been demonstrated that migraine patients present increased GCRP level and that it relates to pain crisis. This opens the door to a new therapeutic target in migraine treatment.

Session 6: New advances in the Pharmacology of Respiratory System

Targeting K⁺ Channels in Pulmonary Hypertension

Ángel Cogolludo

Universidad Complutense, Spain

Pulmonary arterial hypertension (PAH) is a multifactorial and severe disease. PAH pathobiology involves a sustained pulmonary vasoconstriction, endothelial dysfunction, distal pulmonary vessel remodelling, and inflammation. The reduced lumen of pulmonary arteries can cause increased pulmonary vascular resistance leading to right ventricular hypertrophy, heart failure and premature death. Current pharmacological treatments have improved survival rates but annual mortality from PAH remains high. A better understanding of the molecular mechanisms involved in the pathophysiological processes is essential to identify novel drug targets of potential therapeutic interest. Potassium (K⁺) channels represent the largest and most diverse group of ion channels, being expressed in all mammalian cell types. In pulmonary artery smooth muscle cells, K⁺ channels are main contributors in setting membrane potential and hence contraction. The activity of K⁺ channels is finely regulated by a number of vasoactive factors and, in fact, their activation and inhibition has been shown to contribute to the pulmonary vascular effects of main vasoconstrictors and vasodilators, respectively. Among the different K⁺ channels functionally expressed in the pulmonary vasculature special attention has been paid to the voltage-gated K⁺ channel Kv1.5 and the two-pore domain K⁺ channel TASK-1, whose expression or activity is dysregulated in most forms of PAH. A number of mechanisms leading to this K⁺ channel dysfunction in PAH have been proposed. In recent years, the identification of mutations in the genes encoding for TASK-1 (KCNK3) and Kv1.5 (KCNK5) as novel causes of PAH demonstrates their crucial role in the onset of the disease. Likewise, Kv7 channels have recently emerged as key players regulating vascular tone. The availability of selective activators currently indicated for other conditions makes Kv7 channels an attractive target in PAH. In summary, targeting K⁺ channels to increase their activity or prevent their impairment may represent a possible future therapeutic strategy for PAH.

Other targets of interest are the pituitary adenylate cyclase activating polypeptide (PACAP), with a monoclonal antibody in preclinical assays, and its PAC1 receptor, in relation to which a monoclonal antibody already exists in phase II clinical trials that may give rise to a new family of antimigranous. Transient receptor potential (TRP) channels, linked to the release of neuropeptides, constitute another hypothetical target for the control of migraine that is currently under investigation, as well as acid-sensing ion channels (ASICs), implicated in pain perception and who are inhibited by AINE, or NOD-like receptor protein 3 (NLRP3) inflammasome related to neuroinflammation.

ADAM Family Proteases: Friends or Foes in Pulmonary Inflammation?

Andreas Ludwig
RWTH Aachen University, Germany

Several membrane-bound proteins with a single transmembrane domain are subjected to limited proteolysis at the cell surface. This cleavage leads to the release of their biologically active ectodomains, which can trigger different signalling pathways. In many cases, this ectodomain shedding is mediated by members of the a disintegrin and metalloproteinase (ADAM) family. ADAM10, in particular, can be positively and negatively involved in various physiological processes as well as in inflammatory, fibrotic and malignant pathologies. Using mice with cell specific ADAM10 deficiency we could demonstrate a role of ADAM10 in acute pulmonary inflammation. In vivo studies and vitro analysis of primary lung cells demonstrate a critical involvement of ADAM10 in cytokine production, edema formation, vascular permeability and leukocyte recruitment. Endogenous inhibitors such as TIMP1 can control excess ADAM10 activity. Such control can also be achieved by application of synthetic small molecules. In previous studies we have described the small molecule inhibitor GI 254023X (GI) which can rapidly block ADAM10 by binding to the active site. Now, we demonstrate that this inhibitor also induces long term downregulation of the protease from the cell surface associated with degradation and release of the protease in extracellular vesicles. The resulting loss of ADAM10 shedding activity is reversed by de novo synthesis of the protease. Furthermore, intraperitoneal administration of GI in mice led to profound loss of surface expressed ADAM10 on leukocytes derived from peripheral blood or different organs. Finally, GI effectively suppressed induction of acute lung inflammation. Thus, synthetic and natural inhibitors can mediate long term systemic inhibition of ADAM10 by depletion of the protease. These findings are of importance for development of optimal treatment strategies using ADAM10 inhibitors.

Other targets of interest are the pituitary adenylate cyclase activating polypeptide (PACAP), with a monoclonal antibody in preclinical assays, and its PAC1 receptor, in relation to which a monoclonal antibody already exists in phase II clinical trials that may give rise to a new family of antimigranous. Transient receptor potential (TRP) channels, linked to the release of neuropeptides, constitute another hypothetical target for the control of migraine that is currently under investigation, as well as acid-sensing ion channels (ASICs), implicated in pain perception and who are inhibited by AINE, or NOD-like receptor protein 3 (NLRP3) inflammasome related to neuroinflammation.

Session 7: New advances in Neuropharmacology

Inside a Secretory Vesicle. Novel Targets for Modulating the Exocytosis of Neurotransmitters

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Chromaffin granules are similar organelles to the large dense core vesicles (LDCV) present in many cell types including neurons. LDCV accumulate solutes at large concentrations (catecholamines, 1 M; ATP, 200 mM; or Ca²⁺, 40 mM). Solute seem to aggregate into a condensed matrix under the acidic vesicular pH (≈ 5.5) to elude the osmotic lysis. The affinity of solutes for LDCV matrix is responsible for the delaying release of the release of catecholamines after granule fusion. We have accumulated experimental evidence to conclude that the manipulation of intravesicular media largely affects the LDCV cargo and produce changes in the kinetics of exocytosis.

The accumulation of solutes depends on the pH gradient across membrane vesicle that is maintained by a V-ATPase. The alkalinization of secretory vesicles slows down exocytosis and causes the leak out of catecholamines and Ca²⁺.

We have studied the functional contribution of chromogranins (the major intravesicular proteins) and ATP to the exocytosis by manipulation of its expression and using KO animals. Also, we have explored the consequences of the lack of the main catecholamine (adrenaline) stored in LDCV using a PNMT-KO mouse.

Several drugs are accumulated inside LDCV (hydralazine, b-blockers, some antimalarial or tricyclic antidepressants) displacing catecholamines to the cytosol. In addition, these drugs are co-released with catecholamines as false neurotransmitters.

In this presentation we will summarize all of our results obtained along the last years.

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Supported by the Spanish Ministerio de Ciencia, Innovación y Universidades (MICIU; BFU2017-82618) and AGS and ADLI are recipient of FPI fellowships (MICIU); PM has a contract from MICIU.

Pamam Dendrimers Effects on Neuronal Functionality in vitro

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PAMAM (polyamidoamine) dendrimers are hyperbranched macromolecules which have been described as one of the most promising drug nanocarrier systems. We focused in the effects in hippocampal neurons of fourth generation PAMAM dendrimers: with a complete positive charged surface (G4) compared with dendrimers modified in a 25% of their terminal groups with folate (PFO25), polyethylene glycol (PPEG25) and acrylate (PAC). A key process to understand is the cellular internalization mechanism of PAMAM dendrimers because its direct influence on their intracellular distribution, association to organelles, entry kinetics, and cargo release. Despite that these mechanisms have been studied in different cell types, in the case of neurons they are not completely described. According to confocal images both polyethylene glycol (PEG) and acrylate (AC) modified PAMAM dendrimers were not able to enter the neurons. Colocalization studies with specific endocytosis markers and specific inhibitors demonstrate that clathrin-mediated endocytosis would be the main internalization mechanism for G4 dendrimers, whereas clathrin and caveolae-mediated endocytosis would be implicated in folate (PFO) conjugated PAMAM internalization.

Another key aspect to study about PAMAM dendrimers is their biocompatibility. In this sense, we have studied specific alterations of neurons in contact with this polymer. Cell viability assay showed that G4 dendrimer induce a high cytotoxicity, which is attenuated by both chemical modifications, being PPEG25 the most biocompatible of them. The study of their effects in membrane integrity by patch clamp techniques demonstrate the increase of membrane permeability and suggest a loss of its integrity in the presence of G4, but not with other dendrimers tested. Moreover, the treatment with G4 induce a significant increase of intracellular Ca²⁺ with a complete disruption of its normal pattern of transient increments, whereas for both PFO25 and PPEG25 treatments it was possible to observe a normal pattern of Ca²⁺ transients but with an increase of its frequency. Synaptic activity registers by patch clamp showed that G4 treatment promotes the significant increase of frequency but not of amplitude. These results suggest that cationic G4 dendrimer induces excitotoxicity generated by the disruption of membrane integrity and the subsequent increase of its permeability leading to the increase of intracellular Ca²⁺, which is reverted by surface modification of dendrimers with folate or polyethylene glycol.

Programming of Dopaminergic Neurons by Early Exposure to Sex Hormones: is a Vulnerability Factor for Drug Addiction?

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Sex hormones play an important role in regulating reproductive and non-reproductive tissues, such as the brain. In the nervous system, sex hormones are important in its development and neural plasticity, however changes in the sex hormones milieu during fetal or neonatal stages affect brain function and generate persistent changes until adulthood. During last 7 years our lab has been interested in study how neonatal exposure to sex hormones such as estradiol valerate (EV), testosterone propionate (TP) or dihydrotestosterone (DHT) affect the functionality of midbrain dopaminergic neurons of adult male and female rats.

Our results show that the expression of tyrosine hydroxylase (TH: rate limiting enzyme in the production of catecholamines) is increased in substantia nigra (SN) and ventral tegmental area (VTA) of EV and TP adult rats. In addition, dopamine (DA) content in SN-VTA and DA release in Nucleus Accumbens (NAcc) are higher in the same groups of rats. However, neonatal exposure to DHT (a non-aromatizable androgen) does not produce the same effects. At behavioral level the neurochemical changes observed in EV and TP adult rats are significantly associated with an increase in the pharmacological effects of depressant drugs such as alcohol and morphine. However no behavioral changes were observed with psychostimulant drugs (amphetamine and methylphenidate).

Our research suggests that neonatal exposure to estrogenic compounds affects the functionality of midbrain dopaminergic neurons in the adulthood and depending of the action mechanism of the drug of abuse, reprogramming with sex hormones may be a vulnerability factor to addiction.

Supplementation with Melatonin Impedes Cognitive Decline in tau-related Alzheimer Models, by Restoring the Autophagic Flux, once the Pathology is Initiated

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Universidad Autónoma de Madrid, Spain

Lack of effective treatments for such a devastating neurodegenerative disease, like Alzheimer's disease, makes the scientific community to work on the understanding of the mechanisms underlying the pathology and on its potential pharmacology treatment. Alterations in autophagy are increasingly being recognized in the pathogenesis of Alzheimer's disease. Melatonin is a neurohormone whose levels are decreased with aging and, most importantly, in certain neurodegenerative diseases like Alzheimer's disease. The lack of knowledge of how autophagy failure participates in the pathogenesis of Alzheimer's disease and the potential relationship between the reduced levels of melatonin in this patients with this clearance mechanism, prompted us to define this study. Different Tau-related models were performed. For instance, injection of AAV-hTau/GFP viral vectors and treatment/injection with okadaic acid were used to achieve ex vivo, human brain slices and in vivo tau related models. In the in vivo studies, intracerebroventricular injection of AAV-hTau increased oxidative stress, neuroinflammation and tau hyperphosphorylation in the hippocampus 7 days after the injection, without inducing cognitive impairment; however, when animals were maintained for 28 days, cognitive decline was apparent. Interestingly, late melatonin treatment, starting once the alterations mentioned above were established (from day 7 to day 28) reduced oxidative stress, neuroinflammation, tau hyperphosphorylation and caspase-3 activation; these observations correlated with restoration of the autophagy flux and memory improvement. This study highlights the importance of autophagic dysregulation in tauopathy and how melatonin treatment in the prodromal phases of the disease can restore the autophagy flux, and thereby, prevent cognitive decline. We therefore propose the development of drugs that improve the autophagy flux for the treatment of proteinopathies like Alzheimer's disease.

Session 8: Free Radicals in Translational Pharmacology

Relevance of Nrf2 and Heme Oxygenase-1 in Articular Disease

María José Alcaraz
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A wide range of evidence indicates that Nrf2 is able to control oxidative stress and inflammation as well as innate and adaptive immune responses. Nrf2 inhibits the production of reactive oxygen species and mediators driving chronic inflammation and tissue destruction. This transcription factor can decrease differentiation, proliferation and activity of antigen-presenting cells, T cells and B cells while enhancing these processes in regulatory T cells. The appropriate balance of Nrf2 is necessary for cartilage metabolism. In addition, Nrf2 plays a key role in the maintenance of bone microarchitecture. Heme oxygenase-1 (HO-1) induction is a consequence of Nrf2 activation. Therefore, oxidative stress and pro-inflammatory cytokines upregulate HO-1 whereas anti-inflammatory cytokines have the opposite effect. HO-1 upregulation or administration of its metabolites results in anti-inflammatory and antioxidant effects in articular diseases and it may protect against cartilage destruction and inflammatory bone loss. HO-1 decreases the production of oxidative stress, pro-inflammatory agents and catabolic enzymes and controls the dysbalance between anabolic and catabolic processes in articular cells. Both Nrf2 and HO-1 can be targets in the control of joint degradation and bone alterations. There is a need for improved strategies to regulate their activity which may open up new therapeutic opportunities for the treatment of joint conditions.

Free Radicals in translational Pharmacology: NOS, NOX & sGC as a network pharmacology-based approach for brain ischemia

Ana Casas

Maastricht University, Netherlands

Existing drugs fail to provide benefit for most patients. The efficacy of drug discovery is in a constant decline. This poor translational success of biomedical research is due to false incentives, lack of quality/reproducibility and publication bias. In fact, drug discovery faces an efficacy crisis to which ineffective mainly single-target and symptom-based rather than mechanistic approaches have contributed. Systems medicine opened a new concept of therapeutic treatment focused on the so-called network pharmacology, where several targets modulated at the same time lead to the first effective therapy for high unmet medical need indications with no treatment so far.

In stroke, we therefore validated 3 single ROS-related enzymes, i.e. NADPH oxidase, NO Synthase and soluble Guanylate Cyclase as promising therapeutic targets for brain ischemia within a network-pharmacology strategy. Pharmacological modulation of these targets leads to less infarct size, reduced blood-brain barrier leakage, improved neuro-motor functioning and direct neuroprotection. Our therapeutic approach is now in the last pre-clinical step (large animal validation) towards a clinical trial, currently in design.

The Oxidative Stress Theory of Disease

Pietro Ghezzi

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The formulation of theories about mechanisms of disease has led to major advancements in medicine. These include, for instance, the germ theory of disease and the cytokine theory of disease. The oxidative stress (OS) theory of disease implicates that OS is a causal factor in some diseases and that antioxidant molecules, by scavenging reactive oxygen species (ROS) could represent a therapeutic approach. However, although it was first hypothesized in 1956 with the “free radical theory of ageing”, so far there are no antioxidants that have been approved as medicinal products by regulatory agencies. Despite this, and the fact that OS has been implicated practically in every disease, there is a multibillion dollar market of antioxidant supplements. Of course new research will identify new antioxidant molecules and those may be found effective, but there is a possibility that there is an intrinsic weakness in the OS theory of disease.

We will analyze the weakness of this theory highlighting various problems encountered when analyzing causation in biology. These include the epistemological problem of drawing a causal link, the lack of specific experimental tools, the lack of a way of grading experimental evidence that fits the criteria of evidence-based medicine, and the problem of extrapolating conclusions from well-defined experimental model to the clinical situation.

In particular, we will focus on the difficulty of measuring ROS, which forces us to use surrogate biomarkers. Finally, we will discuss the role of OS in diseases with multiple causes and how this impact on the interpretation of results from statistical analysis using significance testing.

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Session 10: Aging from mechanisms to pharmacological perspectives**Aging from mechanisms to pharmacological perspectives**

José Viña

Universidad de Valencia, Spain

Understanding the mechanisms of aging is of primary importance to promote longevity, but also –and critically important- healthy aging.

The concepts of aging, longevity and frailty will be discussed (1). Based on considerable information on the mechanisms of aging gathered in the last twenty years, we can provide basis for the promotion of longevity, but also of healthy aging, a concept closely related to the more clinically accepted idea of frailty, a geriatric syndrome that leads to disability and death.

Healthy aging can be promoted by interventions like programmed exercise, that we consider as a drug (2), and one specially useful for the elderly (3),

Nutritional interventions will also be discussed with special emphasis on the importance of protein. Finally the role of conventional pharmacological interventions – with emphasis on drugs like metformin or antioxidants- will be emphasized.

The main corollary that will be discussed , i.e. that interventions to treat frailty and delay disability, are of the utmost importance for individual health but also for society will serve to close the presentation (4)

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P2xr Overexpression contribute to Beta Amyloid Toxicity: New Pharmacological Target to AD

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Soluble oligomers of amyloid beta peptide (SOA β) have been considered as central factors in Alzheimer's disease (AD). A β peptide is generated through the sequential cleavage of the amyloid precursor protein (APP), a process that requires the previous endocytosis of APP and that can be modulated by the multidomain adaptor protein Fe65. This protein is able to regulate the transcription of key genes directly related to AD pathogenesis, encoding proteins like APP and BACE 1. On the other hand, we have described that chronic SOA β treatment induces an increase in the expression of the P2X2 purinergic receptor in PC12 cells and hippocampal neurons. Additionally, it has been described that the P2X2a isoform has an intracellular domain that can interact with Fe65, a segment which is absent on the P2X2b isoform. We found that SOA β treated cells displayed an increase in evoked ATP currents (C: $100 \pm 50\%$; SOA β : $231 \pm 70\%$; n=9). Additionally, immunocytochemistry (ICC) experiments demonstrated that these cells exhibited an increase in their P2X2R immunoreactivity (C: $100 \pm 1\%$; SOA β : $149 \pm 15\%$; n=5). Moreover, cells treated chronically with SOA β showed a reduction in the Fe65 nuclear-cytoplasmic (N-C) ratio (C: $100 \pm 6\%$; SOA β : $80 \pm 4\%$; n=5). A similar behavior was observed in PC12 cells transfected to express the P2X2a isoform, but not in those transfected with P2X2b (C: $100 \pm 5\%$; P2X2a: $70 \pm 6\%$; P2X2b: $95 \pm 6\%$; n=3). Colocalization analyses demonstrated that SOA β decreased the colocalization of Fe65 with APP (C: $100 \pm 17\%$; SOA β : $47 \pm 12\%$; n=5); results that correlate with the increase observed in the colocalization of APP with clathrin (C: $100 \pm 8\%$; SOA β : $127 \pm 8\%$; n=4) and Rab5 (C: $100 \pm 6\%$; SOA β : $132 \pm 16\%$; n=5). In conclusion, these results suggest that chronic SOA β treatment promotes the endocytosis of APP, potentiating its amyloidogenic processing. Additionally, the calcium dyshomeostasis/overload induced by P2X2R overexpression, alter the activation and localization of CAMKII α , in the context of AD. Using molecular biology techniques, we observed that after chronic SOA β treatments, mice hippocampal neurons showed an increase on the levels of P2X2R compared to the control cells (C: $100.0 \pm 6.4\%$; SOA β : $130.1 \pm 10.7\%$, n=5). This was correlated with increased Ca $^{2+}$ signal evoked by ATP (C: $100.0 \pm 12\%$, SOA β : $194 \pm 24\%$, n=4). Immunocytochemistry approaches on mice hippocampal neurons, showed that the overexpression of P2X2R induced changes on the immunoreactivity pattern of pCAMKII α (in soma and neurites), which induced alterations on the cells morphology, and electrophysiological recordings assessed by Sholl Analysis and Patch Clamp, respectively. These results suggest that P2X2R overexpression can potentiate the toxicity of SOA β , due to the chronic Ca $^{2+}$ overload and inactivation of CAMKII α , and thus, altering the mechanisms of neuronal plasticity, the basis of the pathophysiological mechanism of AD.

Cardiac Fibroblast Role on Inflammatory Process: Interaction with Immune Cells

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Cardiac remodeling and fibrosis by tissue damage, is a disease characterized by a gradual transition of two paradoxically opposed states, inflammation and repair which reside on immune infiltrated cells as well as cardiac fibroblast and myofibroblast which deposit high amounts of collagens. Cardiac fibroblast had been named as sentinel cells, and due their immunomodulatory properties they can quickly react to either pro-inflammatory or pro-fibrotic stimuli; however, they are efficient producers of cytokines, chemokines and also express adhesion molecules necessary to recruit immune cells, therefore it is feasible to propose that fibroblasts could regulate monocytes recruitment.

The abundance and strategic location of cardiac fibroblasts and also macrophages in cardiac tissue damage, suggest the possibility of a highly coordinated interaction between both cell types, in order to orchestrate the different stages of cardiac remodeling. In particular macrophage is able to adapt their phenotype and activity according to the cytokine milieu present in the local cardiac environment. This phenomenon, known as macrophage polarization, contributes to the accumulation of pro-inflammatory M1 macrophages during the onset of cardiac remodelling, while also explaining the high levels of anti-inflammatory/profibrotic M2 macrophages found in the later stages of cardiac repair.

While the effects of macrophages on cardiac fibroblast activity have been extensively studied, the ability of cardiac fibroblasts to modulate macrophage behavior is less understood. LPS, and Heparan sulfate as pro-inflammatory stimulus, triggers on cardiac fibroblast ICAM-1 and VCAM-1 expression levels, which allow spleen mononuclear cells and neutrophils adhesion. LPS triggers high TNF- α /IL-10 ratio, whereas, TGF- β a profibrotic stimulus triggers an increase on ICAM-1 and VCAM-1 expression levels, but low TNF- α /IL-10 ratio. Consequently, cardiac fibroblast under LPS-treatment promote monocytes-macrophages M1 polarization. By contrast, cardiac fibroblast under TGF- β promote monocytes-macrophages M2 polarization. Our results demonstrate that cardiac fibroblasts interact with immune cells and contribute to monocyte recruitment and induce their differentiation to M1 or M2 macrophages.

Session 11: New Advances in the Pharmacology of Cancer

Abietane diterpenoids from *Plectranthus* spp. as Lead Protein Kinase C Modulators for Cancer Therapy

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Cancer is one of the highest causes of death worldwide. Protein kinase C (PKC) is a family of serine/threonine kinases divided into three groups according to their regulatory domain structure and cofactors requirement for activation: classical, novel, and atypical PKCs. Recently, PKC family isoforms have been the focus of intense research, and recognized as therapeutic targets in anticancer drug development. [1]. Diterpenoids are commonly found in the *Plectranthus* spp., and have a widespread spectrum of biological activity, namely anticancer properties [2]. The diterpenoid 7 α -acetoxy-6 β -hydroxyroyleanone (AHR) isolated from *P. grandidentatus* displays low cytotoxicity and the basic requirements approaches for the development of pharmaceutical formulations based on AHR as a lead. These AHR features includes an extraction optimization and structural and thermal properties characterization [3]. These features suggests that AHR can be used as a lead for drug development. Considering this, a small library of abietane derivatives was tested for their ability to activate PKC isoforms from classical (α , α ; β , β), novel (δ , δ ; ϵ , ϵ) and atypical (ζ , ζ) subfamilies, using a previously developed yeast-based screening assay to search for modulators of PKC isoforms [4]. The results obtained revealed potent activators of PKC family proteins, namely: a selective activator of PKC δ , the 7 α -acetoxy-6 β -benzoyloxy-12-O-benzoylroyleanone (Roy-Bz). The patented diterpenoid Roy-Bz was prepared using AHR as starting material. Roy-Bz potently inhibited the proliferation of colon cancer cells by inducing a PKC δ -dependent mitochondrial apoptotic pathway involving caspase-3 activation. The results indicate that Roy-Bz targets drug resistant cancer stem cells, in HCT116 colon cancer cells, preventing tumor dissemination and recurrence. Moreover, these findings support a tumor suppressive function of PKC δ in colon cancer. Overall, these results point to promising activators of PKCs with high potency and isoform-selectivity that may emerge from the exploitation of this new family of abietane diterpenoids [5]. Molecular docking studies are currently ongoing to further identify new selective abietane diterpenoids as new PKC modulators.

Novel biomarkers and therapeutic targets in clear cell renal cell carcinoma (ccRCC)

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Renal cell carcinoma (RCC) is the third most prevalent urological cancer. It accounts for approximately 4% of all new cancer cases and the incidence rates for all stages have been rising steadily over the last 3 decades. Over 65,400 new renal cell carcinomas (RCCs) are annually detected in the USA, and 15,000 people will die from the disease. The clear cell RCC (ccRCC) is the most aggressive and common subtype and accounts for approximately 80% of all renal cancers.

Commonly asymptomatic, most RCCs are discovered incidentally on medical imaging. A great percentage of them may be treated by surgery, but one third of patients will present either with locally advanced tumor or with metastases, resulting in a 95% mortality rate. Moreover, one third of organ-confined cancers treated by nephrectomy develop metastasis during the follow-up. Conventional therapies have little effect on survival in patients with metastatic RCC. Until 2008, the 5-year survival rate for untreated metastatic RCC (mRCC) was 2%, and the median survival was approximately 8 months. In this scenario, our group was focused on the identification of novel diagnostic and prognostic biomarkers that could, eventually, become novel therapeutic targets for ccRCC patients. We first found that HAVRc-1, also known as KIM-1 or TIM-1, was up regulated in ccRCC tumors (60%) and had a role in cellular de-differentiation, scattering- and proliferating-related processes.

HAVRc-1 ectodomain was detected in the urine of ccRCC patients confirming its value as a noninvasive biomarker for early detection of ccRCC and the follow-up of metastatic patients, that was correlating with tumor grade and invasiveness.

HAVRc-1 was expressed in ccRCC and papillary tumors and unexpectedly expressed in tubule cells of adjacent and distal unaffected counterparts of ccRCC suggesting that constitutive expression of HAVRc-1 in kidney could constitute a susceptibility trait for ccRCC tumor development. Clinical trials have confirmed this hypothesis since HAVRc-1 predicts RCC incidence up to 5 years prior to tumor diagnosis and constitutes a good marker for tumor relapse, after nephrectomy. We have also described that HAVRc-1 overexpression up-regulates IL-6 and activates the gp130/STAT-3/HIF-1 pathway in human ccRCC derived cell lines. This is an important pathway because: i) an IL-6 autocrine mechanism correlating with bad prognosis has been described in ccRCC; ii) STAT-3 regulates genes involved in tumor proliferation, apoptosis inhibition, immunosuppression and angiogenesis; iii) HIF-1A is a key element in promoting hypoxia-induced angiogenesis. pSTAT-3 activation studied in a tissue microarray (TMA) including 98 ccRCC patients with 5 to 10 years follow-up showed that pSer727-STAT3 is an independent prognostic factor of survival respect to classical clinic-pathologic features and constitutes a clinically relevant prognostic biomarker that correlates with ccRCC patient overall survival.

Development of targeted agents such as TKI inhibitors targeting angiogenesis and immunotherapies designed to block immune checkpoints such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD-1) has changed the choice of treatment for patients with mRCC. Although new therapies have improved the median survival period of patients with advanced ccRCC there are still many opportunities and challenges for non-responding patients or for those that develop resistance after treatment. We propose that HAVRc-1 and STAT-3 besides being good diagnostic and prognostic biomarkers might also represent novel targets for personalized treatment of ccRCC patients.

Sesquiterpene Lactones as Potential Agents against Cancer

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During the past decades natural products have played a crucial role in the drug discovery process and have contributed to the identification of key therapeutic targets. Among natural products sources, plants have been used as the basis of medicines for a long time even today plant-based systems continue to play an essential role in healthcare. Sesquiterpene lactones are naturally occurring terpenoids - found mainly in plants of Asteraceae family- which display a remarkable spectrum of biological activities. Sesquiterpene lactones modulate multiple cascades and specific signal transduction pathways that target selectively cancer cells. Due to their nature as alkylating agents the interest for these compounds was undermined for a long time. However, it is clear that the highly reactive α -methylene- γ -lactone group of specific sesquiterpene lactones does not react nonspecifically with any nucleophile group. Many sesquiterpene lactones have been described as cytotoxic compounds, cell invasion inhibitors and apoptosis inducers. This kind of cell death is considered to be an important response to most chemotherapeutic agents in cancer cells and it is mediated by molecular pathways that culminate in the activation of a conserved family of aspartate-specific cysteine proteases, known as the caspases. Sesquiterpene lactones have been described as activators of -at least- two fundamental apoptotic pathways, referred to as the extrinsic pathway and the intrinsic pathway. The extrinsic pathway is initiated with the activation of the tumor necrosis factor receptor superfamily, involved in the recruitment and activation of the initiator caspase-8 or -10, which activate the effector caspases (-3, -6 and -7). The intrinsic pathway involves permeabilization of the mitochondrial outer membrane by the activation of the pro-apoptotic Bcl-2 family proteins, cytochrome c release and caspase-9 activation, which cleaves and activates downstream caspases. Parthenolide is a sesquiterpene lactone that induces both the intrinsic and the extrinsic apoptotic pathways, downregulates the expression of genes involved in proliferation, survival and metastasis and has potential for the treatment of leukaemia. Artemisinin and thapsigargin analogues have shown potential anticancer effects. Interest in such compounds is not only as lead compounds to generate new more bioavailable and more potent cytotoxic chemical entities, but also as agents used in combination with other compounds capable of sensitizing cancer cells to chemotherapy. An important issue of sesquiterpene lactones is that they are able to induce cell death in cells that overexpress genes involved in chemoresistance and some analogues have reached the phase of clinical trials. I will illustrate the effects of specific sesquiterpene lactones isolated from endemic plants on cancer cells viability and the signal transduction pathways involved in cell death.

4.3. Oral Communications Index

- (O001)** Systemic Inflammation is reduced in Primary Hypercholesterolemia Patients after an Oral fat load administration. (Aida Collado)
- (O002)** Probiotics Prevent Hypertension in a Murine Model of Systemic Lupus Erythematosus Induced by TLR7 Activation. (Néstor De La Visitación)
- (O003)** Combination of Innovative Teaching Techniques in Pharmacology Seminar Classes. (M. Carmen Montesinos)
- (O004)** Information about Drugs and Related Topics in the Spanish general, Economic and Professional Press. (Gonzalo Casino)
- (O005)** Role of il-11 in Pulmonary Fibrosis Associated to Pulmonary Hypertension. (Inés Roger)
- (O006)** The Role of MUC1 in a Bleomycin induced Pulmonary Fibrosis Mouse Model. (Paula Montero Magalló)
- (O007)** Evaluation of Nrf2 Activators for the Treatment of Chronic Obstructive Pulmonary Disease (COPD). (Cristina Estornut)
- (O008)** Adipose Tissue Mesenchymal Stem Cell-Derived Extracellular Vesicles as a Biological Therapy in Osteoarthritic Cells. (Miguel Tofiño-Vian)
- (O009)** Targeting Soluble Epoxide Hydrolase to Improve Neurodegenerative Traits in SAMP8 Mouse. (Mercè Pallàs)
- (O010)** The Angiotensin-(1-7)/Mas Receptor Axis Attenuates Human Endothelial Cells Senescence through the activation of Klotho and Nrf2. (Alejandra Romero Martínez)
- (O011)** CM-728 as a Potential and Novel Compound for Metastatic Clear Cell Renal Cell Carcinoma (ccRCC) Treatment. (Jazmine Arévalo)
- (O012)** Preliminary Screening for Selective Anticancer Activity of Andalusian Plants. (Víctor Jiménez González)
- (O013)** Methadone is an Analgesic with low addictive Side Effects due to its Weak Potency on Opioid-Galanin Receptor Heteromers. (Verónica Casadó-Anguera)
- (O014)** Identification and Validation of two Small Molecules Targeting the IL-17 Inflammatory Pathway. (Elia Álvarez Coiradas)
- (O015)** Protective Effect of Phosphorus Dendrimers in a Murine Model of Multiple Sclerosis. (Valentín Ceña)
- (O016)** NLRP3 Inflammasome Inhibition Protects the Neurovascular Unit, Reduces Infarct Volume and Inflammation in Cerebral Ischemia. (Alejandra Palomino Antolin)
- (O017)** Cannabidiol Antidepressant-Like Effects in Rats: Decreased Sensitivity in Adolescent versus Adult Rats. (M. Julia García-Fuster)
- (O018)** Essential Role of C148-C227 Disulfide Bridge in Human 5-HT_{2A} Receptor Functionality and Trafficking. (Marta Cimadevila)

4.4.1. Oral Communications by Topics

(O001)

Systemic inflammation is reduced in Primary Hypercholesterolemia patients after an Oral fat load administration

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Introduction and Objectives: Primary hypercholesterolemia (PH) is a lipid disorder characterized by elevated levels of cholesterol and low-density lipoprotein (LDL). Low-grade systemic inflammation is associated with PH, which might explain the higher incidence of cardiovascular diseases. In this study, we have evaluated the effect of an oral fat load (OFL) on different immune parameters and its consequences in PH patients.

Methods: 22 PH patients' whole blood was analyzed by flow cytometry to determine platelet activation (P-selectin+ and PAC-1+), leukocyte activation (CD11b+ and CD69+) and the percentage of circulating platelet-leukocyte aggregates before and 4h after a treatment with a commercial liquid preparation of long-chain triglycerides ($\omega 6/\omega 3$ ratio $>20/1$, Supracal, Nutricia). The parallel-plate flow chamber was employed to study platelet-leukocyte and leukocyte adhesion to the arterial endothelium. Plasma inflammatory markers were determined by ELISA.

Results: Four hours after OFL, PH patients presented a lower percentage of activated platelets. The percentage of circulating eosinophils, type 1 monocytes, platelet- eosinophil, but not platelet-neutrophil aggregates was significantly decreased. Surprisingly, the percentage of regulatory T lymphocytes (Treg) was significantly increased. Moreover, a tendency in reduced percentages of platelet-monocyte (types 1 and 2) aggregates was also observed. Leukocyte adhesion to the dysfunctional endothelium (TNF α -stimulated) was significantly ameliorated together with plasma levels of CXCL4, sP-selectin, CXCL8, CCL2, CCL5 and TNF α .

Conclusion: After 4h OFL, PH patients have a decreased platelet activation state, circulating levels of different chemokines and soluble adhesion molecules as well as diminished platelet-leukocyte and leukocyte adhesion to the dysfunctional arterial endothelium. Therefore, OFL may become a powerful tool to dampen the systemic inflammation associated with PH and the development of further cardiovascular events. **Funding:** This work was supported by the Spanish Ministry of Economy and Competitiveness [grant numbers SAF2014-57845-R, SAF2017-89714-R]; Carlos III Health Institute and the European Regional Development Fund [grant numbers PI15/00082, PIE15/00013, PI18/00209].

(O002)

PROBIOTICS prevent hypertension in a murine model of systemic lupus erythematosus induced by TLR7 activation

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Objective: We tested whether *Lactobacillus fermentum* CECT5716 (LC40) and/or *Bifidobacterium breve* CECT7263 (BFM26) prevent hypertension, endothelial dysfunction and intestinal dysbiosis in an inducible model of systemic lupus erythematosus (SLE).

Materials and methods: Eight-week-old BALB/cByJrj mice were treated with 1.25 mg of the agonist of TLR7 Imiquimod (IMQ) for 8 weeks. At the same time, LC40 (109 UFC/mL) and BFM26 (109 UFC/mL) were gavaged daily.

Results: IMQ induced intestinal dysbiosis characterized by both reduction in the Firmicutes/Bacteroidetes ratio (F/B) and α -diversity measure by Chao-richness and numbers of species. LC40 and BFM26 could not restore these parameters. The three-dimensional principal component analysis of the bacterial taxa in faecal samples showed perfect clustering among groups (CTR and IMQ). The clusters corresponding to LC40 and BFM26 were more similar to IMQ. The short chain fatty acids (SCFA) producing bacteria were also analyzed, the level of acetate-producing bacteria was found elevated but neither of the probiotics restored it. BFM26 and LC40 were found set in the gut microbiota of animals treated respectively. LC40 and BFM26 treatments significantly reduced lupus disease activity assessed by plasma double-stranded DNA autoantibodies, as well as B cell populations in spleen, which were found elevated in the IMQ group. Both probiotics reduced the expression of IFN γ which was found elevated in the SLE model. Also, LC40 and BFM26 treatments reduced Th17 in mesenteric lymph nodes. However, only in the LC40-treated group Treg were elevated. Aortae from IMQ mice showed reduced endothelium dependent vasodilator responses to acetylcholine, which were normalized by both BFM26 and LC40. Furthermore, vascular ROS contents were increased in IMQ mice and reduced by both probiotic strains.

Conclusion: BFM26 and LC40 prevented the development of hypertension in this model, and normalized the endothelial function.

(O003)**Combination of innovative teaching techniques in Pharmacology seminar classes**

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Our main objective, besides promoting critical spirit, was to encourage the study of Pharmacology by relating the subject with real life situations. We proposed to combine two strategies applied successfully in our Department, problem-based learning [1] and peer evaluation, with the production of a video.

Based on a prior project consisting in the critical analysis of pharmacological news accessible through social networks [2], we addressed this strategy to students of Pharmacy Degree naive to the subject of Pharmacology (Year 3). Students were instructed to work in teams of 4 and provide a list of 6-10 internet links of news concerning drugs or treatments related to the year curriculum (Central Nervous System or Immunity) to the supervising teacher, who selected the one to be analyzed. Each team had to perform a critical analysis of the news item regarding its veracity and scientific rigor (supported by scientific evidence), giving an assessment of the interest and potential impact in comparison with the standard treatment or drug of reference. Their analysis was presented in a 5-minute video, which was peer-evaluated using a 5- point rubric during the seminar classes.

In general, the activity was well accepted, although some students preferred more structured conventional activities. Most students rated the activity high or very high in terms of promotion of critical spirit. The usefulness towards the learning of pharmacological concepts or to relate the subject with real life was considered mostly satisfactory. The best valued aspect was the interest of the news items. Among the aspects to improve, the most relevant is time-management, followed by the need of assistance by multimedia service of the university to expedite the viewing of the videos.

[1] Recio MC, et al. EDULEARN11 Proceedings (2011) pp. 2488-2495.

[2] Montesinos MC, et al. Basic Clin Pharmacol Toxicol (2018) 123 (Suppl. 2): 75.

(O004)

Information about drugs and related topics in the Spanish general, economic and professional press^aGonzalo Casino, ^aAndreu Prados, ^bElisabet Serés, ^bFèlix Bosch^aPompeu Fabra University.^bEsteve Foundation

Background News about drugs are relevant for citizens, health professionals and industry, but quantitative and qualitative studies are lacking. This study analyses the evolution, frequency and percentage of information about drugs in the Spanish general, economic and professional press, as well as the most relevant pharmacological topics.

Methods We conducted a content analysis of texts about drugs in 17 Spanish newspapers (13 general, 2 economical and 2 professional) identified in Factiva database for the period 2008-2017. We analysed frequencies, absolute and relative numbers of texts and headlines about drugs, and specifically about 10 pharmacological related topics: clinical trials; preclinical research; adverse effects; cancer; infections; antibiotics; resistances; generics; scientific journals and vaccines.

Results In the 2008-2017 period, the volume of texts about drugs decreased by 10%; the national general press published one article about drugs every two days and the regional one every three days; the economic, three in a week, and the professional, 14. In 10 newspapers analysed for 2017, the most covered topics about drugs (n=2,780) were cancer (n=255; 9.2%) and generics (n=152; 5.5%); the least covered was preclinical research (n=28; 1.0%). The press reported six times more on clinical than preclinical research. The economic press is the one that published the most about clinical (5.6%) and preclinical (2.1%) research. In 2017, the information about drugs represents 0.5% (n=822) of the whole information in the general press (n=178,926); 0.8% (n=468) in the economic press (n=55,800), and 8.5% (n=415) in the medical press (4,856).

Conclusions Information about drugs represents less than 1% of the whole information in general and economic press. The professional press publishes the most articles on drugs, followed by the economic and general press. Clinical research on drugs is more covered than preclinical. Cancer and generics are the most intensive covered topics.

Keywords: press, newspapers, news, drugs

(O005)

Role of il-11 in pulmonary fibrosis associated to pulmonary hypertension

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Background

Pulmonary hypertension (PH) in idiopathic pulmonary fibrosis (IPF) portends a poor prognosis. Currently, no therapy can improve survival of patients diagnosed with this disease. IL-11 molecular pathway is over-expressed in proliferative disorders however, its role in PH- associated IPF is unknown.

Objective

The aim of this study was to evaluate the expression of IL-11 in IPF patients with or without PH. Also we hypothesized that the stimulation of pulmonary artery smooth muscle cells (PASMCS) and human pulmonary artery microvascular endothelial cells (HMVEC-L) with IL-11 induced the transformation into invasive myofibroblast.

Methods

Human pulmonary artery rings, parenchyma tissue, broncho-alveolar lavage (BAL) and serum were obtained from control subjects (n=32), IPF (n=26) and IPF with PH patients (n=22) to study the expression and predominant distribution of IL-11 and IL-11R α . The effect of recombinant human IL-11 on pulmonary artery remodeling was evaluated in isolated PASMCS and HMVEC-L.

Results

IL-11 and IL-11R α were over-expressed in pulmonary arteries, parenchyma, serum and BAL of IPF patients with PH and in a lesser extend in IPF patients compared with control subjects. The immunostaining and immunofluorescence revealed a predominant distribution of IL11 and IL-11R α in remodeled pulmonary arteries of IPF patients and in a greater extend in IPF with PH patients and no expression in control subject. IL-11 induced morphological changes in isolated PASMCS and HMVEC-L characterized by myofibroblast phenotype.

Conclusions

IL-11 and IL-11R α are over expressed in pulmonary arteries of IPF + PH patients contributing to pulmonary artery remodeling. Pharmacologic modulation of this route may be a promising target for the treatment of this disease.

(O006)

The role of MUC1 in a bleomycin induced pulmonary fibrosis mouse model

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Background

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and irreversible form of fibrotic interstitial lung disease. MUC1, a membrane-bound O-glycoprotein, is considered as oncogenic molecule by altering signaling pathways involved in cellular processes related to IPF. In previous studies we have observed an up-regulation of MUC1 and its phosphorylated forms in IPF lung tissue, as well as the in vitro involvement of its cytoplasmatic tail (MUC1-CT) in IPF characteristic cellular processes.

Objective

To analyze the implication of MUC1 in IPF by the use of a bleomycin (BLM)-induced lung fibrosis MUC1-Knock Out (KO-MUC1) mouse model.

Methods

The influence of MUC1 in pulmonary fibrosis was evaluated by the difference in survival rate, lung function and tissue remodeling between BLM-induced pulmonary fibrosis WT and KO-MUC1 mice. Masson trichrome staining, micro-CT-SPECT-PET analysis and right ventricular hypertrophy index were performed. Expression of IPF markers was analysed from lung tissue by Real Time-PCR, Western Blotting and immunohistochemistry.

Results

Mortality was improved in MUC1-KO mice. WT BLM treated animals showed increased Penh values that were significantly lower in the MUC1-KO BLM group. WT BLM treated mice showed higher collagen deposition and lesser air spaces than MUC1-KO mice. Right ventricular hypertrophy and pulmonary metabolism were increased in WT BLM treated mice. By contrast, pulmonary circulation was impaired in WT BLM group. Expression of recognized fibrotic markers and mediators were induced by BLM in lung tissue from WT mice, whilst MUC1-KO animals maintained a minimal elevation. In addition, similarly to previous results, MUC1-CT phosphorylated forms were upregulated after BLM administration.

Conclusions

MUC1-CT and its phosphorylated forms are increased in BLM-induced pulmonary fibrosis mouse model. Unlike WT mice, KO-MUC1 mice are protected against IPF, improving lung function, inflammation and fibrotic lung tissue remodeling. Therefore, pharmacologic targeting of MUC1 may be a promising option for the treatment of IPF.

(O007)

Evaluation of NRF2 activators for the treatment of chronic obstructive pulmonary disease (COPD)

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Background

Chronic Obstructive Pulmonary Disease (COPD) is a progressive inflammatory lung disease primarily caused by chronic exposure to cigarette smoke. Oxidative stress is one of the principal mechanisms involved in the physiopathology of the disease. Nuclear Factor Erythroid 2-related (Nrf2) is critical in protection against oxidative stress by inducing expression of antioxidant enzymes and a significant decrease in its expression has been observed in COPD patients.

Objective

The aim of this study was to characterize the effects of Bardoxolone, Omavexolone and Obacunone as antioxidant drugs in COPD.

Methods

Peripheral blood neutrophils from COPD and healthy volunteers and Primary Human Bronchial Epithelial cells were pre-incubated with the cited drugs at different concentrations and stimulated with cigarette smoke extract (CSE). Expression of antioxidant genes, cytokine release, GSH levels and apoptosis were measured by RT-PCR, ELISA, luminescent assay and flow cytometry, respectively.

Results

Expression assays using lung tissue and neutrophils from COPD and healthy volunteers showed a negative correlation between the expression of antioxidant genes and the severity of the disease. After stimulation with CSE and drug treatment, cells displayed an increase in expression of antioxidant genes, as well as, an inhibition in the release of inflammatory cytokines. In addition, GSH assays showed that Bardoxolone, Omavexolone and Obacunone were able to activate Nrf2 with EC50 values of 6.4nM, 15.3nM and 38.7μM, respectively. Moreover, these drugs were highly effective in apoptosis inhibition.

Conclusions

Bardoxolone, Omavexolone and Obacunone show a huge antioxidant response against CSE-induced COPD by Nrf2 activation. Thus, these drugs may represent a promising therapeutic option in COPD.

(0008)

Adipose tissue mesenchymal stem cell-derived extracellular vesicles as a biological therapy in osteoarthritic cells

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INTRODUCTION: Osteoarthritis (OA) is a joint condition associated with articular cartilage loss, low-grade synovitis and alterations in subchondral bone and periarticular tissues. In the last years, the possible therapeutic application in OA of adipose tissue-derived mesenchymal stem cell (ASC)-extracellular vesicles (EVs) has aroused considerable interest among the scientific community. In this work, we have integrally assessed the immunomodulatory and antisenescent dose-dependent properties of ASC microvesicles (MV) and exosomes (EX) in human chondrocytes (OAC) and osteoblasts (OB) from OA patients.

METHODS: ASC were isolated from fat of patients who undergone abdominoplasty, and were cultured with appropriate media supplemented with EV-free serum. EV were isolated from AD-MSC conditioned medium by differential centrifugation and characterized by resistive pulse sensing, mass spectrometry and electron microscopy. OAC and OB were isolated from knee specimens of advanced osteoarthritis patients, stimulated with IL-1 β (10 ng/mL) and treated with MV (3.6x10⁷ particles/mL) or EX (7.2x10⁷ particles/mL) for 24h. Then, inflammatory, oxidative and senescence markers were measured by several methods such as ELISA, radio-immune assay, fluorometry and confocal microscopy. Data was analysed by ANOVA followed by Dunnett's test.

RESULTS: Both in OAC and OB, EVs were able to downregulate the levels of IL-6, TNF α , PGE₂, MMP activity and NO production, and stimulated the production of the anti-inflammatory IL-10. In OAC, EVs stimulated the production of collagen II, an effect tied to the presence of annexin-A1 in MVs. In OBs, EVs reduced several senescence makers such as lipid peroxidation, inner mitochondrial layer potential and β -galactosidase activity.

CONCLUSION: Administration of EV may have potential pharmacological applications to control inflammatory processes, extracellular matrix degradation and intracellular remodelling in osteoarthritic cells. In this regard, we propose ASC-derived EVs a putative biological therapy for chronic inflammatory conditions such as OA.

(0009)

Targeting soluble epoxide hydrolase to improve neurodegenerative traits in SAMP8 mouse

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Aging is the most important risk factor of neurodegenerative disorders and is associated with cognitive impairment and dementia. Recent studies demonstrate that inflammation is a key event of the transition of normal aging to progressive neurodegeneration. Soluble epoxide hydrolase (sEH) is a key enzyme in the arachidonic acid (AA) cascade. Epoxyeicosatrienoic acids (ETTs) demonstrated anti-inflammatory among others biological effects. sEH hydrolyses ETTs to dihydroxyeicosatrienoic acids (DHETs) that loss beneficial effects. Our interest was to test a new family of sEH inhibitors (sEHi), UB-EV-52, with a new chemical structure, comparing with TPPU and AS-2586114, a well known sEHi, in a murine model of cognitive decline associated with aging, the senescence accelerated mice prone 8 (SAMP8).

Neuroprotective effects of 5mpk UB-EV-52, AS-2586114 and TPPU in 5-months-old SAMP8 were evaluated. Drugs were administered in drinking water for 4 weeks. UB-EV-52, TPPU and donepezil treated SAMP8 improved cognitive impairment (novel object recognition test) compared to SAMP8 control (SAMP8-Ct) mice group. Likewise, a reduction in neuropathological markers such as APP, sAPP α and $-\beta$, ADAM10 and BACE1, sAPP levels and abnormal tau hyperphosphorylation (Ser396 and Ser404) by Western blot were determined in treated SAMP8. Furthermore, changes in gene expression of inflammatory and oxidative stress markers such as Il-6, Cxcl10, Ccl3 and iNOS, Aox1 and Aldh2 were found in SAMP8 mice treated with sEHi compared to SAMP8-Ct. In addition, analysis of cytokine levels of IL-1 β and TNF- α and hydrogen peroxide levels by ELISA showed a significant reduction in treated SAMP8 mice. Finally, a cellular thermal shift assay (CETSA) was performed to verify ex vivo target engagement of UB-EV-52 and TPPU in SAMP8 brain.

In conclusion, we demonstrated that sEHi might be an effective treatment for neurodegenerative disorders associated with aging leading to an improved cognitive impairment, reducing the neuroinflammation and oxidative stress.

Financial support from Ministerio de Economía y Competitividad (Projects SAF2016-33307 and SAF2017-82771) is acknowledged. S. C., R. L. and D.P-I. thank the Universitat de Barcelona (APIF grant), Ministerio de Educación, Cultura y Deporte (FPU program) and the IBUB, respectively, for PhD Grants.

(O010)

The angiotensin-(1-7)/Mas receptor axis attenuates human endothelial cells senescence through the activation of klotho and Nrf2.

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Background and aims: Endothelial cell senescence, is one of the major mechanisms contributing to vascular aging. It is characterized by cell growth arrest and the acquisition of a senescence-associated secretory phenotype (SASP), which predisposes to vascular disease. Here, we explored the capacity of Angiotensin (Ang)-(1-7), a member of renin-angiotensin system (RAS), to counteract human umbilical vein endothelial cell (HUVEC) senescence triggered by RAS-dependent and – independent stressors, such as Angiotensin (Ang) II or interleukin (IL)-1 β . Besides, we aimed to identify intracellular pathways mediating its potential protective actions.

Methods: Cultured HUVEC were stimulated with Ang II (100nM) or IL-1 β (2,5 ng/ml) for 18h. Endothelial cell senescence was assessed by positive senescence-associated β -galactosidase staining (SA- β -gal+) and by total and telomeric DNA damage. Protein levels, leukocyte adhesion to HUVEC monolayers and IL-6 secretion were determined by Western blot, flow chamber assays and ELISA, respectively.

Results: Both Ang II (100 nM) and IL-1 β enhanced the fraction of SA- β -gal+ cells, the expression of ICAM-1 and VCAM-1 adhesion molecules, leukocyte adhesion and IL-6 secretion, used as markers of the SASP. Ang-(1-7) (100 nM) counteracted the pro-senescence action elicited by both Ang II and IL-1 β through a mechanism that was blocked by the Mas receptor antagonist, A779 (1 μ M). Ang-(1-7) also increased endothelial klotho levels, while klotho silencing blunted the anti-senescence capacity of the heptapeptide. Both Ang-(1-7) and recombinant klotho (1 nM) activated the cytoprotective Nrf2/heme oxygenase-1 (HO-1) pathway. HO-1 inhibitor tin protoporphyrin IX (1 μ M) prevented the anti-senescence evoked by Ang-(1-7) or recombinant klotho.

Conclusions: The present study identifies Ang-(1-7) as a tool to protect against human endothelial cells senescence through the consecutive activation of klotho and the Nrf2/HO-1 axis. Developing new Ang-(1-7)/Mas axis activators or mimetic drugs may prove useful to counteract endothelial cell senescence and premature vascular aging.

(O011)**CM-728 as a potential and novel compound for metastatic clear cell renal cell carcinoma (ccRCC) treatment.**

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Clear cell renal cell carcinoma (ccRCC) is the most prevalent and lethal histological subtype of renal cell carcinoma (RCC). If detected early, partial or radical nephrectomy is the first-line treatment with an associated 5-year survival rate of 90%. In contrast, as ccRCC is generally asymptomatic, most patients are diagnosed in a more advanced and metastatic stage of the disease, for which the 5-year survival rate drops to 12%. At present, the first-line treatments for metastatic ccRCC are antiangiogenic agents that only stabilize the disease and are only effective in less than 50% of patients. One of the most prescribed is Sunitinib malate (Sutent®), a tyrosine kinase inhibitor (TKI) which acts partly by preventing the activation of the signal transducer and activator of transcription 3 (STAT3). Interestingly, an abnormal STAT3 activation has been correlated with poor prognosis and low overall survival in ccRCC patients. In consequence and based on the partial effectiveness of first-line treatments for advanced metastatic ccRCC, CEAMED SA is developing the compound CM-728 that has been shown to effectively inhibit STAT3 activation in several tumor cell lines. Therefore, the aim of this work was: i) to determine the sensitivity of human ccRCC derived cell lines (769-P, 786-O) to CM-728, ii) to analyze the effect of CM-728 on STAT3 activation (pTyr705) in these cell lines and, iii) to compare the effect of CM-728 with the first-line treatment Sunitinib, and with established JAK/STAT pathway inhibitors. Our results indicate that both human ccRCC derived cell lines tested (769-P and 786-O) are more sensitive to CM-728 than to Sunitinib and that CM-728 is a strong inhibitor of STAT3 activation in these ccRCC cell lines. In conclusion, our results suggest that CM-728 may have potential, as a novel compound for the treatment of metastatic ccRCC.

(O012)

Preliminary screening for selective anticancer activity of Andalusian plants.

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According to the WHO data, lung cancer (along with bronchus and trachea cancers) is the sixth leading cause of global deaths in the last years. These cancers kill almost 2 million people every year. Despite the recent approval of new anticancer therapies, the tolerable doses of the current treatments are not yet enough to kill all cancer cells without not killing the normal health cells. As consequence, the treatments delay the progression of cancer but not the fatal ending. Therefore, it is necessary to find more selective anticancer drugs. Since plant kingdom is an important source of useful anticancer drugs, as paclitaxel used for treatment of lung cancer, we have started a random screening for selective anticancer activity of plants collected in Andalusia. Andalusia is autonomous community in southern Spain of high plant diversity and endemism. In this work, we show a preliminary results for selective anticancer activity of 32 extracts from 30 plants collected in western Andalucía. Using lung cancer cells A549 and non-malignant skin cells HaCaT, we demonstrated that some extracts were more cytotoxic and selective against cancer cells than the standard anticancer drug cisplatin. The highest anticancer selective activity was found on *Daphne gnidium* and *Thymelaea hirsuta*, two plants from Thymelaeaceae family. Further studies are needed to understand and evaluate the potential anticancer of these plants.

(O013)

Methadone is an analgesic with low addictive side effects due to its weak potency on opioid-galanin receptor heteromers

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mu-Opioid receptor (MOR) agonists offer the most effective treatment for severe pain, making the search for a non-addictive opioid medication a high priority in medical sciences. MOR is a G protein-coupled receptor (GPCR) that is essential for opioid-induced analgesia, but also responsible for adverse effects, including respiratory depression, reduced gastrointestinal motility and euphoria that can lead to addiction. Thus, MORs mediate both the analgesic and addictive effects of opioids, responsible of the well-known opioid epidemic, which represents a severe public health crisis. The neuropeptide galanin acts as a modulator of neurotransmission in the CNS and in the peripheral nervous system, acting on three subtypes of GPCRs (Gal1R, Gal2R and Gal3R). Galanin is co-expressed with different neurotransmitters and co-released by the major ascending noradrenergic, serotonergic, histaminergic and cholinergic systems. Biochemical and behavioral studies also indicated the functional presence of galanin and galanin receptors in dopaminergic areas, including the ventral tegmental area (VTA) and the nucleus accumbens (NAc), where they mediate an antagonistic effect on opioid reward. Here, we have found a significant pharmacodynamic difference between methadone versus morphine and fentanyl that entirely depends on the heteromerization of MOR with Gal1R, rendering a profound decrease in the potency of methadone. This explains methadone's weaker ability to activate the dopaminergic system as compared to morphine and fentanyl and predicts a dissociation of therapeutic versus euphoric effects of methadone, that preferably binds to peripheral MOR which does not form heteromers with Gal1R. In conclusion, these results suggest that MOR-Gal1R heteromers mediate the dopaminergic effects of opioids and that opioids with selective low potency for the these heteromers, such as methadone, may have lower addictive side effects.

(O014)

Identification and validation of two small molecules targeting the IL-17 inflammatory pathway

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Interleukin 17 (IL-17) is a proinflammatory cytokine that not only plays a pivotal role in host defence against extracellular pathogens, but also acts as an immune checkpoint in several autoimmune diseases. As a result, the IL-17 family has become an attractive pharmacological target for inflammatory autoimmune diseases such as psoriasis, for which therapeutic antibodies have been approved for clinical use.

Targeting IL-17 pathway with small molecules is feasible, although both novel methods and novel chemical matter are needed. We employed a virtual screening of our chemical library of 60 000 compounds to identify 67 potential ligands against IL-17 available structures. Then, we developed a biophysical label-free assay based on Dynamic Mass Redistribution technology, which allows the detection of the interaction between proteins and small molecules. We immobilized the extracellular domain of IL-17RA and we screened the ligands identified before in the label-free assay. 2 molecules sharing a common chemical scaffold were found active as potential binders with micromolar binding affinity and were confirmed by a Surface Plasmon Resonance assay. The functional activity of these ligands as inhibitors of the IL-17A-IL17RA interaction was first tested by a cytokine CXCL1 release assay based on blocking IL-17A proinflammatory stimulation of HT-29 colorectal cancer cells. Confirmation of this biological activity was tested in a keratinocyte cell line (HaCaT), showing that both compounds inhibit IL-17A proinflammatory activity as they decrease the release of CCL20 and IL-8.

Combining biochemical and cell-based assays with structure-based design from this novel chemotype can facilitate the identification of compounds functionally targeting IL-17 inflammatory pathway. Further characterization of the efficacy of these molecules can facilitate their progress as preclinical therapeutic agents for psoriasis.

(O015)**Protective effect of phosphorus dendrimers in a murine model of multiple sclerosis**^aValentín Ceña, ^aInmaculada Posadas, ^aLaura Romero-Castillo^aUniversidad de Castilla-La Mancha.

PROTECTIVE EFFECT OF NEUTRAL PHOSPHORUS DENDRIMERS IN A MURINE MODEL OF MULTIPLE SCLEROSIS

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Background.

Inflammation is a physio-pathological process raised in response to different exogenous and endogenous stimulus. Following the recognition of these stimuli, inflammatory cells including macrophages and lymphocytes, activate several pathways allowing the inflammatory response to eliminate the disturbing element, and resolve the inflammation. However, in certain pathologies, inflammation is not resolved resulting in chronic inflammatory diseases such as arthritis rheumatoid or multiple sclerosis. Phosphorus dendrimers have recently emerged as interesting immunomodulatory nanoparticles able to modulate macrophages polarization and reduce the peripheral inflammatory response.

Material and Methods.

Cortical neurons or astrocytes were obtained from C57BL/6j mouse embryonic fetuses 17 days or from 1-5 days old pups, respectively. Lymphocytes were obtained from spleen and thymus from adult C57BL/6j mice. Lack of toxicity was studied by MTT, LDH and proliferation assays.

Lymphocytes-derived cytokines were determined by ProcartaPlex Multiplex immunoassay. Experimental Autoimmune Encephalitis (EAE) was induced by standard procedures sensitizing the mice with a MOG peptide. The animal's clinical score was determined using a generally accepted scale ranging from 0 to 5.

Results.

Phosphodendrimers (generations 3 and 4) displayed a good security profile in all cells studied. Both prevented CD3: CD28-induced cytokine production in a concentration dependent-manner without affecting lymphocytes viability. G3b and G4b dendrimers significantly prevented MOG-induced disability in the experimental model of EAE, displaying a potency similar to the reference compound fingolimod.

Conclusion.

Neutral phosphorous dendrimers display promising features for the treatment of autoimmune diseases.

Supported by MINECO (grant SAF2017-89288-R to VC).

(O016)

NLRP3 inflammasome inhibition protects the neurovascular unit, reduces infarct volume and inflammation in cerebral ischemia.

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Cerebral ischemia is the third cause of death and the main cause of adult disability worldwide. Currently the only pharmacological treatment for acute ischemic stroke is intravenous tissue plasminogen activator (tPA). However, only 10% of patients benefit from tPA administration, due to its limited therapeutic window and the risk of intracerebral hemorrhage. Inflammation in ischemic injury is crucially mediated by NLRP3, a key component of immune system. In this study, we investigated the role of NLRP3 in post-ischemic inflammation, using MCC950, a potent inhibitor of NLRP3 inflammasome. For that purpose, we used transient middle cerebral artery occlusion (tMCAO) during 1 hour in mice as a model of cerebral ischemia. Administration of MCC950 1h after reperfusion reduced infarct volume in a dose-dependent manner (1, 3, 10 mg/kg). As a clinical outcome parameter, MCC950 at 3 mg/kg improved neuro-motor function and reduced expression of different pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6), chemokines (CCL2) and NLRP3 inflammasome components (pro-caspase-1, NLRP3, pro-IL-1 β). We observed that tMCAO produced Blood Brain Barrier (BBB) disruption that was improved in animals treated with MCC950 (3 mg/kg). In MCC950-treated animals, we observed a functional recovery of endothelial proteins that forms the tight junctions of BBB (VE-cadherina, Claudin-5, ZO-1), together with a reduction of the expression of adhesion molecules (ICAM, VCAM) and matrix metalloproteases (MMP9), that leads to a lower peripheral immune cells infiltration in the infarction area (Ly6G+ neutrophils, leukocytes CD45^{high}, microglia CD11⁺, Ly6C⁺ macrophages). From these results we can conclude that inhibition of NLRP3 inflammasome with MCC950 significantly reduces infarct volume and improves neuro-motor function and protects the neurovascular unit by improving the BBB integrity through stabilization of the tight junctions. Hence, the inhibition of NLRP3 may be a promising target in cerebral ischemia.

(O017)

Cannabidiol antidepressant-like effects in rats: Decreased sensitivity in adolescent versus adult rats^aM. Julia García-Fuster, ^aRubén García-Cabrerizo, ^aCristian Bis-Humbert^aIUNICS, University of the Balearic Islands, and IdISBa.

Cannabidiol is a non-psychoactive phytocannabinoid with great therapeutic potential in diverse psychiatric disorders, however its beneficial effects have been mainly ascertained in adult rats. This study aimed at comparing the antidepressant-like effects induced by cannabidiol in adolescent and adult rats and the possible parallel modulation of hippocampal neurogenesis. Male Sprague-Dawley rats were repeatedly treated with cannabidiol (3, 10 and 30 mg/kg) or vehicle (1 ml/kg) during adolescence (postnatal days, PND 27-33) or adulthood (PND 141-147) and exposed to 3 consecutive tests, 2-3 days apart (forced-swim, open field, sucrose preference) that quantified different aspects of affective-like behavior (behavioral despair, anxiety- and hedonic-like responses). Cannabidiol induced differential effects depending on the age and dose administered when compared to control rats: (1) cannabidiol (30 mg/kg) decreased body weight only in adult rats following 6 (-34±15 g, p<0.05) and 7 (-40±15 g, p<0.05) days of treatment; (2) cannabidiol improved behavioral despair in adolescent and adult rats, but with a different dose sensitivity (10 vs. 30 mg/kg), and with a different extent (2 vs. 21 days post-treatment: -59±19 sec immobile, p<0.05 and -55±20 sec immobile, p<0.01 respectively); (3) cannabidiol did not modulate anxiety-like behavior at any dose tested in adolescent or adult rats; and (4) cannabidiol induced prohedonic-like effects in adult rats following 10 mg/kg (+50±18 g, p<0.05) and 30 mg/kg (+32±5 g, p<0.05). Our findings support the notion that cannabidiol exerts antidepressant-like (improved behavioral despair and prohedonic) and anorexigenic-like effects in adult rats, and demonstrate a decreased sensitivity when administered in adolescent rats. Moreover, since cannabidiol did not modulate hippocampal neurogenesis (cell proliferation and early cell survival) in adolescent or adult rats, the results revealed potential antidepressant-like effects induced by cannabidiol without the need of regulating hippocampal neurogenesis. Supported by 'Delegación del Gobierno para el Plan Nacional sobre Drogas' (2016/002, MSSSI) and by RTA-RD16/0017/0010.

(O018)

Essential role of C148-C227 disulfide bridge in human 5-HT_{2A} receptor functionality and trafficking

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G Protein Coupled Receptors extracellular domains are emerging as a determining factor in receptor functionality, not only for orthosteric ligands, but also as an allosteric modulation site (Wooley MJ, et al., 2017). The recent crystallization of serotonin 2A (5-HT_{2A}) receptor showed a critical role of extracellular loop 2 (ECL-2) in receptor selectivity. This finding is in agreement with previous studies, where dithiothreitol was used to nonspecifically break a conserved disulfide bridge between C148 at transmembrane domain 3 (TM-3) and C227 at ECL-2 (Iglesias A, et al. 2017).

Our hypothesis is that the C148-C227 disulfide bridge in 5-HT_{2A} receptor may be critical for ligand binding, receptor activation and trafficking. Thus, we aimed to generate three constructions incapable of maintaining this bridge and study their binding, function and subcellular expression.

The two cysteines involved in the aforementioned disulfide bridge were mutated into alanines, obtaining three constructs: pcDNA5/FRT/TO myc-5-HT_{2A}C148A-eYFP (C148A), pcDNA5/FRT/TO myc-5-HT_{2A}C227A-eYFP (C227A) and pcDNA5/FRT/TO myc-5-HT_{2A}C148A/C227A-eYFP (C148A/C227A). These three constructions, together with the parental one (pcDNA5/FRT/TO myc-5-HT_{2A}eYFP, WT) were employed to generate four stable cell lines that were characterized by means of binding and functional assays. For subcellular localization studies, cell nucleus and the endoplasmic reticulum were stained with fluorescent probes, and images were obtained in Operetta High Content Imaging System (Perkin Elmer).

When compared with WT cell line, C148A, C227A and C148A/C227A mutants were unable to bind [³H]LSD and to respond to (±)DOI neither when measuring IP₃ accumulation, nor calcium mobilization. Furthermore, all the mutants showed around a 50% decrease of expression of receptor in the cell membrane.

In conclusion, these results show that the disulfide bridge formed between TM-3 and ECL-2 is critical to maintain human 5-HT_{2A} receptor proper conformation for ligand binding and receptor activation, but also to facilitate receptor trafficking to cell membrane.

4.5. Posters Index

Posters Session 1

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(P003) Apoptosis induction by an α -Bromoacryloylamido Indolyl-Pyridinyl-Propenone Derivative on Human Melanoma Cells. (Irene Rodríguez)

(P004) Signal Transduction Pathways involved in the Stimulation of Melanogenesis by Melatonin in the Human Melanoma Cell Line SK-MEL-1. (Juan Perdomo)

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Posters Session 2

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(P042) Essential Role of C148-C227 Disulfide Bridge in Human 5-HT_{2A} Receptor Functionality and Trafficking. (Marta Cimadevila)

(P043) The IUPHAR/BPS Guide to PHARMACOLOGY in 2019: new Features and Updates. (Michael Spedding)

(P044) Functional Differences between Dopamine D_{4.4} and D_{4.7} Receptor Variants within the Dopamine D₄-Adrenergic α _{2A} Receptor Heteromer in the Brain. (Verónica Casadó-Anguera)

Posters Session 3

Pharmacology of Pain and Inflammation

(P045) Lupine (*Lupinus angustifolius* L.) Peptide Ameliorates the Non-Alcoholic Steatohepatitis in Mice. (Ana Lemus Conejo)

(P046) Development of a Translational In Vitro Assay for Iatrogenic Neuropathic Pain. (Antón Leandro Martínez Rodríguez)

(P047) Adenosine A2B Receptor Involvement in Skin Protection: from Epidermal Barrier Homeostasis to Regulation of Inflammation. (Asunción Marín Castejón)

(P048) CCL11/CCR3 Axis is involved Atherosclerosis Development. (Elena Domingo)

(P049) Osteostatin Down-Regulates Osteoclast Differentiation. (Josep Nacher Juan)

(P050) A Capsaicin-Based TRPV1 Soft Antagonist as an Anti-Pruritic Drug Candidate. (Laura Butrón García)

(P051) Evaluation of Wheat Gluten Protein Hydrolysates on Inflammation and Oxidative Stress in Primary Human Monocytes. (M^a Carmen Millán-Linares)

(P052) A Cellular Study of Different Methods for Lipoaspirate Processing in the Surgical Treatment of Dysphonia. (M^a José Vazquez)

(P053) In Vitro Characterization of a TRPV1 Soft Antagonist. (Magdalena Nikolaeva Koleva)

(P054) Modulation of the Chondrocytic Inflammatory Response by Formononetin. (María Guillán-Fresco)

(P055) Hydroxytyrosol and its Acetylated Derivatives Prevent M-CSF/RANKL-Induced Osteoclastogenesis in Human Monocytes. (María Angeles Rosillo)

(P057) Dietary Oleuropein and its New Acyl-Derivate, attenuate Murine Lupus Nephritis through HO-1/Nrf2 Activation And Suppressing JAK/STAT, NF- κ B and MAPK Signaling Pathways. (María Luisa Castejón Martínez)

(P058) Mitochondrial Na⁺/Ca²⁺ exchanger (NCLX) Participates in NLRP3 Inflammasome Activation. (Paloma Narros Fernández)

(P059) Characterization and Evaluation of Hemp Protein Hydrolysates On Neuroprotection. (Sergio Montserrat De La Paz)

(P060) Oleocanthal Modulates LPS-induced Murine Peritoneal Macrophages Activation Via Regulation Of Inflammasome and Nrf-2/HO-1 Signaling Pathways. (Tatiana Montoya García)

(P067) Antiinflammatory Effect of Osteostatin in Monosodium Urate Crystal-Induced Gouty Arthritis Model. (Laura Catalán)

Teaching

(P061) Promoting in- and out-class engagement in a subject of Pharmacology. (Ana M. Sahagun)

(P062) Flipped Classroom based on Objective Structured Clinical Examinations Analysis by Undergraduate Students of Pharmacology course from the Podiatry Degree improve their Learning And Assessment Communication Skills about Medicines. (Inmaculada Bellido Estevez)

(P063) Preventing Online Cheating with Snowl in a subject of a Master Degree: a Pilot Study. (M Jose Diez)

(P064) Service-Learning, a good Methodology for doing Final Degree And Master's Projects. (M^a Luisa Ferrándiz)

(P065) Robotics Applied to Research and Teaching of Pharmacology: Opinions of Students of Different Degrees. (M^a Nélica Fernández)

(P066) Use of Experimental Animals in Research and Teaching of Pharmacology: Opinions of Students of Different Degrees. (Matilde Sierra)

4.5.1. Posters by Topics

Cancer

(P001)

New findings in molecular mechanisms responsible of the antitumoral in vitro effect of CM728 in MDA-MB-231 cell line

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Background:

Triple negative breast cancer (TNBC) is an aggressive kind of breast cancer. At present, there are no drugs aimed at specific targets in this disease so it is necessary to keep investigating to find new options of treatment. Here, we study the effect of CM728, a naphthoquinone derivative synthesized by CEAMED, in TNBC. Previous results, demonstrate the antiproliferative in vitro effect of CM728 in TNBC through cell cycle arrest and induction of apoptosis. Furthermore, it activated MAPK pathway and increased ROS levels.

Results:

The growth inhibition and decrease of cell viability caused by CM728 were shown to be produced in a dose and time dependent manner. At 48 hours, 250 nM of CM728 produced a significant effect on cell proliferation but not on cell viability which indicated a cytostatic effect of the compound. At higher doses, CM728 provoked both effects. Pulse-exposed experiments showed that 15 minutes of exposition to CM728 (1 μ M) were enough to induce the same effect on these events than the produced by 48 hours.

N-acetyl cysteine (NAC) avoided the effect of CM728 on cell proliferation and viability. Catalase completely prevented the death but just partially the growth inhibition in response to the compound. This data suggested the implication of ROS on the phenotypic changes induced by CM728.

CM728 provoked caspase-3 and -8 cleavage and activation which indicated the involvement of the extrinsic pathway in the activation of apoptosis. The effect on caspase -9 was not clear. The pan-caspase inhibitor, z-VAD-FMK, confirmed the implication of caspases in the apoptotic effect of CM728.

Conclusions:

The molecular mechanisms of action of CM728 that lead to growth inhibition and cell death occur into the first 15 minutes of exposition to the compound through ROS regulation. Caspases -3 and -8 are implicated in the activation of apoptosis produced by CM728.

(P002)**Apoptosis induction and activation of the mitogen-activated protein kinase pathway in human U-937 leukaemia cells by the synthetic flavanone 6-methoxy-2-(naphthalen-1-yl)-chroman-4-one.**

^aFrancisco Estévez, ^aEster Saavedra, ^aHenoc Del Rosario, ^bIgnacio Brouard, ^cJudith Hernández-Garcés, ^cCelina García, ^aJosé Quintana, ^h, ⁱ

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Chalcones (1,3-diphenyl-2-propen-1-ones) are biosynthetic precursors of flavonoids and some of them are potential anticancer agents. In this communication we report the synthesis of a new series of chalcones and their corresponding flavanones as well as their antiproliferative activity against the human tumour cell line U-937. This series of chalcone derivatives was characterized by the presence of a naphthalene ring as the second aryl system - which was kept unaltered. The structure-activity relationship of these chalcone derivatives and their corresponding cyclic compounds was investigated by the introduction of different substituents (methyl, methoxy, benzyloxy, chlorine) or by varying the position of the methoxy or benzyloxy groups on the A ring. The chalcone containing the methoxy group at 5' position of the A ring and its corresponding flavanone were the most cytotoxic compounds, with IC50 values of $2.8 \pm 0.2 \mu\text{M}$ and $1.3 \pm 0.2 \mu\text{M}$, respectively. Synthetic flavanone was as cytotoxic as the antitumor agent etoposide against human leukaemia cells, but human peripheral blood mononuclear cells were more resistant than leukaemia cells to the cytotoxic effects of the flavanone. This compound induced (i) G2-M cell cycle arrest, (ii) apoptosis which was blocked by overexpression of the anti-apoptotic protein Bcl-2, and (iii) phosphorylation of p38 MAPK, extracellular-signal regulated kinases and c-jun N-terminal kinases / stress-activated protein kinases (JNK/SAPK) following different kinetics. Moreover, cell death was attenuated by the inhibition of mitogen-activated extracellular kinases and JNK/SAPK and was independent on reactive oxygen species generation.

(P003)

Apoptosis induction by an α -bromoacryloylamido indolyl-pyridinyl-propenone derivative on human melanoma cells

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World Health Organization estimates that proximately 132,000 new cases of melanoma skin cancer are diagnosed globally each year, and the incidence of this kind of disease has been increasing over the past decades. It has been proved that mutations that transform healthy cells into cancer cells usually involve the inactivation of apoptosis, a physiological process responsible of programmed cell death. Naturally occurring compounds or their semisynthetic derivatives represent a realistic option in the fight against the disease. Among them, chalcones have received considerable attention due to their potential anticancer properties. We have previously described a chalcone containing two aromatic heterocyclic rings (indole and pyridine moieties) and an α -bromoacryloylamido moiety at 5'-position of the indole nucleus which displays a potent antiproliferative activity against several cancer cell lines. In this communication we wish to disclose the effect of this chalcone on human melanoma cells viability. We found that this compound is highly cytotoxic (IC50 value of 130 ± 26 nM) and a potent apoptotic inducer, it induces activation of multiple caspases, poly(ADP-ribose)polymerase cleavage and upregulation of TRAIL, DR4 and DR5.

(P004)

Signal transduction pathways involved in the stimulation of melanogenesis by melatonin in the human melanoma cell line SK-MEL-1

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Melatonin is a methoxyindole synthesized from tryptophan that is present in almost all living organisms, including plants, and exhibits a variety of biological properties. Previous studies on human melanoma cell line SK-MEL-1 have revealed that melatonin, at concentrations higher than the observed at the night-time in the blood, reduces the cell growth and stimulates melanin production and tyrosinase activity, the key-enzyme involved in the biosynthesis of melanin, via a receptor-independent mechanism. In the present study, the cells were incubated with melatonin at different time points and the role of diverse signalling pathways that are usually involved in melanogenesis was evaluated. The immunoblotting analysis indicates that the wnt/ β -catenin pathway is not activated by melatonin since levels of cytosolic β -catenin were similar in all experimental groups. In contrast, GSK-3 β seems to play an important role since melatonin reduced the amount of phospho-GSK-3 β and the pre-treatment of the cells with specific inhibitors of the kinase (LiCl or BIO) significantly blocked the expression and activity of tyrosinase. Inhibition of the PI3K/AKT pathway using the PI3K inhibitor Ly294002 stimulated melanogenesis in SK-MEL-1 cells, and the effect was abrogated by the inhibitors of GSK-3 β . Together, these results suggest that melatonin activates melanogenesis via PI3K/AKT/GSK-3 β pathway. To evaluate the role of cAMP/PKA pathway the effect of a PKA inhibitor (H-89) was also investigated. Interestingly, H-89 alone had no influence on melanogenesis, however in combination with melatonin enhanced the levels and activity of tyrosinase. Although melatonin is a well-known antioxidant, it also induces reactive oxygen species in some human tumour cells, including SK-MEL-1 cells. Stress oxidative seems to be an important signal in triggering melanogenesis since N-acetyl-cysteine reduced both the levels and activity of tyrosinase in response to melatonin. These results reinforce the concept that regulation of melanin synthesis results from a cross talk between several signalling pathways.

(P005)

Effect of *Sideritis hyssopifolia* on cell cycle distribution in human prostate cancer PC-3 cells

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Introduction: Prostate cancer is the most common cancer diagnosed in men in Europe. *Sideritis hyssopifolia* may be a possible candidate as chemopreventive for prostate cancer due to its antioxidant effects. The aim of the study was to evaluate the effects of the ether, methanol and chloroform extracts, obtained from the aerial parts of this plant on cell cycle in human prostate cancer PC-3 cells.

Material and Methods: PC-3 cell line were seeded (1.5x10⁵ cells/well) in 2 mL of culture medium. After 24h, the cells were treated with the extracts and incubated at 37°C for 72h. Cells harvested by trypsinization were collected, centrifuged and resuspended in cold PBS. To determine cell cycle distribution, cells were incubated for 15 min in the dark with Igepal CA-630 (1%), RNase (10 mg/mL) dissolved in sodium acetate (0.01 M at pH 5.2) and propidium iodide (1mg/mL). Cells were quantified by flow cytometry using Summit 4.3 software, followed by data analysis using Flowing Software 2.5.1.

Results and Conclusions: The results were expressed as the percentage of cells in subG0/G1 (apoptotic cells), G0/G1, S and G2/M phases of the cell cycle. The treatment with the extracts induced cell accumulation in the subG0/G1 phase: 2.83% in the control group, 8.41%, 7.39% and 12.31% in ether, methanol and chloroform extracts (significant differences, $p \leq 0.05$), respectively. A reduction in the percentage of cells in the phases G0/G1, S and G2/M phases, respectively, was also observed: 67.49, 10.87 and 17.31% in the control group; 70.09, 7.93 and 12.03% in ether extract; 65.55, 11.30 and 14.36% with methanol extract and 56.59, 13.66 and 15.80% with chloroform extract (no significant differences, $p \leq 0.05$).

(P006)

Mechanisms of Resistance to Egfr-Targeted Therapy induced by Nicotine in Human Lung Cancer^aMaría Extremera Mazuela, ^aAnna Bordas Sánchez, ^aCarmen Montiel López^aDepartment of Pharmacology and Therapeutics. School of Medicine. Universidad Autónoma de Madrid.

Cigarette smoking is not only correlated with the onset and progression of a variety of human tumors, including lung cancer, but continued smoking after cancer diagnosis is known to reduce the effectiveness of conventional chemotherapy and EGFR-targeted therapy. Certain nicotinic acetylcholine receptor (nAChR) subtypes expressed in lung tumor cells play an essential role in the acquisition of the above resistance to anti-tumor drugs after their activation by certain tobacco-specific constituents, like nicotine. Although the mechanism implicated in the nicotine-induced resistance to conventional chemotherapy has been widely investigated, the mechanism that underlies resistance to EGFR-targeted therapy remains unexplored. Here, by using a combination of molecular biology, biochemical, pharmacological and flow cytometry techniques, we evaluate the nicotine effect on cell viability, apoptosis, cell cycle distribution and gene expression levels of several nAChR subunits in the human lung adenocarcinoma cell line HCC827 (EGFR mutated) exposed to erlotinib or osimertinib (72 h). Our results show that nicotine reduces the cytotoxicity of erlotinib ($IC_{50} = 0.153 \pm 0.01 \mu M$ vs. $1.93 \pm 0.26 \mu M$; $p \leq 0.05$) and osimertinib ($IC_{50} = 3.87 \pm 0.10 nM$ vs. $20.06 \pm 0.10 nM$; $p \leq 0.05$) through the inhibition of their apoptotic effects. Moreover, nicotine partially prevents the erlotinib-mediated reduction of the cell fraction in the S phase. Both drugs up-regulate the expression of most of the nAChR subunits tested, including the $\alpha 7$, leading to an increase in the number of nicotine binding sites in the tumor cells. Taken together, our results identify various mechanisms by which nicotine causes tobacco-mediated resistance to EGFR-targeted therapy in lung cancer. Although further studies will be required to elucidate the nAChR subtype responsible of the above nicotine effects, our findings may be clinically relevant and increase our understanding of the mechanisms involved in tobacco-mediated resistance to new cancer-targeted therapy.

(P007)

Natural occurring sesquiterpene lactone Spiciformin induces apoptosis and activation of mitogen-activated protein kinase pathway on human tumour cell lines.

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Sesquiterpene lactones are naturally occurring compounds that have attracted considerable attention because to their vast array of biological activities. Many of them exhibit cancer cell cytotoxicity and are promising anticancer agents. Spiciformin is a sesquiterpene lactone belonging to the group of germacranolides which is obtained in good yield from several Canary Islands endemic plants. In this communication we wish to disclose the effects of spiciformin and its acetyl derivative on cell viability of human leukaemia and melanoma cell lines.

Spiciformin and its acetate inhibit the proliferation of two human leukaemia and one melanoma cell lines (U-937, HL-60 and SK-MEL-1). The naturally occurring sesquiterpene lactone was also cytotoxic against Bcl-2-overexpressing cells, but it was less cytotoxic against human peripheral blood mononuclear cells. Cell growth inhibition triggered by both sesquiterpene lactones does not appear to be mediated by alterations in the cell cycle. However, both sesquiterpene lactones were able to increase the percentage of apoptotic cells (hypodiploid) and also led to the exposure of phosphatidylserine on the outside of the plasma membrane as detected by annexin V-FITC staining. Spiciformin acetate is a fast apoptotic inducer in U-937 cells and this effect was prevented by the pan-caspase inhibitor z-VAD-fmk and by the specific caspase inhibitors against caspase-3/7, caspase-8 and caspase-9. Cell death was associated with a decrease in the mitochondrial membrane potential, cytochrome c release, PARP cleavage and a downregulation of the anti-apoptotic factor Bcl-2. In addition, spiciformin and its acetate induced the activation of MAPK-pathway, including c-Jun N-terminal kinases/stress-activated protein kinases and p38 MAPK and generation of reactive oxygen species.

This study was prepared with the support of FEDER and Agencia Canaria de Investigación, Innovación y Sociedad de la Información (PROID2017010095 FEDER/ACIISI).

Cardiovascular**(P008)****The adipokine visfatin induces endothelial dysfunction through TLR-4 and NLRP3 inflammasome activation.**

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Introduction: Visfatin is a multi-faceted adipokine whose expression and secretion are enhanced in obesity. It has been suggested that visfatin can promote vascular inflammation and endothelial dysfunction through its enzymatic Nampt activity.

Aims: At present, it remains mostly unknown whether visfatin directly promotes vascular complications *in vivo*, as well as the possible mechanisms involved.

Methods: 4 month-old C57BL/6 mice were exposed for 7 days to osmotic minipump infusion of saline, visfatin (100 ng/kg/day), and/or the specific Nampt inhibitor FK866 (2,4 mg/kg/day). Some animals also received the blocker of TLR4 receptors CLI-095 (3 mg/kg/day), the inhibitor of NLRP3-inflammasome expression MCC950 (10 mg/kg on days 2, 4, and 6), or the IL-1-receptor antagonist anakinra (AK; 100 mg/kg on days 4, 5, and 6). After end-point, endothelium-dependent and independent relaxations to acetylcholine (ACh; 0.1 nmol/L to 1 μ mol/L) or sodium nitroprusside (SNP; 1 nmol/L to 1 μ mol/L), respectively, were studied in isolated mesenteric microvessels. Cultured human umbilical endothelial cells (HUVEC), were exposed to visfatin (50 ng/mL) to analyze its effects on the expression of phosphorylated-p65, an indicator of NF- κ B activation, as well as the proteins NLRP3-inflammasome and pro-interleukin(pro-IL)-1 β , either with or without FK866 (10 μ mol/L), CLI-095 (1 μ mol/L), or MCC950 (1 μ mol/L).

Results: The microvessels obtained from visfatin-treated mice showed endothelial dysfunction to ACh but not changes to SNP. This endothelial dysfunction was blunted when the animals received also FK866, CLI-095, MCC950, or anakinra. In HUVEC, visfatin enhanced p-p65, NLRP3-inflammasome, and pro-IL-1 β , these effects being antagonized by FK866 and CLI-095.

Conclusion: We propose that visfatin produce endothelial dysfunction in mice by a TLR4-mediated pathway involving NLRP3-inflammasome expression and through the paracrine production of IL-1 β . Interestingly, the vascular deleterious effects by visfatin were prevented by CLI-095 and anakinra. Indeed, targeting IL-1-receptors or TLR4 may represent therapeutic strategies to treat and/or prevent obesity-related vascular dysfunction.

(P009)

Utilization of cardiovascular medicines in Castilla y Leon in 2011-2017

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Cardiovascular disease remains the leading cause of death in Castilla y Leon, and represents a major population burden. Practice guidelines emphasize primary and secondary prevention of cardiovascular disease with medication to prevent future adverse cardiac events and mortality. The increasing age of population may also be related to a higher use of this group of drugs. The objective of the present study was to define the trends in the outpatient utilization and expenditures for cardiovascular drugs in this autonomous region. Data on outpatient drug utilization in Castilla y Leon for the period 2011-2017 were used for this study (Concylia database). Anatomical Therapeutic Chemical (ATC) classification was followed to group drugs. Patterns of drug consumption and expenditures were described for the different cardiovascular drug groups (group C, cardiovascular system): number of units prescribed, Defined Daily Doses (DDD) per 1000 inhabitants per day (DID), overall cost, and cost per treatment per day. Consumption of group C (cardiovascular system) increased over the period studied in terms of number of units prescribed (2.7%) and Defined Daily Doses (DDD) per 1000 inhabitants per day (DID) (6.2%), whereas both overall cost and cost per treatment per day diminished (16.5% and 11.9%, respectively). Agents acting on the renin-angiotensin system (subgroup C09) and lipid modifying agents (subgroup C10) account for most of cardiovascular drugs consumed in 2017 (65.0% of the units dispensed in group C; 69.4% of DID; and 80.2% of both overall cost and cost per treatment per day). Cardiovascular drug utilization rised slightly in Castilla y Leon during the period studied (number of units prescribed and DID), dropping at the same time treatment costs. The highest consumption is mainly concentrated in two subgroups: agents acting on the renin-angiotensin system and lipid modifying agents.

(P010)**P144, an anti-TGF- β signaling peptide does not prevent aortic aneurysm in Marfan syndrome.**

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Introduction: Marfan syndrome (MFS) is characterized by the formation of ascending aortic aneurysms. MFS is caused by mutations in the protein fibrillin-1 (FBN1), which affects the integrity of connective tissue elastic fibers and causes a dysfunction in the transforming growth factor (TGF- β) signaling (1). Recent data from mouse model of MFS suggest that aortic-root enlargement is caused by excessive signaling by TGF- β , which can be mitigated with angiotensin II-receptor blocker losartan. Additionally, angiotensin II – receptor blockade induces a significant decrease in TGF- β signaling (2, 3). In the research project, we studied the use of anti-TGF- β peptide as a therapeutic approach for MFS-associated ascending aorta aneurysm.

Methods: All experiments were performed in MFS Fbn1C1039G/+ or wild-type mice. Losartan was prepared at 0.6 g/L in drinking water and P144 was injected at 2.72×10^{13} genome copies/mice. Two-dimensional transthoracic echocardiography was carried out using an ultrasound scanner (Vivid Q, GE Healthcare, Madrid, Spain) with a 10–13 MHz phased array linear transducer (IL12i GE Healthcare, Madrid, Spain) to determine the diastolic aortic root diameter. Verhoeff-Van Gieson Staining was performed to quantify the elastic fibers breaks in paraffin-embedded ascending aorta sections.

Results: The results shown that P144 decreases the number of elastic fibres breaks, but not ameliorates the aortic aneurysm progression in MFS mice. As previously reported, losartan significantly reduces both the number of elastic fibers breaks and the aortic root dilatation. Therefore, P144 results suggest that TGF- β hypersignaling seems not to be the main responsible for the aortic root dilatation in MFS mice.

Conclusions: The anti-TGF- β peptide P144 does not prevent or mitigate aortic aneurysm progression in MFS and therefore it is not suitable as therapeutic tool.

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(P011)

Soluble dipeptidyl peptidase 4 triggers endothelial cell senescence: which role for thromboxane A2 (TXA2)?

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Introduction. The soluble form of dipeptidyl peptidase 4 (sDPP4) has been identified as a novel adipokine, whose serum concentrations are upregulated in obese patients. We have previously shown that sDPP4 may have a direct deleterious effect on the vasculature, promoting endothelial dysfunction. In endothelial cells, sDPP4 upregulates TXA2 release which induces vascular smooth muscle cell contractility and defective vasorelaxation. In the present study, we aimed to investigate whether TXA2 mediates other deleterious effects evoked by sDPP4 in the vascular wall. We focused on the direct effect of sDPP4 to promote endothelial senescence, as an important hallmark of vascular ageing that has been related to endothelial dysfunction and atherosclerosis.

Methods. In human umbilical vein endothelial cells (HUVEC), senescence was assessed by senescence associated- β -galactosidase staining (SA- β -Gal+). The expression of the pro-senescence protein p53, the DNA-damage indicator γ H2AX and interleukin-(IL)-1 β , as a component of the senescent-associated secretory phenotype (SASP), were determined by Western Blot.

Results. sDPP4 (200 ng/ml) increased SA- β -Gal+ staining in a concentration-dependent manner and induced the expression of both γ H2AX, p53 and IL-1 β . Such effects seemed to be dependent on DPP4 enzymatic activity, since they were attenuated by the DPP4 inhibitors K579 (100 nM) and linagliptin (10 nM). In addition, the specific proteinase-activated receptor (PAR)-2 antagonist GB83 (10 μ M), the COX-2 antagonist celecoxib (3 μ M) and the antagonist of the TP receptor SQ-29,548 (10 μ M) prevented the cell senescence evoked by sDPP4. The TXA2 stable analogue U46619 (1 μ M) mimicked the effects of sDPP4, increasing SA- β -Gal+ staining and γ H2AX via TP receptor.

Conclusion. Here we identified sDPP4 as capable of directly promoting endothelial senescence via PAR-2 receptor and TXA2 formation. Our results present TXA2 as mediating not only sDPP4-induced endothelial dysfunction but also endothelial senescence. sDPP4 and TXA2 therefore arise as pharmacological targets for the treatment of vascular ageing.

(P012)

5-HT₂ receptor blockade modifies serotonergic responses on the vascular sympathetic neurotransmission in experimental diabetes

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It is well established that 5-HT system inhibits vascular sympathetic neurotransmission, and 5-HT₂ receptor antagonism potentiates this 5-HT sympatho-inhibition. 5-HT₂ receptors are associated with platelet aggregation, vasoconstriction, adrenaline release and tachycardia, playing an important role in the pathophysiology of diabetes. The aim of this study was to evaluate whether 5-HT₂ receptor blockade, in experimental diabetes, modifies the vascular sympathetic neurotransmission and the 5-HT influence. Diabetes was induced by alloxan (150 mg/kg, s.c.) in male Wistar rats and maintained for 4 weeks. Diabetic rats were treated for 2 weeks with sarpogrelate (5-HT₂ receptor antagonist) in drinking water (30 mg/kg.day; sarpogrelate-treated group) or nothing (control group). Glycaemia and body weight (BW) were monitored periodically. After 4 weeks of diabetes induction, rats were anaesthetized and pithed. Electrical stimulation of the sympathetic outflow from the spinal cord (0.1, 0.5, 1.0 and 5.0 Hz; 27.5 ± 2.5 V) resulted in frequency-dependent increases in mean blood pressure (MBP), without modifying heart rate (HR). After 4 weeks, glycaemia was slightly different in diabetic sarpogrelate-treated group compared with control group (446.2 ± 12.4 mg/dl and 487.5 ± 12.3 mg/dl, respectively), while BW was similar (347 ± 7 g and 357 ± 3 g, respectively). Additionally, MBP or HR were not modified by sarpogrelate treatment vs control in diabetic pithed rats (56.9 ± 0.8 mmHg and 297 ± 3 bpm in sarpogrelate-treated group; 58.5 ± 3.1 mmHg and 290 ± 9 bpm in control group). However, 5-HT₂ blockade decreased the vasoconstrictions induced by sympathetic stimulation in diabetic pithed rats. Continuous infusion of 5-HT (1, 10 and 80 μ g/kg.min; i.v.) exerted a higher degree of inhibition of sympathetic outflow in diabetic rats receiving sarpogrelate treatment. In conclusion, oral treatment with sarpogrelate reduces the vascular noradrenergic neurotransmission, potentiating the sympatho-inhibition evoked by 5-HT in diabetic rats. Therefore, 5-HT₂ receptor blockade may open a new pharmacological target treating cardiovascular disorders such as diabetes.

(P013)**Changes in gut microbiota composition induced by losartan are involved in its antihypertensive effects**

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Objective: Hypertension is associated with gut dysbiosis. We aimed to evaluate the effects of the angiotensin receptor blocker losartan on gut microbiota in spontaneously hypertensive rats (SHR), and to test if this modification contributes to its blood pressure (BP) reducing properties.

Materials and methods: Twenty-weeks old Wistar Kyoto rats (WKY) and SHR were assigned to three groups: untreated WKY (WKY), untreated SHR (SHR), and SHR treated with losartan for 5 weeks (SHR-losartan). A faecal microbiota transplantation (FMT) experiment was also performed by gavage of faecal content from donor SHR-losartan group to SHR recipient.

Results: Faeces from SHR showed gut dysbiosis, characterised by higher Firmicutes/Bacteroidetes ratio, lower acetate- and higher lactate-producing bacteria, and lower strict anaerobic bacteria, which was restored by losartan. The improvement of gut dysbiosis was linked to higher colonic integrity and lower sympathetic drive in the gut. In contrast, hydralazine reduced BP but it did neither restore gut dysbiosis nor colonic integrity. Interestingly, FMT from SHR-losartan to SHR reduced BP, improved the aortic endothelium-dependent relaxation to acetylcholine and reduced NADPH oxidase activity. These vascular changes were linked to both increased Treg and decreased Th17 cells population in the vascular wall.

Conclusions: Losartan treatment reduced gut dysbiosis in SHR. This effect seems to be related to its capacity to reduce sympathetic drive in the gut, improving gut integrity. The changes induced by losartan in gut microbiota contributed, at least in part, to protect the vasculature and reduce BP, possibly by modulating gut immune system.

(P014)**Consumption of statins in the province of Zamora (2013-2017)**

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Statin prescription has increased during the last years in all developed countries, as hypercholesterolemia is one of the most common modifiable risk factors among population, and the treatment with this pharmacological group helps reduce cardiovascular events. The aim of this study was to characterize the statin consumption pattern over a 5-year-period (2013-2017) in the province of Zamora, which has a high rate of ageing population. Data regarding consumption of the chemical subgroup C10AA (HMG CoA reductase inhibitors) (Anatomical Therapeutic Chemical classification) in this province were obtained from Concyliat database (Department of Health of Castilla y Leon). Results were expressed as number of units dispensed, total cost, Defined Daily Doses (DDD) per 1000 inhabitants per day (DID), and cost per treatment per day. The highest increase in the 5 years studied is reported for DID (20.9%), from 114.124 DID in year 2013 to 137.979 DID in 2017, being more moderate for the number of units dispensed (8.5%). Regarding the costs of treatment, the overall costs have hardly changed during the period studied (1.5%), whereas the cost per treatment per day did 7.8% (from 44.14€ to 47.56€, respectively), evidencing the control on spending carried out the last years. Atorvastatin was the most frequently prescribed drug in this subgroup, representing approximately 56% of DID and 53% of the cost per treatment per day for C10AA in 2017. Statin consumption increased in the province of Zamora during the last five years, especially in terms of DID, being this increase more moderate for the number of units dispensed, overall cost and cost per treatment per day. Atorvastatin was the most consumed active ingredient.

(P015)

Experimental hypertension modifies the 5-HT receptor/s inhibiting mesenteric noradrenergic outflow

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Mesenteric vasculature plays a pivotal role in regulating cardiovascular homeostasis. Structural and functional alterations in the mesentery have been evidenced to contribute to the hypertensive process, in which sympathetic overdrive is a key player. Our group has already shown that serotonergic system downregulates noradrenergic neurotransmission in the rat mesenteric vascular bed, via 5-HT₁ receptor activation. In this work, we investigated whether hypertension alters the 5-HT-induced modulation of mesenteric sympathetic neurotransmission. Hypertension was induced in male Wistar rats by N-nitro-L-arginine methyl ester (L-NAME) administration (30 mg/kg per day; 21 days) in drinking water. Rats were anesthetized and prepared for the in situ autoperfused rat mesentery, monitoring their systemic blood pressure (SBP), heart rate (HR), and mesenteric perfusion pressure (MPP). Electrical stimulation of perivascular nerves from the superior mesenteric artery (SMA) resulted in frequency-dependent increases in MPP, which were higher in hypertensive rats than in normotensive rats at all frequencies tested (16.0 ± 1.5 , 41.4 ± 3.5 and 90.1 ± 3.6 mmHg in hypertensive rats versus 9 ± 1.6 , 25.7 ± 3.9 and 60.2 ± 5 mmHg in normotensive animals for 2, 4 and 8 Hz and, respectively), without altering SBP or HR. 5-HT (1-25 µg/kg) and cisapride (5-HT₄ agonist; 1-25 µg/kg) i.a. bolus inhibited the vasopressor responses by mesenteric sympathetic nerves electrical stimulation, unlike i.a. bolus of agonists 5-carboxamidotryptamine (5-HT_{1/7}), α-methyl-5-HT (5-HT₂) and 1-PBG (5-HT₃) (25 µg/kg each). Pretreatment with selective 5-HT₄ receptor antagonist, GR 125487 (1 mg/kg, i.v.) blocked cisapride- and 5-HT-induced mesenteric sympathoinhibition. In conclusion, hypertension modifies serotonergic modulation: 5-HT₄ receptors inhibit noradrenergic neurotransmission in the in situ autoperfused mesentery of hypertensive rats. Thus, acting on noradrenergic overactivity in the mesenteric vasculature through serotonergic system might establish novel pharmacological strategies for the development of new antihypertensive drugs.

(P016)

PCSK9 inhibition, a potential target to reduce cardiovascular events in patients with familial hypercholesterolemia

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Introduction and Objectives: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme ubiquitously expressed in many tissues and cell types. It binds to LDL-C receptor and promotes its degradation. Gain-of-function mutations in PCSK9 gene are found in Familial Hypercholesterolemia (FH) and PCSK9 inhibitors are used to reduce LDL levels when other treatments are not effective. Given that FH has been associated to a systemic inflammatory state and the development of cardiovascular disease (CVD), we have investigated the consequences of PCSK9 inhibition in the FH inflammatory state.

Material/Methods: Human umbilical arterial endothelial cells (HUAEC) were stimulated with TNF α (20 ng/mL) for 1, 4, 24 or 48h. PCSK9 mRNA expression was determined by qRT-PCR and protein expression by flow cytometry, western-blot and immunofluorescence. Targeted siRNA for PCSK9 in HUAECs was performed and the parallel-plate flow chamber was employed to study mononuclear cell adhesion to HUAEC. Chemokines were determined by ELISA in cell supernatants.

Results: TNF α induced increased PCSK9 mRNA expression in HUAEC at 24h and protein expression 48h later. Silencing of PCSK9 resulted in a dramatic reduction of mononuclear cell adhesion to the dysfunctional arterial endothelium. This effect was partly due to decreased expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and CXCR6 expression. Furthermore, decreased release of inflammatory chemokines (CXCL8, CXCL16, CCL2 and CX3CL1) were found in siRNA PCSK9 HUAEC stimulated with TNF α .

Conclusions: This is the first evidence detecting a functional role of PCSK9 expression in HUAEC. These results also suggest that inhibition of PCSK9 might constitute a promising therapeutic target to control the inflammatory state and prevent further cardiovascular events in FH patients.

Funding: This work was supported by the Spanish Ministry of Economy and Competiveness [grant numbers SAF2014-57845-R, SAF2017-89714-R]; Carlos III Health Institute and the European Regional Development Fund [grant numbers PI15/00082, PIE15/00013, PI18/00209].

(P017)**Sodium-glucose cotransporter-2 inhibition reduces abdominal aortic aneurysm in mice.**

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Background: Abdominal aortic aneurysm (AAA) is a vascular degenerative disease characterised by a local dilatation of the abdominal aorta. Since AAA is associated with high morbidity and mortality in male over 65 years old, new effective treatments are needed to prevent AAA pathogenesis. Empagliflozin is a selective inhibitor of sodium-glucose cotransporter-2 (SGLT-2) used to treat type 2 diabetes. Recent studies have shown that SGLT-2 inhibitors could have beneficial effects in the treatment of different cardiovascular and metabolic diseases such as myocardial infarction, atherosclerosis and obesity. However, the effect of SGLT-2 inhibition in AAA remains unknown.

Objective: The aim of this study is to evaluate the effect of oral treatment with empagliflozin on AAA induced by angiotensin-II (Ang-II) infusion for 28 days in apolipoprotein E knockout (apoE^{-/-}) mice.

Approach and Results: Ang-II infused apo E ^{-/-} mice developed AAA (p<0.05). By contrast, oral cotreatment with the SGLT-2 inhibitor, empagliflozin, reduced Ang-II-induced AAA formation in apoE^{-/-} mice (p<0.05). It also decreased macrophage infiltration, neovessel formation and matrix metalloproteinase-2, matrix metalloproteinase-9, chemokine CCL2 [(C-C motif) ligand 2], CCL5 [(C-C motif) ligand 5] and vascular endothelial growth factor (VEGF) expression (p<0.05) in suprarenal aortic walls of apoE^{-/-} mice infused with Ang-II.

Conclusion: SGLT-2 inhibition reduces dissecting AAA formation induced by Ang-II in apoE^{-/-} mice and may constitute a novel therapeutic strategy to prevent AAA progression in humans.

This study was supported by grants PI15/00082, PIE15/00013, PI18/00209 and SAF2017-89714-R from the Carlos III Health Institute and co-financed by the European Regional Development Fund (FEDER).

Clinic Pharmacology

(P019)

Study in vitro of PDE4 as a possible therapeutic target in cutaneous fibrosis

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Background

Fibrosis is defined as increased proliferation of fibroblasts and deposition of extracellular matrix (ECM) with possible clinical manifestations, including organ dysfunction. Cutaneous fibrosis is considered to be the result of abnormal repair in response to tissue damage. The PDE4 inhibitor, Roflumilast, has shown to reduce numerous functions of human lung fibroblasts in in vivo studies, such as chemotaxis, collagen gel contraction, proliferation, fibroblast to myofibroblast transition (FMT) and ECM generation. However, roflumilast has not been tested with human dermal fibroblasts.

Objective

To study the involvement of PDE4 in cutaneous fibrosis.

To explore the mechanisms which explain the effect of Roflumilast in cutaneous fibrosis and its role in tissue remodeling in different cellular models.

Methods

The influence of PDE4 in cutaneous fibrosis was evaluated by PCR and co-immunofluorescence of cutaneous tissue from human healthy/cutaneous fibrosis patients. The mechanisms which explain the effect of Roflumilast in cutaneous fibrosis and its role in tissue remodeling was evaluated in vitro by co-immunoprecipitation and western blot in keloid primary fibroblasts, Normal Human Dermal Fibroblasts (NHDF) and Normal Human Epidermal Keratinocytes (NHEK).

Results

Expression assays and co-immunofluorescence with p ERK1/2 have shown that, PDE4A, PDE4B and PDE4D isoforms are overexpressed in keloid tissue vs healthy skin. Furthermore, pERK1/2 increases its expression in keloid tissue. Roflumilast decreases the expression of collagen type 1 and alfa-SMA, FMT markers, and also decreases the expression of pSMAD2/3 and pERK1/2. In addition, we confirm the interaction of PDE4B and pERK1/2 thanks to co-immunoprecipitation and its decrease after treatment with Roflumilast.

Conclusion

PDE4 is involved in the pathogenesis of cutaneous fibrosis and the PDE4B isoform is the most overexpressed. This could lead to the study of a more specific pharmacological target of said isoform. Roflumilast mitigates the tissue remodeling characteristic of fibrosis and also acts through the pERK1/2 and pSMAD2/3 pathways.

Drug Safety and Toxicology

(P020)

Analysis of drug-drug interactions in a nursing home in León

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Due to the progressive aging of the population, the drug safety for the elderly are gradually gaining attention. The aim of this study was to assess the occurrence of drug-drug interactions (DDIs) and the association between the number of drugs and DDIs in a nursing home of León (Spain) with 330 residents. Sex and age differences were also assessed. Bot Plus[®] software was used to classify interactions. The study showed that the mean number of medications per resident was 6.9 ± 3.1 . 76.4% of the residents suffered some type of DDIs, with an average of 3.4 per person. Of these DDIs, 50% had a moderate level of evidence, in 40% the evidence of DDIs was theoretical, 8% showed a major level of evidence, and 2% were those for which administration should be spaced. Residents were classified in non-polymedicated (< 5 drugs), minor polymedicated (5-9 drugs), and major polymedicated (> 9 drugs) to assess DDIs prevalence. DDIs with theoretical and moderate level of evidence were present in 83.8% and 83.0% of major polymedicated residents. Significant differences were found between DDIs with theoretical, moderate and major level of evidence and the 3 groups of polymedicated residents ($p < 0.001$, test χ^2). Regarding gender, significant differences were also found between all DDIs groups except for those in which administration should be spaced. Finally, with respect to age, significant differences were found between those DDIs with moderate and major level of evidence and those groups of oldest age: 75-84 years ($p < 0.001$ and $p = 0.005$ respectively, test χ^2) and ≥ 85 years ($p < 0.001$ in both cases, test χ^2).

Endocrinology**(P021)****ROR α receptor expression is increased in visceral fat from severe morbid obesity patients with diabetes.**

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Background: Obesity is a chronic multifactorial disease that constitutes a major health problem worldwide, with associated cardiovascular risk and increased morbidity and mortality. The retinoic acid receptor-related orphan receptor alpha (ROR α) is a member of the nuclear receptor superfamily involved in many pathological and physiological processes. However, the role of ROR α in obesity is not well known and we believe that it deserves further investigation.

Objective: In the present study we investigated the expression of ROR α in the visceral and subcutaneous fat tissue of patients with severe morbid obesity and its relationship with the development or not of diabetes.

Methods: We have analysed by immunohistochemical, Western Blot and RT-PCR studies the expression levels of ROR α in both visceral and subcutaneous adipose tissue explants of 43 patients with severe morbid obesity which underwent bariatric surgery (Roux-en-Y gastric bypass).

Results: RT-PCR and Western Blot analysis have shown that ROR α at both mRNA and protein level are significantly increased in visceral fat compared to subcutaneous fat ($p < 0.05$) in patients with severe morbid obesity. In addition, the expression ROR α is increased in the visceral fat of diabetic patients compared with the visceral fat of non-diabetic patients ($p < 0.05$). Immunohistochemical studies have shown that ROR α is located mainly in endothelial cells and visceral fat lymphocytes.

Conclusions: Although more functional studies are necessary, this preliminary results suggests that ROR α could play an important role in severe morbid obesity and represent a possible pharmacological target for the treatment of diabetes.

This study was supported by grants PI15/00082, PIE15/00013, PI18/00209 and SAF2017-89714-R from the Carlos III Health Institute and co-financed by the European Regional Development Fund (FEDER).

(P022)

Obesity-induced hypogonadism: Pathophysiological roles of a novel hypothalamic miRNA/Kisspeptin pathway and potential therapeutic implications

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Obesity is a life threatening condition associated with numerous comorbidities. Among them, central hypogonadism, i.e., low circulating levels of testosterone, has been recently suggested as a putative contributor to the metabolic complications of obesity, especially in men. Yet, the mechanisms for obesity-induced hypogonadism (OIH), and its actual roles in the generation/evolution of the metabolic alterations of obesity remain ill defined. Yet, recent data has suggested that OIH may be caused by suppression of hypothalamic Kiss1 system; kisspeptins being potent activators of the reproductive axis that ultimately stimulate testosterone secretion. However, the mechanisms for Kiss1 suppression in obesity remains unknown.

Our recent evidence suggests that microRNAs (miRNAs) are putative regulators of the Kiss1 system. In this work, we aimed to identify novel miRNAs capable of regulating kisspeptin expression and to evaluate their potential contribution to OIH. Bioinformatic analyses on KISS1 gene were conducted with different algorithms (<http://www.targetscan.org/>; <http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/>; <http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>; <http://www.microrna.org/microrna/home.do>) to seek for potential miRNA regulators. Selection of miRNA candidate(s) was based on the following criteria: 1) to be identified in at least two different databases; 2) to show an evolutionary conserved seed region (rat, mouse, human); and 3) to be deregulated by metabolic alterations, associated with changes in hypothalamic kisspeptin expression. Using these criteria, miR-A (anonymized for reasons of ongoing patent protection) was selected as a robust putative modulator of KISS1. This was tested biochemically using a luciferase reporter assay, which documented a repressive interaction of miR-A at the 3'UTR of KISS1. Further confirmation was obtained in vivo, using a Target Site Blocker (TSB) approach. TSB, tailored to block the repressive interaction of miR-A selectively at Kiss1 3'UTR, kisspeptin-10 and/or testosterone (T) were administered to a male rat model of OIH, with severe suppression of T and gonadotropin (LH) levels, and marked metabolic alterations: increased systolic blood pressure (SBP), glucose intolerance and insulin resistance. TSB administration not only restored T and LH levels and increased hypothalamic kisspeptin, but also ameliorated insulin resistance, glucose intolerance, SBP and cardiac hypertrophy, without detectable changes in absolute body weight. In contrast, treatments with kisspeptin-10 or T failed to restore the alterations induced by OIH. Our results are the first to provide conclusive evidence about the relevant role (and eventual therapeutic value) of a novel central miRNA/ kisspeptin pathway in OIH, and strongly suggest that central hypogonadism is a major contributor for the metabolic complications of obesity.

(P023)

ANGPTL8 protein expression could be regulated by metformin in explants of human visceral adipose tissue

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ANGPTL8 is a protein mainly expressed in the liver and weakly in adipose tissue. This protein had awakened great expectations, but the results in humans are contradictory. Nowadays, there are clear evidences that relate ANGPTL8 with the lipid metabolism and scientific discussion on whether ANGPTL8 regulate insulin resistance has been ongoing. The aim of this study was to analyze the role of ANGPTL8 in explants culture of visceral adipose tissue treated with metformin coming from obese-morbid patients and to assess if the regulation of this protein could be applied as a potential clinical biomarker to predict insulin resistance.

The explants of adipose tissue were obtained from 54 obese women undergoing bariatric surgery with BMI >45 and 10 non-obese women recruited at surgery service with informed consent. HOMA-IR test showed values > 2.5 in obese-patients demonstrating the insulin resistance. The explants samples were cultured 24 hours in M199 medium supplied with dexamentasone (0.77 μ M) and insulin (0.07mM), posteriorly treated at different times (3-12 and 24 hours) and doses with metformin (1-20mM) to reduce the insulin resistance. Western blotting and inmunohistochemical analysis showed the presence of ANGPTL8 in non-treated explants of adipose tissue, which slightly increased in obese patients but not statistically significant. The samples that received 1 and 20mM of metformin increased pAMPK expression in this tissue and reached a maximum increase at 24 hours (3-4 fold). Alike, 1 and 20mM of metformin stimulate the expression of ANGPTL8, reaching maximum values at 24 hours with 20mM. This increase in ANGPTL8 expression could alleviate the insulin resistance process, since has been showed in transfected cells that the phosphorylation of Akt, GSK-3 β and FoxO1 is associated with an increasing of ANGPTL8 mRNA. In summary, the results reveal that metformin increase ANGPTL8 expression in explants of visceral adipose tissue a way dose and time dependent.

(P024)

A novel synthetic benzopyran with dual PPAR α / γ agonist activity and anti-inflammatory effect in vitro, prevents dyslipidaemia in ob/ob mice

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Introduction and Objectives: A novel synthetic benzopyran 1 displayed full hPPAR α activation and partial hPPAR γ agonism. Molecular modeling studies identified those amino acid residues relevant for ligand binding. To know its potential anti-inflammatory and metabolic effects, compound 1 was evaluated on TNF α -induced leukocyte adhesion and investigated after administration in obese mice. **Material/Methods:** Compound 1 was analysed by parallel-plate flow chamber assay to evaluate leukocyte adhesion to TNF α -stimulated endothelium. Flow cytometry was employed to determine ICAM-1, VCAM-1, fractalkine (CX3CL1) and CXCL16 expression and p38MAPK and NF-kB activation. Immunoprecipitation assays were used to evaluate RXR α /PPAR γ interactions. Anti-inflammatory effect in adipocytes was studied by quantification of Mcp1 expression and immunohistochemical staining of anti-F4/80+ cells in WAT of ob/ob mice. Biochemical lipid parameters were determined by commercial kits in plasma of ob/ob mice. **Results:** Compound 1 concentration-dependently reduced TNF α -induced endothelial mononuclear cell adhesion via RXR α /PPAR γ interaction. Compound 1 down-regulated TNF α -induced endothelial VCAM-1, fractalkine (CX3CL1) and CXCL16 expression and inhibited p38MAPK and NF-kB activation. In addition, compound 1 was able to reduce inflammation in WAT and to ameliorate lipid parameters in ob/ob mice without inducing liver toxicity. **Conclusions:** Compound 1 emerges as a novel lead candidate with dual PPAR α / γ agonist activity, low toxicity and high effectiveness in the treatment of cardiometabolic disorders. This compound may prevent the development of dyslipidaemia and associated cardiovascular disease.

Funding: This work was supported by grants SAF2017-89714-R, SAF2014-57845-R, CP15/00150, PI18/01450 from the Spanish Ministry of Economy and Competiveness, Carlos III Health Institute (ISCIII) and co-funded by European Regional Development Fund and European Social Fund ("Investing in your future").

Gastrointestinal / Respiratory Pharmacology**(P025)****MUC1-CT as an IPF potential druggable target**

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Background

MUC1 overexpression is one of the most common alterations in human cancers. This overexpression is associated with accumulation of MUC1-C in the cytoplasm and targeting of MUC1-C to the nucleus by MUC1-C oligomerization by a CQC motif in MUC1-cytoplasmic tail (CT). In previous studies we showed that MUC1 is also overexpressed in Idiopathic pulmonary fibrosis (IPF) lungs, and play an essential role in the cellular transformations and processes related to IPF. However, the participation of MUC1-CT signalling in IPF is currently unknown.

Objective

To analyze the implication of MUC1-CT in IPF

Methods

The intracellular mechanism of MUC1 in IPF was evaluated by immunoprecipitation and immunofluorescence studies in IPF lung tissue and alveolar type II cells and lung fibroblasts stimulated with TGF β 1, and by the use of the peptide GO-201 (an inhibitor of MUC1-CT CQC motif that attenuates targeting of MUC1-C to the nucleus) in these cells. This inhibitor was used to study the effect of MUC1-CT on epithelial to mesenchymal (EMT) and fibroblast to myofibroblasts (FMT) transitions, cellular senescence and cellular proliferation. Attenuation of lung fibrosis development in a GO-201 treated bleomycin-induced pulmonary fibrosis mouse model was also assessed.

Results

Immunoprecipitation and immunofluorescence studies showed that under fibrotic conditions MUC1-CT forms a complex with p-Smad2/3 and active- β -catenin, translocating into the nucleus to activate fibrotic genes in human cells and human lung tissue. Furthermore, inhibition of nuclear MUC1-CT translocation by GO-201 indicated that MUC1-CT activation and translocation into the nucleus is essential to induce EMT and FMT cellular transformations, as well as, ATII and fibroblast cell senescence and fibroblast proliferation. Finally, lung tissue remodeling was attenuated in GO-201 treated mice.

Conclusions

MUC1-CT is essential to induce IPF progression. Therefore, pharmacologic targeting of MUC1-CT may be a promising option for the treatment of IPF.

(P026)

Role of Kv7 channels and KCNE ancillary subunits in the pulmonary vasculature: possible implication in pulmonary hypertension.

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Pulmonary arterial hypertension (PAH) is characterised by vasoconstriction, in situ thrombosis and vascular remodelling in pulmonary arteries (PA). K⁺ channels play a fundamental role regulating membrane potential (Em) of PA smooth muscle cells (PASMCs). Recently, a key role of Kv7 channels (Kcnq1-5) and KCNE subunits (Kcne1-5) in the control of vascular tone has been demonstrated. Moreover, reduced Kv7 channel activity has been observed in different cardiovascular pathologies such as diabetes, hypertension or long QT syndrome. Our objective was to study the role of Kv7 channels/KCNE subunits in the pulmonary vasculature and their possible alteration in PAH.

Kv7 channel activity was analyzed in PASMCs or PA using the whole-cell configuration of the patch-clamp technique and vascular reactivity. The characterization of Kv7 channels was performed using a blocker (XE-991) and selective enhancers (ML214, ML277, retigabine and S1). The expression of Kv7 channels and KCNE subunits was analyzed by qRT-PCR and Western blot in lungs from PAH model. Cell localization studies were analyzed using immunocytochemistry and proximity ligation assay.

In control-PA, the Kv7 channels enhancers produce a negligible relaxation. These data were supported by the cellular localization study, which showed that Kv7 channels/KCNEs subunits expression in PA was essentially cytosolic. Interestingly, the vascular reactivity data showed an enhanced response to Kv7 channels modulators in PAH-PA. The electrophysiological data showed a decrease in K⁺ current and a more depolarized Em in PAH-PASMCs compared to controls. Despite, the contribution of Kv7 channels to the total current was higher in PAH-PASMCs than in controls. We also found an altered expression of Kv7 channels/KCNE subunits in PAH-lungs; with a decrease in Kv7.4 α and KCNE3 and an increase in KCNE4 subunit.

In conclusion, there is a Kv7.4 channels downregulation in PAH; but paradoxically, this seems to be associated with a higher response to Kv7 channel modulators. Our data suggest that the ionic remodeling produced in the PAH pathophysiology makes Kv7 channels more suitable for pharmacological intervention.

(P027)

Pharmacological Modulation of Autophagy as a novel strategy for Intestinal Fibrosis

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Keywords: Autophagy, intestinal fibrosis, inflammation

Background: Intestinal fibrosis is a common complication of Crohn's Disease (CD) patients which requires surgery. Several single nucleotide polymorphisms in autophagy-related genes have been associated to CD. Although autophagy is impaired in CD-patients, the relevance of autophagy in intestinal fibrosis remains unclear. We aim to analyze the effect of pharmacological modulation of autophagy in intestinal fibrosis.

Methods: Murine intestinal fibrosis was induced using the heterotopic transplant model. Segments of 1cm murine colon were transplanted subcutaneously into the neck of a recipient mice and collected 7 days after transplantation. Recipient mice were treated daily with an injection of 3-MA (10mg/kg) or rapamycin (1.25mg/kg). Expression of inflammation, fibrosis, EMT and autophagic markers were analyzed by qPCR and WesternBlot. Collagen layer was evaluated by Sirius Red. Intestinal resections from CD-patients were obtained and expression of p62, Col1a1, α -SMA, Snail1 and Snail2 was analyzed by qPCR. Results are expressed as fold induction (mean \pm SEM, n \geq 5).

Results: Grafts at day 7 exhibited a reduced autophagic flux and the autophagy inhibition by 3-MA and autophagy activation by rapamycin were confirmed by WesternBlot. Grafts at day 7 from 3-MA-treated mice exhibited a significant increase in the expression of proinflammatory, profibrotic and EMT genes and a thicker collagen layer. Interestingly, grafts at day 7 from rapamycin-treated mice showed a significant reduction in the expression of proinflammatory, profibrotic and EMT genes and a significant reduction in the thickness of the collagen layer (Table 1). In intestinal resections from CD-patients, the expression of p62 positively correlates with the expression of Col1a1 (rSpearman=0.6098, P=0.004), α -sma (rSpearman=0.5168, P=0.041), Snail1 (rSpearman=0.4112, P=0.0003) and Snail2 (rSpearman=0.4410, P=0.0009).

Conclusion: Pharmacological modulation of autophagy modulates intestinal inflammation and fibrosis. In intestinal resections from CD-patients autophagy markers positively correlates with pro-fibrotic and pro-EMT genes, which led us to suggest that pharmacological modulation of autophagy might be a new therapeutic strategy for intestinal fibrosis.

(P028)

Impact of vitamin D deficiency on the rat gut microbiome^aMaria Callejo, ^bIñaki Robles-Vera, ^bJuan Duarte, ^aFrancisco Pérez-Vizcaíno^aUniversidad Complutense de Madrid.^bUniversidad de Granada.

Multiple recent studies suggest a relationship between vitamin D deficiency and several disorders such as deregulation of the immune system, alterations of metabolic homeostasis, cardiovascular diseases among others. Herein, we have analysed if vitamin D deficiency alters the rat gut microbiota. For that purpose, male rats were fed a standard or vitamin D-free diet for seven weeks. The microbiome composition was determined in faecal samples by 16S rRNA gene sequencing and bioinformatics analysis. After seven weeks, animals presented a severe vitamin D deficiency. Markers of gut dysbiosis such as Firmicutes-to-Bacteroidetes ratio or short chain fatty acid producing bacterial genera were not significantly affected by vitamin D deficiency. Moreover, vitamin D-free diet produced no changes in the total number of species, neither the Chaos index nor β -diversity. But, paradoxically, it produced a modest increase in some indicators of α diversity such as the Pielou, the Simpsons and the Shannon indices. Notably, there was an increase in the abundance of the Enterobacteriaceae family, with significant rises in its associated genera *Escherichia*, *Candidatusblochmannia* and *Enterobacter*. Besides, *Prevotella* and *Actinomycetes* were also increased and *Odoribacteraceae* and its genus *Butyricimonas* were decreased in rats with vitamin D deficiency.

Despite vitamin D deficiency did not induce gut dysbiosis, this hypovitaminosis produced some specific changes in bacterial taxa, which may play a pathophysiological role in the immunologic dysregulation.

Neuro / Psycho Pharmacology**(P029)****Fingolimod phosphate protection against mitochondrial damage in neuronal cells**

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Background: Major role of oxidative stress in the pathogenesis of neurodegenerative diseases have been suggested, being mitochondria one of the main sources of ROS.

Aim: In the present work, we have studied the antioxidant effect of fingolimod phosphate (FP) on neuronal mitochondrial function and morphology using a model of mitochondrial oxidative damage induced by menadione (Vitk3).

Methods: SN4741 neuronal cells were grown (70-80% confluence) and used as control (non-treated cells) or treated cells with Vitk3 15 μ M alone or in presence of FP 50 nM during 4 hours. Mitochondrial membrane potential (MMP), cytochrome c oxidase (COX) activity, mitochondrial oxygen consumption rate (OCR), mitochondrial distribution and morphology were analysed. Statistical differences were determined using one-way ANOVA.

Results: Vitk3 incubation produces a dramatical decrease in MMP compared to control (43.7 %); this can be almost totally reverted by the co-incubation of Vitk3 in presence of FP ($p < 0.05$). A 20.7 % decrease in COX activity has been found after Vitk3 incubation, again this effect was counteracted when Vitk3 and FP are combined, restoring COX activity to control levels ($p < 0.05$). Vitk3 incubation triggers initially an increase in OCR, decreasing dramatically (61%) after 4 hours. In experiments co-incubating Vitk3 in presence of FP, the OCR decrease found was reduced to only 17% ($p < 0.05$). In experiments with MitoTracker™ Green, we found a change in the network pattern distribution after Vitk3 administration that partially disappears when co-incubated in presence of FP. Almost all the mitochondria treated with Vitk3 show ultrastructural alterations at the electron microscopy level while normal mitochondria can be found when Vitk3 and FP are combined.

Conclusion: FP protects against the mitochondrial damage induced by Vitk3, as seen by the results obtained in mitochondrial functional markers, distribution and morphology.

Keywords: Fingolimod phosphate, Mitochondria, Oxidative stress.

(P032)

Minor compounds from virgin olive oil promote M2 microglia polarization and neuroprotection

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Microglia are the primary cells that exert immune function in the central nervous system, and accumulating evidence suggests that microglia act as key players in the initiation of neurodegenerative diseases. It is now well recognized that microglia have functional plasticity and dual phenotypes, proinflammatory M1 and anti-inflammatory M2 phenotypes. Inhibiting the M1 phenotype while stimulating the M2 phenotype has been suggested as a potential therapeutic approach for the treatment of neuroinflammation-related diseases. Our aim was to evaluate the effects of minor compounds found in the unsaponifiable fraction (UF) and in the phenolic fraction (PF) of virgin olive oil (VOO) could modulate the plasticity of microglia. We observed that UF and PF enhance M2 microglia polarization, whereas postprandial LPS made polarized microglia prone to an M1 phenotype. In addition, in contrast to dietary SFAs, dietary VOO primed for a reduced pro-inflammatory profile in the brain of mice. These findings unveil a potential beneficial role for minor compounds of VOO in regulating the plasticity of microglia. These exciting findings open opportunities for developing nutraceutical strategies with olive oil as the principal source of fat to prevent development and progression of neuroinflammation-related diseases.

(P033)**Sports performance declines with ageing by a single exponential: implications for drug targets**^aMichael Spedding^aSpedding Research Solutions SAS.

Multiple drug targets have been proposed for neurodegenerative diseases, but little progress for Alzheimers Disease (AD) and Amyotrophic lateral sclerosis (ALS) has been made. Recent human evolutionary history, and the molecular drivers that are involved, need to be taken into account for drug targets. First, human beings evolved to run in Africa, over a relatively short evolutionary time scale, markedly increasing metabolic rate and VO₂max, compared with other primates, and at the same time increasing brain size, and lifespan – thus humans are already remarkably optimized. Lipid metabolism was crucial in this evolution. There is a specific, highly precise, decline with ageing, essentially following the second law of thermodynamics, in world-best athletic performances, and metabolic capacity, in multiple sports. Furthermore, this process appears to be already peaked, in that little progression in age-related records has been made over the last three decades (Marck et al., 2018, J Ger A Biol Sci Med Sci. doi: 10.1093/gerona/gly165). It is highly probable that age-related diseases are a result of a stochastic decline in regulatory function.

Using this approach, we have performed basic research to obtain a new concept for ALS, based on lipid metabolism, high content lipidomics (3000 lipids) and transcriptomics in animal models, backed up by extensive human enzyme workup. Glucosylceramidase (GBA2) was found to be critical for neuromuscular function, resulting in the identification of a modulator compound, a generic, which has obtained ALS orphan drug designation from the EMA and is phase II ready. Integration of evolution and metabolomics may therefore yield new directions for difficult diseases.

(P034)

Gamma-decanolactone as selective MAO-B inhibitor in Parkinson's disease treatment

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Introduction: Several studies point to the neuroprotective role of monoamine oxidase-B (MAO-B) inhibitors in Parkinson's disease (PD). Gamma-decanolactone (GD) was active in acute and chronic epilepsy models and demonstrate to decrease neuroinflammation in vivo and in vitro studies indicating that has a neuroprotective therapeutic potential.

Aims: In this work we have evaluated the possible effect of GD (300mg/Kg), alone or associated with Levodopa/Benserazida (LD/BZ, 100:25 mg/Kg), in vivo PD model. Also, evaluated the effect the GD in vitro to inhibit the A and B isoforms of hMAO enzyme.

Methods: Male CD-1 mice (22±3g) reserpinized (1.5 mg/Kg) previously (18 h) were used and motor coordination (rota-rod test, 180 sec cut off time) and modification of body temperature were evaluated after 30, 60, 90, 120 min and 24h. The effects of GD on MAO-A and B isoform enzymatic activity were evaluated by a fluorimetric method.

Results: Reserpinized animals showed marked ataxia (57.88± 21.39 sec at 90 min, p<0.001) and almost all measurement times compared to the control group (180 sec, all measurement). Treatment with LD/BZ partially reversed ataxia after 60 min (158.70±14.49 sec, p<0.001) and also other times, as did GD alone as 60 and 90 min (128.80±22.30, p<0.05 and 126.00±20.18, p<0.05; respectively). Concomitant treatment of GD with LD/BZ, at 90 min, showed a potentiation of the effect 162.90±12.89, p<0.001). Animals showed a marked decrease in body temperature compared to untreated animals, and only GD facilitated the reversal of hypothermia at all times. In vitro, GD inhibited the activity of MAO-B, with IC50 ≈ 100µM demonstrating an important selectivity profile against the MAO-B isoform.

Conclusion: These results suggest that GD is a promising multifunctional agent for effective therapy for PD.

(P035)

Consumption of central nervous system drugs in a nursing home in León

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The use of central nervous system (CNS) drugs among old people is common, and has increased markedly during the last few decades. The purpose of this study was to provide preliminary evidence on the frequency of consumption of those drugs included in ATC group N (nervous system) in a nursing home residents in León.

This was a observational, descriptive, and retrospective pharmacoepidemiological study of the use of ATC group N by 330 nursing home residents in León.

64.2% were women and 35.8% men, the mean age of the residents participating in the study was 86.5 ± 8.1 years old. Nervous system diseases were one of the most prevalent in the center (55.9%) with higher prevalence in women (61.1%) and in those with ages under 75 years (54.2%). The three most diagnosed pathologies were cognitive impairment (37.9%), depression (24.5%), and psychological disorders (11.7%). Drugs included in ATC group N were consumed by 82.4% of the residents, being their use higher in women (85.4%) than in men (77.1%) and in the group that were 85 or more years old (85.2%). Considering ATC subgroups, psycholeptics (subgroup N05) were the most commonly used (39.1%), followed by subgroup N06 psychoanaleptics (37.4%) and subgroup N02 analgesics (14.1%). In subgroup N05, therapeutic subgroup N05B anxiolytics (54.4%) was the most consumed and, in subgroup N06, therapeutic subgroup N06A antidepressants (76.6%). The three most commonly employed drugs were lorazepam (subgroup N05B, 12.4%), trazodone (subgroup N06A, 9.2%) and paracetamol (subgroup N02B, 8.3%).

(P038)

TLR4 blockade reduces SAA1 levels and improves neurological function in mice after traumatic brain injury

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Traumatic brain injury (TBI) is a common serious problem and its consequences are unknown. In this context, neuroinflammation that takes place after TBI plays a key role in the development of secondary lesions, which can become chronic. Thus, the main aim of this study is to detect inflammation-related biomarkers that could contribute to the progression of the lesion. In TBI patients, we have discovered that SAA1 protein increases dramatically in serum at 24 hours after hospital admission, reaching a peak at 72h. At 1 week SAA1 levels decrease to similar values than 24h subjects. We have studied in vitro and in the mice model "Closed Head Injury" (CHI) the role of SAA1 in TBI. We have observed in mixed glial cultures that SAA1 activates the TLR4 pathway and induces the release of IL-1 β and TNF- α and the expression of iNOS; furthermore, TLR4 agonist, TNF- α and IL-1 β induce the release of SAA1 by glial cells. All these effects were reverted by TAK242 (1 μ M), a TLR4 inhibitor. In mice subjected to the CHI model, we have detected an increase of SAA1 serum and brain levels after 24h, which returned to basal levels 1 week after trauma. Treatment with TAK242 (3 mg/kg) 1h after TBI significantly reduced cerebral SAA1 at 24h, while serum levels did not change. We assessed the neurological functions of the animals 1h and 24h after TBI by the Neurological Severity Score (NSS) test. Mice treated with TAK242 obtained a lower score than non-treated animals, which means an improvement in motor and behavioral functions. In addition, TAK242 prevented blood-brain barrier impairment, evaluated by Evans Blue dye. Taken together, these results suggest that TLR4 could be a potential pharmacological target to treat the detrimental effects of inflammation in traumatic brain injury.

Pharmacogenetics**(P039)****Response to fluoxetine in children and adolescents: a weighted gene co-expression network analysis of peripheral blood**

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In view of the lack of replicated candidate gene studies, together with the inconclusive and non-replicated results of pharmacogenetic GWA studies on antidepressant response, gene expression studies provide an interesting insight into this complex phenotype. Our study uses a method based on gene expression changes in peripheral blood after treatment with fluoxetine in a sample of naive children and adolescents. Expression changes caused by fluoxetine treatment were found to be related to several biological processes. Those related to neurogenesis were especially remarkable because, for the first time in this kind of study, they were found to be associated with the SSRI response. This makes sense from a biological point of view. Through other methods, the scientific literature had already indicated neurogenesis as a phenomenon involved in the response to SSRI, which shows that this strategy can be used to obtain new candidate pathways and genes. In addition, an exploratory pharmacogenetic study of a relevant gene in neurogenesis (PAK2) was conducted, and a significant association with the CGI scale was observed.

Receptors

(P040)

Quantification of functional bias of serotonin 5-HT_{2A} receptor agonists and validation of structural hypotheses on functional selectivity at 5-HT_{2A} receptor

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5-HT_{2A} receptor (5-HT_{2AR}) was one of the first G protein-coupled receptors (GPCRs) for which functional selectivity was described.¹ A recent computational study proposed agonist interaction with residue S242 (S5.46) of 5-HT_{2AR} as relevant for ligand bias towards the inositol phosphate (IP) signalling pathway modulated by the receptor, whereas interaction with residue N343 (N6.55) would favour bias towards the arachidonic acid (AA) pathway.² These observations allowed to select the serotonin (5-HT) derivatives 5-nitro-1H-indole-3-ethanamine (Nitrol), silent at the AA pathway, and 3-(aminoethyl)1-methylindol-5-ol (Metl), which preferentially activated the AA pathway. Here we aimed at quantitatively determining the functional bias of these agonists as well as at directly testing by site-directed mutagenesis the structural hypotheses formulated on functional selectivity at 5-HT_{2AR}.

IP and AA signalling of 5-HT, Nitrol and Metl was evaluated at 5-HT_{2A}-WT, 5-HT_{2A}-S242A and 5-HT_{2A}-N343A receptors. Functional bias was quantified applying the operational model of functional selectivity.

The point-mutations did not compromise the affinity of [3H]-Ketanserin for 5-HT_{2AR}. At 5-HT_{2A}-WT, Metl showed bias towards the AA pathway with $\Delta\Delta\log(\tau/K_A)$ (IC 95%) = 1.27 (0.73 – 1.82), in agreement with the prediction for this derivative. This bias diminished at 5-HT_{2A}-S242A ($\Delta\Delta\log(\tau/K_A)$ (IC 95%) = 0.55 (-0.16 – 1.27)), consistent with residue S242 as relevant for IP bias. However, the improvement in affinity and potency of Metl specifically at the IP pathway supports changes in its binding mode in this mutant, with other interactions contributing to IP signalling. The N343A mutation preserved AA signalling of 5-HT and Metl as well as Metl bias towards AA ($\Delta\Delta\log(\tau/K_A)$ (IC 95%) = 1.59 (0.63 – 2.56)), whereas unexpectedly reduced the IP potency of both agonists. Even considering possible limitations of our experimental approach, the results suggest that other agonist properties indirectly related to the interaction patterns investigated may contribute to the bias of compounds Nitrol and Metl.

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(P041)**Typical and atypical antipsychotics differentially modulates D2/5-HT2A heterooligomer signaling**

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G-protein coupled receptors (GPCRs) oligomerization has proved to modify the individual receptors function and consequently their pharmacology [1]. The 5-HT2A receptors have been observed to be expressed as heterodimers with the D2 receptor, causing alterations in intracellular signaling [2]. In previous works we showed the hallucinogenic 5-HT2A agonist (\pm)DOI [1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine] modulated dopamine-mediated D2 receptor activation. Our hypothesis was that D2/5-HT2A oligomerization may play a role in antipsychotic drugs signaling modulation. Thus, we aimed to study the modulation of D2 and 5-HT2A signaling exerted by both typical and atypical antipsychotics.

We employed a cell line constitutively overexpressing D2 receptors and expressing 5-HT2A receptor in a doxycycline-dependent inducible way. Dopamine D2 signaling was determined by measuring cAMP levels by employing cAMP-Gs Dynamic Kit (Cisbio). 5-HT2A signaling was determined by measuring IP₃ levels by employing [³H]myo inositol.

We observed that when both receptors were coexpressed (\pm)DOI activates cAMP production in presence of forskolin (an activator of adenylyl cyclase). This effect can be explained by the inhibition of PLC that enhances PKA [3]; or by a crosstalk between G_q and G_i from 5-HT2A or D2 coupled G proteins, respectively. Related to that, typical antipsychotics haloperidol and chlorpromazine had a different effect in the efficacy of dopamine to inhibit the production of cAMP in absence and presence of (\pm)DOI with E_{max} values of 103 \pm 12.9% and 182 \pm 31.6%* and 114 \pm 12.7% and 159 \pm 29.5%*, respectively. The atypical antipsychotics clozapine and risperidone showed a differential effect with E_{max} values of 84.3 \pm 19% and 105 \pm 29.1%* and 88.4 \pm 3.1% and 99.5 \pm 2.3%*, respectively (*p<0.001 compared with each compound control curve in absence of (\pm)DOI).

We conclude that the activation of 5-HT2A receptors by the hallucinogenic agonist (\pm)DOI modulated the D2 receptor signaling in HEK293 cells co-expressing both receptors. This modulation condition the response of both receptors to antipsychotic drugs.

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(P042)

Essential role of C148-C227 disulfide bridge in human 5-HT_{2A} receptor functionality and trafficking^aMarta Cimadevila, ^bAnton L Martínez, ^bAlba Iglesias, ^bMaría I Cadavid, ^bJose Brea, ^bMaría I Loza^aUniversidade de Santiago de Compostela.^bBioFarma Research Group, Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CiMUS), Universidade de Santiago de Compostela.

G Protein Coupled Receptors extracellular domains are emerging as a determining factor in receptor functionality, not only for orthosteric ligands, but also as an allosteric modulation site (Wooley MJ, et al., 2017). The recent crystallization of serotonin 2A (5-HT_{2A}) receptor showed a critical role of extracellular loop 2 (ECL-2) in receptor selectivity. This finding is in agreement with previous studies, where dithiothreitol was used to nonspecifically break a conserved disulfide bridge between C148 at transmembrane domain 3 (TM-3) and C227 at ECL-2 (Iglesias A, et al. 2017).

Our hypothesis is that the C148-C227 disulfide bridge in 5-HT_{2A} receptor may be critical for ligand binding, receptor activation and trafficking. Thus, we aimed to generate three constructions incapable of maintaining this bridge and study their binding, function and subcellular expression.

The two cysteines involved in the aforementioned disulfide bridge were mutated into alanines, obtaining three constructs: pcDNA5/FRT/TO myc-5-HT_{2A}C148A-eYFP (C148A), pcDNA5/FRT/TO myc-5-HT_{2A}C227A-eYFP (C227A) and pcDNA5/FRT/TO myc-5-HT_{2A}C148A/C227A-eYFP (C148A/C227A). These three constructions, together with the parental one (pcDNA5/FRT/TO myc-5-HT_{2A}eYFP, WT) were employed to generate four stable cell lines that were characterized by means of binding and functional assays. For subcellular localization studies, cell nucleus and the endoplasmic reticulum were stained with fluorescent probes, and images were obtained in Operetta High Content Imaging System (Perkin Elmer).

When compared with WT cell line, C148A, C227A and C148A/C227A mutants were unable to bind [3H]LSD and to respond to (±)DOI neither when measuring IPs accumulation, nor calcium mobilization. Furthermore, all the mutants showed around a 50% decrease of expression of receptor in the cell membrane.

In conclusion, these results show that the disulfide bridge formed between TM-3 and ECL-2 is critical to maintain human 5-HT_{2A} receptor proper conformation for ligand binding and receptor activation, but also to facilitate receptor trafficking to cell membrane.

(P043)**The IUPHAR/BPS Guide to PHARMACOLOGY in 2019: new features and updates**

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The IUPHAR/BPS Guide to PHARMACOLOGY (GtoPdb) (1), is an open-access, expert-curated, online database of human drug targets and their ligands (2). It provides succinct overviews, key references and recommended experimental ligands for 2,920 drug targets and related proteins organised into families. The database includes 9662 ligands molecules, including approved drugs. The development of GtoPdb is overseen by NC-IUPHAR (3) with data selected by its 90 subcommittees and 800 expert curators covering established drug targets as well as those of emerging interest for drug discovery. NC-IUPHAR has published 125 papers, h-index>80. A major recent effort has seen expansion in the area of immunopharmacology, with the public release of the IUPHAR Guide to IMMUNOPHARMACOLOGY. Relevant targets and ligands have been added and linked to immunological cell types, processes and diseases. All this information has been gathered into a new portal aimed at immunologists wishing to search pharmacological data (5). Another new extension under development is the Guide to MALARIA PHARMACOLOGY (GtoMPdb) supported by funding from Medicines for Malaria Venture (MMV). This resource has the aim of providing optimised access to GtoPdb data for the malaria research community. We continue to develop web services and an RDF linked-data format providing computational access to the database, and social media.

GtoPdb is a useful resource for scientists looking for expert-curated information on drug targets and recommended ligands, and we hope all pharmacologists will use it and contribute to this world-wide effort which has structured modern pharmacology.

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(P044)

Functional differences between dopamine D4.4 and D4.7 receptor variants within the dopamine D4-adrenergic α 2A receptor heteromer in the brain

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Most medications used for the treatment of attention deficit hyperactivity disorder (ADHD) are directed to modulate NE and DA neurotransmission in the brain. Genetic studies have indicated linkage of vulnerability for developing ADHD and substance use disorders (SUD) and polymorphisms of α 2AR-adrenoceptors (α 2AR) and dopamine D4 receptors (D4R). There are evidences demonstrating that α 2ARs are involved in the correct function of working memory and behavioral inhibition and in the protection against distractibility at the level of prefrontal cortex (PFC). α 2ARs are also involved in the basal ganglia motor control inhibiting DA release from the substantia nigra. D4Rs have also been related to the control of glutamate release both in the PFC and in the basal ganglia, thus in the control of PFC excitability and in the "Go" and "NoGo" GABAergic striatal efferent pathways. However, very little is known and there is controversy about the molecular mechanisms and functional significance of the polymorphisms of the human DRD4 gene. Our group, using a combination of approaches including biophysical, pharmacological, functional and immunochemical assays in transfected cells and in cortex and striatum brain slices, has investigated the possibility that D4R might modify adrenergic receptor function through direct receptor-receptor interaction. We report, to our knowledge, the first heteromer between D4R and α 2AR, which also shows functional differences between both products of D4R polymorphic variants that are only evident upon heteromerization with α 2AR, as reported for D4R-D2R heteromers. Concretely, there is a negative cross-talk and a cross-antagonism within the α 2AR-D4R heteromer only evident with D4.4R but not with D4.7R variant. These differences may be responsible for the pathophysiology of ADHD disorder and can give new clues for the rational design of drugs targeting α 2AR-D4R for the treatment of ADHD and SUD.

Pharmacology of Pain and Inflammation**(P045)****Lupine (*Lupinus angustifolius* L.) peptide ameliorates the non-alcoholic steatohepatitis in mice**

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Bioactive peptides are related to the prevention and treatment of many diseases. GPETAFLR is an octapeptide isolated from lupine (*Lupinus angustifolius* L.) that had shown anti-inflammatory properties. The aim of this study was to evaluate the potential activity of GPETAFLR to prevent non-alcoholic fatty liver disease (NAFLD) in high-fat diet (HFD)-induced obese mice. C57BL/6J mice were fed a standard diet or an HFD. Two of the groups fed HFD diet were treated with GPETAFLR in drinking water at 0,5 mg/kg/day or 1 mg/kg/day. To determine the ability of GPETAFLR to improve the onset and progression of NAFLD, histological studies, hepatic enzyme profile, inflammatory cytokine and lipid metabolism-related genes and proteins were analysed. Our results suggest that HFD-induced inflammatory metabolic disorders were alleviated by treatment with GPETAFLR. In conclusion, dietary lupine consumption could repair HFD-induced hepatic damage, possibly via modifications in the liver lipid signalling pathways.

(P046)**Development of a translational in vitro assay for iatrogenic neuropathic pain**

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Iatrogenic neuropathic pain induced by antitumor and antiretroviral drugs remains as an unsolved therapeutic need due to the limited efficacy of the current analgesics. This is partially due to the lack of translationality of the assays used in screening campaigns for hit finding (i. e., not taking into consideration the physical damage caused by these drugs to sensory neurons).

Our aim was to develop an assay to assess iatrogenic damage useful for the identification of new analgesics to treat pain of such etiology. To do so, we developed a novel in vitro translational assay for the screening of analgesic drugs for neuropathic pain that allows the measurement of the length of the neurites of immortalized dorsal root ganglia (DRG) neurons by employing an Operetta High Content Imaging system. This assay allowed us to assess the efficacy of drugs against iatrogenic neurite shortening. We assessed in this novel assay the efficacy of hits previously identified from Prestwick chemical library in a primary HTS in which they reduced the hyperexcitability of DRG neurons pre-exposed to inflammatory mediators [1].

Using this new assay, we were able to identify two of these compounds (felodipine and nitrendipine, both with $p < 0.01$), which partially avoided the shortening of neurites induced by the antitumor (vincristine and paclitaxel) and antiretroviral (rilpivirine) drugs on immortalized DRG neurons.

References:

- [1] Martínez, AL et al. SLAS Discovery 2019, 24(2), 158.

(P047)

Adenosine A2B receptor involvement in skin protection: from epidermal barrier homeostasis to regulation of inflammation

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Adenosine presents anti-inflammatory properties through interaction with its receptors in multiple tissues [1]. There are four known cell surface receptors, namely A1, A2A, A2B and A3, coupled to G proteins. Particularly in keratinocytes, the A2B receptor is highly expressed, suggesting a protective physiological role in the skin [2]. In the present study, we assessed the effects of A2BR agonists and antagonists on inflammation and epidermal barrier integrity in the skin hyperplasia murine model induced by 12-O-tetradecanoylphorbol-13-acetate (TPA).

The A2BR agonist BAY60-6583 (BAY) (1 µg/site), the A2BR antagonist PSB-1115 (5µg/site), and both together were applied on the shaved backs of female Swiss mice 30 minutes before TPA (2nmol/site) for three consecutive days. Evolution of skin lesions was visually scored, using a scale of 0-4. On day four, animals were sacrificed and punch biopsies were collected, weighed and either homogenized to measure inflammatory mediators or processed for histological analysis.

Immunohistochemical study showed that BAY treatment normalized the expression of proteins involved in the epidermal barrier maintenance such as filaggrin, involucrin or loricrin. The expression of cytokeratin-6, a specific marker of hyperproliferative keratinocytes, was also decreased after application of BAY. Concerning the inflammatory response, BAY significantly reduced several parameters when compared to TPA-treated mice: edema (104.5±8.2 mg vs. 150.4±5.9 mg), myeloperoxidase activity (0.2±0.1 AU vs. 0.6±1.5 AU) and cytokines such as IL-6 (26.7±2.9 pg/ml vs. 51.9±7.4 pg/ml) or CXCL-1 (41.1±2.7 pg/ml vs. 119.3±8.2 pg/ml). Interestingly, PSB application blunted the beneficial effect observed after treatment with BAY. Therefore, activation of A2BR could represent a novel pharmacological target in pathologies of the skin characterised by inflammation and hyperproliferation such as psoriasis.

[1] Burnstock et al., J Invest Dermatol (2012) 132:526-46.

[2] Andrés et al., J Invest Dermatol (2017) 137:123-31.

(P048)

CCL11/CCR3 axis is involved atherosclerosis development

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Introduction: Primary hypercholesterolemia (PH) is a lipid disorder characterized by elevated levels of cholesterol and low-density lipoprotein (LDL). Generally, deleterious environmental factors such as hypercholesterolemic diets and obesity are linked to the disease. A low-grade systemic inflammation is associated with PH, which might explain the higher incidence of cardiovascular diseases such as atherosclerosis, one of the leading causes of morbidity and mortality in Western countries. The early atherosclerotic lesion involves an inflammatory response, the intimal accumulation of T lymphocytes and lipid-laden macrophages, continuing throughout the atherogenic process. Eotaxin-1/CCL11 has been detected in human and mouse atherosclerotic aortas; however, its role in the atherosclerotic lesion development remains elusive. **Objective:** To evaluate the impact of eotaxin-1 receptor (CCR3) in PH patients and in the lesion formation in apoE^{-/-}-CCR3^{+/+} mice versus apoE^{-/-}-CCR3^{-/-} mice. **Materials and Methods:** Whole blood from 22 PH patients, 21 age-matched controls and two-month-old apoE^{-/-}-CCR3^{+/+} or apoE^{-/-}-CCR3^{-/-} mice subjected or not to an atherogenic diet (10.8% fat, 0.75% cholesterol) during two months, was analysed by flow cytometry to determine CCR3-expression in different leukocyte subsets. Circulating chemokines were determined by ELISA. Lesion formation, inflammatory infiltration, collagen, necrotic core, vascular smooth muscle cells, mast cells, eosinophils and eotaxin-1 content were determined through histological and immunohistochemical techniques. **Results:** PH patients and atherogenic diet-fed apoE^{-/-}-CCR3^{+/+} mice show elevated percentage of CCR3-expressing leukocytes. Atherogenic diet-fed apoE^{-/-}-CCR3^{-/-} mice showed increased lesion formation, collagen content and macrophage, T lymphocyte and mast cells infiltration respect to apoE^{-/-}-CCR3^{+/+}. Lesion eotaxin-1 expression of atherogenic-fed apoE^{-/-}-CCR3^{+/+} mice was much higher than that detected in apoE^{-/-}-CCR3^{-/-}. **Conclusion:** CCL11/CCR3 axis may exert a protective effect during the development of atherosclerotic processes. **Funding:** This work was supported by the Spanish Ministry of Economy and Competiveness [grant numbers SAF2014-57845-R, SAF2017-89714-R]; Carlos III Health Institute and the European Regional Development Fund [grant numbers PI15/00082, PIE15/00013, PI18/00209].

(P049)

Osteostatin down-regulates osteoclast differentiation

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In rheumatoid arthritis there is an imbalance in bone remodelling associated to the progression of disease. It is known that PTHrP has a main role in different articular cells metabolism which may be modulated by related peptides. In this sense, we have demonstrate that PTHrP (107-111) C-terminal peptide (Osteostatin) is able to enhance osteoblast proliferation and downregulate senescence and inflammatory mediators in vitro (1), as well as chronic inflammation in the collagen-induced arthritis model. However, it is unknown whether this peptide exerts any effect in bone-resorbing cells (osteoclasts). Hence, our objective is to investigate if osteostatin is able to reduce osteoclast differentiation. We first differentiate human osteoclast from peripheral blood monocytes from healthy donors which were isolated by density-gradient centrifugation and were enriched by plate adherence. Cells were harvested and seeded 105cells/well (96 wells plate) in α -MEM completed with 5% Hyclone FBS, 1% streptomycin/penicillin and 0.05% β -mercaptoethanol. Furthermore, RANKL (50ng/mL) and MCSF (25ng/mL) were added for a week to induce osteoclast differentiation. Osteostatin was added to treated groups at 100, 250 and 500 ng/mL from day 0 of the experiment. When control group had 80% osteoclasts confluence, the experiments were stopped and TRAP staining was performed. Cells were considered osteoclasts when they were TRAP+ and 3 nuclei or more were present. Experiments were performed with cells from 9 patients. Our results show a concentration-dependent decrease of osteoclasts differentiation being statistically significant at 250nM and 500nM. Therefore, these results suggest that osteostatin exhibits a dual profile in bone cells metabolism enhancing osteoblast proliferation and decreasing osteoclast differentiation.

1. Platas J, Guillén MI, Gomar F, Castejón MA, Esbrit P, Alcaraz MJ. 2017;72(5):624–31.

(P050)

A capsaicin-based TRPV1 soft antagonist as an anti-pruritic drug candidate

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TRPV1 is a polymodal ion channel that is activated by noxious stimuli including high temperature, acid pH, membrane voltage changes and capsaicin. TRPV1 is widely expressed in several cell types from sensory neurons to non-neuronal skin cells where it plays a pivotal role in the aetiology of inflammation and/or chronic pain as well as dermatological disorders. Indeed, capsaicin is widely used in the clinics for the treatment of pain and pruritus. However, the burning sensation produced by capsaicin is a major clinical complain that leads to treatment dropout. We set to obtain capsaicin analogues devoid of the undesired capsaicin side effects (i.e. burning and poor elimination). As a strategy, we designed capsaicin-based, soft TRPV1 antagonists that incorporate an ester group in the aliphatic chain that is sensitive to hydrolysis by cutaneous esterases, thus preventing their dermal accumulation. We show the functional evaluation in vitro and in vivo of AG1529, a potent TRPV1 soft antagonist. The neuronal activity of AG1529 was studied on capsaicin-evoked action potential firing from primary sensory neuronal networks established onto planar multielectrode arrays (MEA). AG1529 significantly attenuated capsaicin induced neuronal excitability in both basal and algescially-sensitized nociceptor cultures. Consistent with the similarities among thermoTRP channels, AG1529 marginally reduced neuronal firing induced by AITC and menthol, agonists of TRPA1 and TRPM8 respectively. Notably, AG1529 displayed an in vivo strong, dose-dependent anti-pruritic activity in a rodent model of histaminergic itch when administered systemically or topically. This anti-pruritus effect was devoid of the normal nocifensive action evoked by capsaicin. In vitro enzymatic assay show that AG1529 is hydrolysed by esterases. Thus, AG1529 is a promising capsaicin-based soft antagonist candidate for drug development as an anti-pruritic drug. Funded, SAF2015-66275-C2-1, RTC-2017-6507-1 (MCIU), PAR (UMH).

(P051)

Evaluation of wheat gluten protein hydrolysates on inflammation and oxidative stress in primary human monocytes

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Wheat gluten, a Pro-rich dietary protein, was studied for its potential to produce functional protein hydrolysates during enzymatic hydrolysis with Alcalase 2.4 L. The immunomodulatory properties of those hydrolysates were tested by measuring the inhibition of thrombin and angiotensin-converting enzyme (ACE). The best results were found in hydrolysate obtained after 45 min of hydrolysis with Alcalase (A45), which inhibited 66% of ACE activity and 25% of thrombin activity. The anti-inflammatory activity of this hydrolysate (at 50 and 100 µg/mL) was studied in monocytes stimulated with lipopolysaccharide, finding that it reduced the mRNA expression of IL-1β in a dose-dependent manner. Moreover, TNF mRNA expression was suppressed at the highest concentration of hydrolysate. Regarding expression of the anti-inflammatory cytokine IL-10, it was only increased at 100 µg/mL. In conclusion, wheat protein hydrolysates showed potential anti-inflammatory activity by modulation of cytokines expression.

(P052)**A cellular study of different methods for lipoaspirate processing in the surgical treatment of dysphonia**

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INTRODUCTION: Autologous fat is an extensive resource used in reconstructive surgery by the ease of obtaining and the cellular volume it provides. Liposuction techniques provide samples containing adipocytes and lower concentrations of other cells such as mesenchymal stem cells. The objective of this study is the characterization of the cellular content of liposuction samples processed by seven methods for the surgical treatment of dysphonia.

METHODS: Lipoaspirate samples were obtained from the upper periumbilical region of 10 voluntary patients. Each sample was processed using seven different techniques: decanting, decanting and washing with physiological saline, centrifugation at different speeds, combination with lidocaine, and emulsion with different passes between syringes. Liposuction samples were processed with collagenase, filtered and centrifuged. Cell viability was evaluated, and the cells cultured in standard conditions. Morphology and cellular proliferation were evaluated by hematoxylin staining, and fat production by red oil, at 24, 48 and 72 h. Cellular phenotype of initial cell suspension and cultured cells was evaluated by flow cytometry with specific antibodies for CD90, CD105, CD34, CD31 and CD45 markers.

RESULTS: In decantation procedures, more cells and preadipocytes were obtained versus centrifugation. However, proliferation at 48h of culture was higher (3 fold) with the cells obtained by centrifugation. The initial cell suspension contained 3.7% CD90+, 3.7% CD31+ and 0.1% CD45+ cells respect to total cells. The percentage of mesenchymal stem cells in culture (CD90+, CD34+, CD45-, CD31-) was significantly different among patients, but similar among the different methods of processing.

CONCLUSION: Lipoaspirate cells show different cellular markers between the initial suspension and the culture. This study indicates minimal differences in the number of mesenchymal stem cells obtained by different fat processing for the surgical treatment of dysphonia.

(P053)

In vitro characterization of a TRPV1 soft antagonist

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TRPV1, a member of the transient receptor potential (TRP) family, is a nonselective calcium permeable ion channel gated by diverse stimuli (capsaicin, T, pH and voltage). TRPV1 plays a fundamental role in the pathogenesis of dermatological diseases specially related to inflammation, pain and pruritus. Accordingly, the development of new TRPV1 antagonists is yet a high priority. To address this issue, we have designed capsaicinoid molecules that incorporate an ester group in the lipophilic moiety. These compounds have the advantage of undergoing deactivation (soft drug concept) once their desired local effect has been exerted, thus preventing its long-term dermal accumulation. Here, we report the pharmacological evaluation of AG1529 on heterologously expressed human TRPV1 by patch clamp using whole cell configuration. Electrophysiological characterization of AG1529 was further analysed on primary sensory neuronal networks established onto planar multielectrode arrays (MEA). We found a mean IC₅₀ value of 0.92 (0.28 to 3.01) μ M. AG1529 reversibly blocked the channels, with completed washout after 5 min of stopping its instillation. Furthermore, AG1529 is a capsaicin competitive antagonist as it displaced the agonist EC₅₀ to higher values. Molecular modelling substantiates an interaction with the capsaicin binding site in TRPV1. AG1529 also blocked other modalities of TRPV1 activation, and had a marginal inhibitory activity of TRPA1 and TRPM8 as compared with that exerted on TRPV1. MEA measurements show that AG1529 attenuated capsaicin induced neuronal firing in both basal and algescially sensitized neuronal cultures. These results unveil the mode of action of AG1529, and substantiate the tenet that this compound is a promising lead for skin therapeutics.

(P054)**Modulation of the chondrocytic inflammatory response by formononetin**

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Introduction: Osteoarthritis (OA) is a pathology characterized by a reduction of the joint space due to a degradation of the cartilage. The predisposing factors are mechanical stress, age, metabolic diseases and inflammation. However, there is no treatment for this disease.

Beer may have anabolic properties in musculoskeletal tissues due to the estrogenic properties of flavonoids such as formononetin (FNT). Catabolic properties are also attributed to it. Accordingly, its consumption was associated with a higher incidence of OA. However, its direct effect on the modulation of chondrocytes' vitality and inflammatory responses are unknown.

Aim: To study the toxicity of FNT and its effect on the inflammatory and catabolic response of chondrocytes.

Methodology: Chondrocytes (ATDC5) were cultured for 24-48 hours in the presence and absence of the inflammatory stimulus (IL-1 0.1ng / mL) and FNT (5, 12.5, and 25uM). The release of nitric oxide was determined through the Griess reaction. The study of the inflammatory markers IL-6, LCN2 and CCL2, and the cartilage structural proteins COL2A1 and ACAN were made by RT-PCR. In the same way, the expression of the transcription factor SOX9 and the metalloproteinase MMP13 were evaluated. For the study of toxicity, the MTT colorimetric technique was used.

Results: FNT did not affect cell vitality or reduce the release of nitric oxide induced by IL-1. The FNT 25uM did not modify the expression of the inflammatory markers studied. In contrast, it decreased the expression of ACAN mRNA and enhanced the expression of MMP13 mRNA. Likewise, FNT potentiated the inhibitory effect of IL-1 on the mRNA of COL2A1, ACAN and SOX9.

Conclusions: In chondrocytes, FNT has no toxic or anti-inflammatory effect. However, it is a potent catabolic agent. Given that FNT is one of the main flavonoids present in beer, its consumption should be evaluated in patients with OA.

(P055)

Hydroxytyrosol and its acetylated derivatives prevent M-CSF/RANKL-induced osteoclastogenesis in human monocytes

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During inflammation, several growth factors and cytokines are increased inducing osteoclast (OC) differentiation and activation. Chronic inflammation is a condition that initiates systemic bone loss. Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterised by active synovitis and associated with early peri-articular bone loss. Hydroxytyrosol (HTy) is an olive oil polyphenol with antioxidant and anti-inflammatory properties.

The present study was designed to investigate the effects of HTy and its derivatives hydroxytyrosol acetate (Ac-HTy) and peracetylated hydroxytyrosol (Per-HTy) on osteoclast differentiation induced by the macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor κ -B (RANK) ligand (RANKL) in freshly isolated human circulating monocytes.

Tartrate-resistant acid phosphatase (TRAP) staining was used as a marker of OC activity. Cell viability was determined using MTT assay. OC-related gene expression and cytokines were assessed by RT-qPCR and ELISA, respectively.

HTy and its acetylated derivatives inhibited osteoclastogenesis by means of a reduction of the TRAP activity and a downregulation of OC marker gene expression (TRAP, RANK, OSCAR and CATHK), while promoted the transcriptional activation of OPG gene in the monocyte-derived OCs. HTy, Ac-HTy and Per-HTy also induced a downregulation of MMP-9 gene expression and an upregulation in the expression of HO-1 gen. Additionally, HTy, Ac-HTy and Per-HTy decreased the release of osteoclastogenic cytokines (TNF- α , IL-1 β and IL-6) and increased the release of IL-10 (anti-osteoclastogenic cytokine) in the medium of the monocyte-derived OCs.

Overall, these results suggest that HTy and its acetylated derivatives are effective inhibitors of M-CSF/RANKL-induced osteoclastogenesis in human monocytes in vitro and may be potent therapeutic agents for bone-related diseases such as RA.

(P057)**Dietary oleuropein and its new acyl-derivate, attenuate murine lupus nephritis through HO-1/Nrf2 activation and suppressing JAK/STAT, NF- κ B and MAPK signaling pathways.**

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Systemic Lupus Erythematosus (SLE) is a chronic inflammatory and autoimmune disease which can affect multiple organ system, without an effective and safe treatment. Olive leaf extracts are of special interest for their therapeutic effects. Oleuropein (OL) is the most abundant constituents of olive leaf extract and possess many beneficial properties. In this study, we evaluated the effects of dietary OL and its new derivate, Peracetylated oleuropein (Per-OL), in a pristane-induced SLE model. Mice received an injection of pristane or saline solution and were fed with experimental diets: enriched with OL and Per-OL. The levels of proinflammatory cytokines and markers were evaluated by enzyme linked immunosorbent assay (ELISA). The protein expressions of nitric oxide synthase inducible (iNOS), microsomal prostaglandin E synthase 1 (mPGES-1), heme oxygenase (HO-1), nuclear factor E2-related factor 2 (Nrf2), mitogen-activated protein kinases (MAPKs), Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear transcription factor-kappa B (NF- κ B) pathways activation were determined in kidneys by Western Blot. OL and Per-OL significantly reduced renal damage and decreased serum matrix metalloproteinase 3 (MMP-3) and prostaglandine E2 kidneys levels. Our findings indicate that Nrf2 and HO-1 antioxidant protein expressions were up-regulated in mice fed with OL and Per-OL diets, whereas the activation of JAK/STAT, MAPK and NF- κ B pathways were significantly ameliorated. These results suggest that OL and Per-OL supplementation might provide a new alternative approach as a preventive/palliative treatment of nephritis in SLE management.

(P058)**Mitochondrial Na⁺/Ca²⁺ exchanger (NCLX) participates in NLRP3 inflammasome activation.**

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Inflammasomes are multi-protein complexes that process and release interleukins 1 β and 18. Among the different families of inflammasomes, NLRP3 is the most relevant and best described in inflammatory diseases. Mitochondria have been proposed as key elements in NLRP3 activation, however, the mechanisms that underlie this process are poorly understood. The mitochondrial Na⁺/Ca²⁺ exchanger (NCLX) regulates mitochondrial calcium homeostasis and its inhibition in hypoxic and neurotoxic conditions is beneficial in different cell types. In this context, we asked if NCLX inhibition could affect NLRP3 inflammasome activation. To this end we study NLRP3 activation in mouse bone marrow-derived macrophages (BMDMs) using the compound ITH12575, a specific inhibitor of NCLX. After priming BMDMs with LPS, we stimulated the cells with ATP and we observed that NCLX inhibition by ITH12575 reduces IL-1 β release in a concentration-dependent manner. This reduction was over a 70% with ITH12575 treatment at 10 μ M. We had previously observed this effect in mouse peritoneal macrophages and in mouse microglial cells. To assess if the effect of NCLX inhibition was specific over the NLRP3 inflammasome, we treated LPS-primed BMDMs with Flagellin and Poly dA/dT to activate NLRC4 and AIM2 inflammasomes, respectively. In these conditions, IL-1 β release in BMDMs was not reduced by co-treatment with ITH12575, which suggests a specific effect of this compound on NLRP3 inflammasome. We also studied the effect of ITH12575 on the priming phase of NLRP3 activation. BMDMs were treated with LPS \pm ITH1275 10 μ M and TNF- α and NLRP3 inflammasome protein expression levels were measured. LPS induced an increase in NLRP3, pro-Caspase 1 and pro-IL1 β expression levels that was reduced by ITH12575. However, TNF- α release was not reduced by ITH12575. From these results we can conclude that NCLX inhibition by ITH12575 reduces IL-1 β release, being this effect specific over the NLRP3 inflammasome.

(P059)

Characterization and evaluation of hemp protein hydrolysates on neuroprotection

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Hemp (*Cannabis sativa* L.) seeds are well known for their potential use as a source of food-nutrients, dietary fiber, and bioactive compounds. A hemp seed protein isolate (HPI), prepared from defatted hemp seeds, was hydrolyzed by Alcalase or Alcalase-flaoryzyme under specific conditions. The resulting hydrolysates were evaluated to the selection of potentially bioactive hemp seed protein hydrolysates (HPHs) through DPPH scavenging and angiotensin-converting enzyme-inhibitory activities. In vitro cell-free experiments led to the identification of two 2 bioactive HPHs, HPH3 and HPH7, which were used at 50 and 100 $\mu\text{g}/\mu\text{L}$ on BV2 microglial cells in order to evaluate the anti-inflammatory and neuroprotective activities. RT-qPCR techniques were used to analyse the mRNA transcriptional levels. Our results showed that HPH3 and HPH7 down-regulated the mRNA transcriptional levels of IL-1 β , and TNF- α in BV2 microglial cells stimulated with lipopolysaccharide. In addition, HPH3 and HPH7 promote M2 microglia polarization, down-regulating iNOS and up-regulating Arg-1. This study suggests for the first time that HPHs consumption may improve neuroinflammatory and chronic inflammatory states, confirming that hemp seeds are a valuable source of bioactive compounds.

(P060)

Oleocanthal modulates LPS-induced murine peritoneal macrophages activation via regulation of inflammasome and Nrf-2/HO-1 signaling pathways

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Oleocanthal represents approximately 10% of total phenolic compounds in extra virgin olive oil and may contribute, in part, to reported health benefits of following a Mediterranean style diet. The present study was designed to investigate the role of the canonical and non-canonical inflammasome and NRF-2/HO-1 signalling pathways involved in the antioxidant and anti-inflammatory activities of oleocanthal in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages. Isolated cells were treated with oleocanthal in the presence or absence of LPS (5 µg mL⁻¹) for 18 h. Oleocanthal showed a potent reduction of reactive oxygen species (ROS) (25 µM, 50,612±0,02; 50 µM, 53,665±0,09; 100 µM, 52,839±0,02) when compared with LPS-DMSO treated control cells. In term of enzymes protein expression, oleocanthal was able to up-regulated Nrf-2 (25 µM, 1,57±0,01; 50 µM, 1,54±0,01; 100 µM, 1,63±0,05; p<0.05) and HO-1 (25 µM, 2,12±0,03; 50 µM, 2,24±0,01; 100 µM, 1,92±0,05; p<0.01) expression in comparison with LPS-DMSO cells. Moreover, oleocanthal inhibited canonical (NLRP-3, ASC, pro-caspase-1 and cleaved caspase-1) and non-canonical (pro-caspase-11, partially-cleaved and cleaved caspase-11) inflammasome signalling pathway. Thus, oleocanthal might be a promising natural agent for future treatment of immune-inflammatory diseases.

(P067)

Antiinflammatory effect of osteostatin in monosodium urate crystal-induced gouty arthritis model

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The development an inflammatory response in gouty arthritis is associated with articular and periarticular deposition of monosodium urate (MSU) crystals, which induce cytokine production by recruited and tissue resident cells. IL-1 β is one of the main pro-inflammatory cytokines involved in the process as a consequence of caspase-1 activation by MSU[1].

Osteostatin (OT) is a fragment of the C-terminal parathyroid hormone-related protein (PTHrP) (111-117) which previously demonstrated anti-inflammatory properties in the mouse air pouch model by inhibition of cytokine release and caspase-1 activation[2]. In the present study, we have evaluated the effect of OT in a mouse gouty arthritis model induced by MSU in order to confirm the potential anti-inflammatory profile of this peptide.

MSU crystals (2mg/50 μ l PBS) were subcutaneously injected under the plantar surface of the right hind paw. 1h before and 12h after MSU injection, OT (80 and 120 μ g/kg in 0.1ml saline) or vehicle were subcutaneously administered into the back area. Inflammatory paw swelling was quantified at 1, 3, 6, 9 and 24h after MSU injection using a plethysmometer. Then, mice were sacrificed and paw tissue was homogenized for cytokine detection by ELISA, MPO assay, and western blot study of active p20 subunit of caspase-1.

Results demonstrated that administration of OT at the highest dose significantly reduced paw swelling as well as IL-1 β (82%), TNF- α (39 %), IL-6 (75%) and CXCL-1 (81%) levels. MPO activity was also significantly inhibited (96%). All these results and the decrease of p20 subunit in caspase-1 after OT administration, demonstrate the interesting profile of this peptide in the treatment of gouty arthritis and other acute inflammatory processes.

[1] H.E.Lee, G.Yang, N.D.Kim, S.Jeong, Y.Jung, J.Y.Choi, H.H.Park, J.Y.Lee. Scientific Reports. 2016, 9; 38622.

[2] L.Catalan; J.Nacher-Juan; M.C.Terencio; M.L.Ferrándiz; M.J.Alcaraz. II National Congress of Young Researchers in Biomedicine, Valencia. 2017, 179.

Teaching

(P061)

Promoting in- and out-class engagement in a subject of Pharmacology

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In the last decades new tools have emerged and applied to stimulate an active participation of students, facilitate a continuous follow-up of their activities, and improve teacher-student interaction. The aim of this study was to assess the impact of different in- and out-class strategies on learning performance of students in the course Food-Drug Interactions (Degree in Food Science and Technology). Several strategies were integrated and carried out in this course: out-class Moodle questionnaires to prepare for the exams, articles to discuss in the classroom, the use of Socrative application at the beginning and at the end of every theoretical class, and the design of a triptic on a specific food-drug interaction. A satisfaction survey was also performed to know their opinion on the strategies developed. 25 students took part in this study. All of them discussed the articles, designed in pairs a triptic, and answered Socrative questions. 96% used Moodle questionnaires to review the lessons learned (most used the three attempts allowed). Regarding Socrative, mean scores increased significantly from 2.8 points before explaining the topic to 8.2 at the end of the class. 56% of students filled the satisfaction survey. They considered that Moodle questionnaires had favoured to study the subject. As for Socrative, they thought that questions made them be more attentive in the classroom. Moodle questionnaires were the best activities for 57.1%; Socrative for 28.6%, and triptics for 14.3%. They thought that these strategies improved their interest and engagement with the subject, and all of them were satisfied (21.4%) or very satisfied (78.6%) with the activities developed. Implementing different in- and out-class activities helps promote the interest and motivation of students, especially when they are addressed to favour the study of the subject.

(P062)**Flipped classroom based on objective structured clinical examinations analysis by undergraduate students of Pharmacology course from the Podiatry Degree improve their learning and assessment communication skills about medicines.**

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Background and Aims: The flipped classroom (FC) combine online learning and face-to-face classroom activities. In FC students engage in content learning before class, thereby maximising in-class time for active learning. Objective Structured Clinical Examinations (OSCEs) are versatile multipurpose evaluative tools usually utilized to assess health care professionals in clinical setting. OSCE include communication skills and ability to handle unpredictable patient behaviour usually not included in the traditional clinical exam, and it can be quite useful used as simulation for students' analysis and learning. We aim to evaluate the impact of the individual and in-group analysis of OSCEs (filmed by other students) looking for correct and incorrect behaviours and contents in their learning of pharmacology.

Summary of work and outcomes: A 5-year prospective study in which students of Pharmacology course from the Podiatry Degree analysed filmed OSCEs individually and in a group in a flipped classroom. Each group (max 5 students) analysed along 1 week a filmed OSCE, prepare a summary of correct and incorrect items related to clinical events, medicines uses, and people behaviours. The students presented their analysis results to the rest of the class. After each presentation, the other students of the class were encouraged to ask questions and after that, the students voluntarily answered a satisfaction survey.

Result and Discussion: 405 students, 65.2% female, 20±5.3 years old were included. Students spend 13.4±5.4 h on making the filmed-OSCE analysis. The percentage of students satisfied with this way of studying pharmacology was 96.5%. OSCEs analysis by students increased their percentage of success in the final assessment in both OSCEs-related and OSCEs-non-related questions (+18.5% and +10.1%).

Conclusion: Filmed Objective Structured Clinical Examinations analysis by undergraduate students on the Pharmacology course of Podiatry Degree improved their knowledge about medicines use and their communication skills during the assessment.

(P063)**Preventing online cheating with Smowl in a subject of a Master Degree: a pilot study**

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Online education programs at Universities are growing in popularity, as they give students more flexibility to manage their study time at their convenience, and to balance their work and family commitments. Universities are also facing the challenge of a positive identification of students to avoid the occurrence of cheating. In this survey a proctoring tool (Smowl) was assessed in a subject (Cerebrovascular disease) of a Master Degree (Master Degree in Innovation in Biomedical and Health Sciences, University of Leon). Students who voluntarily decided to take part in the pilot-study were asked to register in the tool before answering online questionnaires through Moodle. Acceptance was high among students: 100% students from the face-to-face modality and 95.7% from the online one collaborated in the survey. The tool identify students by taking photos at defined time intervals. There was no incident in 95.5% (face-to-face modality) and 84.1% (online modality) of the photos taken. In the face-to-face modality, 3.6% of these photos showed that students had opened another window during completion of questionnaires, and in 0.9% there was a mistake with the camera. Regarding online modality, the most frequently incidents reported by the tool were camera disabling because it was being used by another application (7.7%), and the presence of more of one person in front of the camera (3.6%) A satisfaction survey was also used to assess students' opinion. Those from face-to-face modality showed a higher satisfaction than students from online modality, although in both modalities they conceded that it would be useful to track them in online tasks. Proctoring tools are interesting to verify remote student identity, but there are certain weaknesses (camera disabling) that is necessary to solve.

(P064)**Service-Learning, a good methodology for doing final degree and master's projects**

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The Final Degree Project (FDP), as well as the Final Master Project (FMP), intend to help the students implement the skills that they have gained during their studies and ensure that they acquire the relevant competences associated with their Degree or Master. The service-learning is an educational and research proposal that integrates the service to the community and academic learning in a common project that allows students to work on real needs directly related to their future profession. The student, supervised by the teacher, selected the context of the service and defined both the service and the learning objectives, stressing how important it was that paid attention to the real needs of their environment. The student design the project and plan all the activities in detail; develop the different planned phases and write the FDP or FMP memory. The evaluation of each project is performed by both, the supervisor and a teachers' court. Up to now, 8 projects have been carried out with this methodology, which have addressed different problems related to health education for people with diabetes or with AIDS as well as for people at risk of social exclusion. Other projects have been oriented to review the use of medication for elderly patients living alone. The comprehensive training of university students focuses on a competency-based learning approach that integrates all dimensions of personal, academic, social and professional development. Service-learning methodology can have benefits for students at all these levels and is very attractive to them. The development of these projects reinforces their learning and helps them to get involved in the health needs of their immediate environment. The incorporation of this teaching methodology in the last step of university education is very positive as it enables the students to work both cross-cutting and specific skills acquired along all their studies, as protagonists of their learning.

(P065)**Robotics applied to research and teaching of Pharmacology: opinions of students of different Degrees**

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Introduction: An interdisciplinary collaboration between Pharmacology and Computer Architecture was developed. Teachers and students from the Robotics course programmed robots trying to simulate the usual behavior of a mouse by using artificial vision and backtracking navigation.

Material and methods: A free response survey was passed to the students of the Biotechnology, Veterinary and Nursing degrees after they completed a practice in which experimental animals and alternative method were used in order to assess their opinion. In the practice, the legislation on the use of experimental animals was also reviewed.

Results and conclusions: 91% of Biotechnology students considered positive the use of robots instead mice. In Veterinary and Nursing, respectively, 87% and 97% of students gave a similar answer. 35% of Biotechnology students thought that robots could be a good model to evaluate drugs, and in the same way, 21% and 22%, respectively, of Veterinary and Nursing students believed this.

As negative aspects, 96% Biotechnology indicated that robots did not equal animal behavior, the real effect cannot be seen or robots are unreliable. Nursing (97%) and Veterinary students (88%) thought that robots cannot act like a living being. No significant differences were found in the answers ($p \leq 0.05$).

(P066)**Use of experimental animals in research and teaching of Pharmacology: opinions of students of different Degrees**

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Introduction: The same Pharmacology practice was taught to Biotechnology, Veterinary and Nursing students. Legislation on experimental animals and the use of alternative methods was reviewed

The objective of the study was to know the opinion of students of different degrees on the use of experimental animals.

Material and methods: To carry out the study a free response survey was passed to the students of the Biotechnology, Veterinary and Nursing degrees after they completed a practice in which the neuroleptic action of promazine in mice was evaluated and the legislation on the use of experimental animals reviewed.

Results and conclusions: 96% of Biotechnology students considered the use of mice positive because they bring the results obtained closer to reality. In Veterinary and Nursing, respectively, 87% and 90% of students gave similar answers (no significant differences, chi-square test, $p \leq 0.05$). As a negative aspect, all students of Biotechnology considered that damage or stress can be caused to mice. 71% and 91% of Veterinary and Nursing students agreed with this opinion (significant differences, chi-square test, $p \leq 0.05$). 32% (Biotechnology), 27% (Veterinary) and 7% (Nursing) thought that the use of experimental animals was good for learning (significant differences, chi-square test, $p \leq 0.05$).

5. Collaborators & Sponsors

Collaborators



Sponsors

