CONFERENCES

1
INFLAMMATORY PATHWAYS AS NEW THERAPEUTIC TARGETS FOR THE TREATMENT OF SCHIZOPHRENIA

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In the past decade, there has been renewed interest in immune/inflammatory changes and their associated oxidative/nitrosative consequences as key pathophysiological mechanisms in schizophrenia. Both brain cell components (microglia, astrocytes and neurons) and peripheral immune cells have been implicated in inflammation and the resulting oxidative/nitrosative stress (O&NS) in schizophrenia. Also, down-regulation of endogenous antioxidant and anti-inflammatory mechanisms has been identified in biological samples from patients, although the degree and progression of the inflammatory process and the nature of its auto-regulatory mechanisms vary in the different stages of the illness, from the early onset to full-blown disease. This oral presentation focuses on the mechanisms whereby inflammation and O&NS damage brain cells in schizophrenia, the possible origins of increased O&NS and inflammation in the disease, and current pharmacological strategies to deal with these processes (mainly treatments with antioxidants or anti-inflammatory drugs as add-ons to antipsychotics).

2
ADIPOKINES AND ENDOTHELIAL DYSFUNCTION

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In the last years, the adipose tissue has been identified as a true endocrine organ releasing a heterogeneous series of compounds globally termed adipokines. The adipose tissue expansion observed in obesity and type 2 diabetes is associated with inflammation and imbalanced secretion of adipokines. This has been in turn linked to local insulin resistance and systemic endothelial dysfunction, which is considered as an early marker of vascular disease and atherosclerosis. In this context, growing attention has been paid to the potential role of adipokines in altering vascular homeostasis and promoting vascular complications associated with obesity and type 2 diabetes.

Visfatin was identified in 2005 as an adipokine preferentially released by visceral fat. Visfatin is structurally identical to the extracellular form of nicotinamide phosphoribosyltransferase (Nampt), which enzymatically converts nicotinamide into nicotinamide mononucleotide (NMN). In metabolic disorders, such as diabetes mellitus, obesity and the metabolic syndrome, an over-production and release of visfatin/Nampt has been described. Indeed, elevated circulating levels of visfatin/Nampt have been found in association with endothelial dysfunction, carotid atherosclerosis and acute coronary syndromes, suggesting that this molecule could be a marker of vascular damage. Moreover, it has been proposed that visfatin/Nampt could also be an effector molecule in altering vascular homeostasis and promoting endothelial dysfunction. We have indeed demonstrated that visfatin/Nampt does not alter the contractility to noradrenaline but it impairs the endothelium-dependent relaxation evoked by acetylcholine in both murine and human isolated microvascular segments.

Visfatin/Nampt can directly activate NADPH oxidase in murine microvessls and cultured human umbilical vein endothelial cells, and the NADPH oxidase inhibitor apocynin can indeed prevent the impaired relaxation induced by the adipokine.

Moreover, visfatin/Nampt was able to promote inflammation in human vascular smooth muscle cells by consecutively activating the ERK 1/2-NF-κB-iNOS axis. Since obesity and type 2 diabetes are considered as progeric diseases causing accelerated vascular aging, we also explored the impact of visfatin/Nampt on human endothelial cell senescence, a condition associated to endothelial dysfunction and the development of atherosclerosis. Indeed, visfatin/Nampt promoted cell senescence in cultured human umbilical vein endothelial cells in a concentration and time-dependent manner.

Hydrogen peroxide mimicked the pro-senescence effect of visfatin/Nampt. In contrast, inhibiting NADPH oxidase with apocynin prevented visfatin/Nampt-induced senescence. Consistent with the activation of a pro-senescence signalling mechanism, visfatin/Nampt increased total and telomeric DNA damage and p53 levels. All these deleterious actions of visfatin relied on its intrinsic nicotinamide phophorybosil transferase (Nampt) enzymatic activity, since they were blocked by the pharmacological Nampt inhibitor FK866 and mimicked by the product of the reaction NMN.

Globally, these findings indicate that visfatin/Nampt may impair vascular homeostasis in terms of reactivity, inflammation and senescence. Therefore, this adipokine may represent a potential pharmacological target to prevent the development of vascular complications in the context of metabolic diseases.

3
AGEING, INFLAMMATION, AND VASCULAR DYSFUNCTION

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Vascular ageing is a key process determining health status of aged population. Ageing is an independent cardiovascular risk factor associated to an impairment of endothelial function representing a crucial event in the development of many different vasculopathies. Vascular ageing, formerly being considered an immutable and inexorable risk factor, is now viewed as a target process for intervention in order to achieve a healthier old age. A further knowledge of the mechanisms underlying the agerelated vascular dysfunction is required to design an adequate therapeutic strategy to prevent or restore this impairment of vascular functionality. Among the proposed mechanisms that may contribute to the age-dependent vascular dysfunction, this communication is focused on the following aspects occurring into the vascular wall: (1) the reduction of nitric oxide (NO) bioavailability, caused by diminished NO synthesis and/or by augmented NO scavenging due to oxidative stress, leading to peroxynitrite formation (ONOO-); (2) the role for other vasodilator mechanisms, such as the endothelium-dependent hyperpolarising factor; (3) the possible sources involved in the enhancement of oxidative stress, such as an enhanced activity of NADPH oxidase or the uncoupling of NO synthases (NOS); (4) the increased activity of vasoconstrictor factors, produced by alterations of the cyclooxigenase (COX) pathway or by the reninangiotensin system; (5) the development of a low-grade pro-inflammatory environment, due to an enhanced role for inflammatory cytokines, activation of nuclear factor-κB, and expression of enzymes, such as inducible NOS or COX isoforms. Moreover, the possible synergisms and interactions between all these pathways are also analysed. A brief summary of some cellular mechanisms related to endothelial cell senescence are also implemented, as they are likely involved in the age-dependent

vascular dysfunction, as well as in the lower vascular repairing capacity observed in the elderly. Finally, some new pharmacological interventions that could interfere with some of those mechanisms leading to vascular dysfunction are discussed, in order to suggest some future therapeutic approaches that can improve the cardiovascular health in older people.

4
BIOLOGICAL MEDICINAL PRODUCTS: CONCEPT AND REGULATION

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Biological medicinal products (BMP) are those in which the participation of living organisms or their extracts (cells, tissues, fluids) are necessary to produce the active ingredient and are obtained by biotechnological methods from recombinant DNA and hybridization processes (1).

To obtain biological medicinal products, recombinant DNA and hybridoma techniques are usually used, mainly incorporating genetic material from living organisms (bacteria, fungi, etc..). In this way these organisms synthesize a product.

The basic chemical structure of these drugs are glycoproteins, because the amino acids necessary for their production are linked forming sequences essential for its correct functions and, therefore, a minimum change in folding cause alterations in their efficacy and tolerability. This explains that throughout the production process strict control in order to preserve the inherent pharmacokinetic and pharmacodynamic product also ensuring their effectiveness, tolerability and safety are required (2).

Moreover, the immunogenicity plays a critical role in the effectiveness of biological medicine, because it can induce a loss of effectiveness, causing it to have to adjust the dose or prolonging treatment administration time (3, 4).

Due to the complex and precise process involving the development and production of biological drugs, they have substantial differences from the standard drugs. These latter consist of molecules of smaller size, have a more simple chemical structure, its molecular weight is smaller and are obtained by physicochemical synthesis (5).

There are several points in the production process of a biological drug that must be carefully considered, especially stability, compatibility and biological activity in pre-formulation or formulation studies.

Characterization, on the nature and properties of the active substance and the excipients in the formulation of the product: molecular size, charge and surface properties. Furthermore, it must describe the structural elements responsible for the biological activity, active sites, receptor and ligand binding sites and the features responsible for signal transduction. They should also be evaluated interactions between the active ingredient and excipients, and to identify and characterize the existence of immunogenicity.

Manufacturing process. The quality of biological products is defined by the kind of production and manufacturing process. Small changes in the process may significantly affect the quality of the product. The development of the manufacturing process is very important in this type of products. Therefore, the parameters defining the manufacturing processes must be well specified for assess the issues related to product quality. Essential part in the process is to maintain aseptic conditions during the manufacturing process, since in most cases it is not possible to subject the products to sterilization.

Compatibility. It is very important to establish the possible interaction of the active substance with the excipients of the final pharmaceutical formulation form of the product. The appearance of adducts or modifications in the activity of the protein surface could be the explanation of some immunogenicity events and possible changes in their biological activities.

Stability of the active substance must be clearly defined, including degradation pathways, and the formulation and the conditions of manufacture and storage, they can influence their possible degradation. The stability of the formulated product or active substance should be evaluated under different process conditions.

The development and administration of the BMP is subject to specific regulations and requirements by regulatory agencies to ensure their effectiveness and safety (3,6). Due to its characteristics and the increasingly frequent use of molecular biology techniques, regulatory agencies have imposed new demands on the manufacturing technology of these drugs (7). The special regulation is conditioned by the lability and complexity inherent in the biotechnological and biological products.

References:

1. Ferro A, Boyce M. Biological therapies: a long way on from Jenner. Br J Clin Pharmacol. 2013;76:161–3.
2. Brown KR. The regulation of biological products. Eur J Clin Microbiol Infect Dis. 1990;9:502–5.
3. Chirino AJ, Mire-Sluis A. Characterizing biological products and assessing comparability following manufacturing changes. Nat Biotechnol. 2004 Nov;22(11):1383–91.
4. Schellekens H. Bioequivalence and the immunogenicity of biopharmaceuticals. Nat Rev Drug Discov. 2002;1(6):457–62.
5. Honorato J. Diferencias entre medicamentos de síntesis química y de origen biotecnológico. Rev Esp Econ Salud. 2007;6:334–8.
6. Johnson IS. The trials and tribulations of producing the first genetically engineered drug. Nat Rev Drug Discov. 2003;2:747–51.
7. Iglesias MC, González-Correa JA, Medina U, Tejerina T. Desarrollo y Regulación de Medicamentos Biotecnológicos. Actualidad en Farmacología y Terapéutica. 2013;11:223–8.

5
CONTRIBUTION AND CLINICAL USE OF BIOLOGICAL DRUGS: MILESTONES AND PROSPECTS

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A biological drug is a medical product of biotechnological origin developed from DNA-derived proteins and hybridization processes, which require living organisms as a key component in the production process. The importance of biological drugs can easily be established by its simple enumeration. The list of biologicals include blood factors, thrombolytic agents, hormones, as insulin or growth hormone, hematopoietic growth factors, including erythropoietin and colony stimulating factors, interferon, interleukins, vaccines, monoclonal antibodies and some additional products as tumor necrosis factor or therapeutic enzymes. The biologicals permit a wide access to own biological components as insulin or erythropoietin, a fundamental change in the equitative access to health market, but especially are very innovative in the topic of new therapeutic targets based on advanced biotechnology.

A good example of the relevance of biologicals is the use of monoclonal antibodies against tumor necrosis factor alpha in the field of inflammatory bowel diseases (IBD). This treatment has represented a therapeutic revolution for the control of IBD. At present in Spain, two biologicals are largely used in this indication: Infliximab (IFX) and Adalimumab (ADA). Effectiveness data are strong for both therapies, with maximum levels of scientific evidence. Recently, Golimumab, a third monoclonal antibody, has also been approved for ulcerative colitis. These monoclonal antibodies have changed the lives of millions of people around the world that signifies more life, more quality of life and less adverse effects. As prospect, a better understanding of IBD pathogenesis is facilitating the research of novel therapeutic targets not only focused on the inhibition of inflammation mediators but also intended to enhance cell repair mechanisms.

The research and clinical application of biologicals require a strong cooperation between biotechnological industry, basic researchers, clinical researchers, practitioners and regulatory and authorities agencies. The biologicals are very complex and expensive treatments. We need excellent evidence, a very careful patient selection, good registries and specially a validated system to evaluate its results and costs.

The approved biologicals have demonstrated efficacy in clinical trials, but their true long term effectiveness only can be demonstrated by the clinical use. And that implies a strong clinical vigilance. This clinical pharmacovigilance is especially important for the biosimilars. A biosimilar is not identical to its original. If a biosimilar is approved only in the basis of this biosimilarity without the traditional extended clinical research, the possible differences in practice need to be tested in the clinical supervision. The practical conclusion is that the clinicians must always take the decision about its use.

6
CHALLENGE AND OPPORTUNITIES OF MODEL BASED DRUG DEVELOPMENT

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The concept of model-based drug development has evolved as a way to improve drug development knowledge management and decision making processes through the use modeling and simulation techniques. This approach has been recognized by pharmaceutical industry as one of the emerging technologies that can contribute to improve the business model for developing new therapeutically and commercially successful drugs, which ultimately will benefit the patients. In recent years, the field of pharmacokinetic and pharmacodynamic (PK/PD) modeling has advanced from using empirical functions to describe and summarize the data to the utilization of mechanism-based models. Mechanism-based PKPD modeling is expected to provide meaningful model parameter estimates and improved predictions of the drug disposition and drug effects, as it incorporates underlying principles of pharmacology, physiology and pathology. Rigorous management of the knowledge generated with the modeling techniques requires the exploration of different scenarios of interest via simulations, which can be utilized to inform many key decisions during drug development.
The added value of implementing MBDD with pharmaceutical industry is illustrated in several case studies. Each of these examples reflects an actual situation with key decisions made based on the knowledge obtained from these models and illustrates the state-of-the art developments that pave the way for the future application of MBDD in pharmaceutical industry. This approach will facilitate the development of better dosing regimens of new medicines, which may lead to enhanced benefit of the drug therapy and improvements in the quality of life of the patients.

7
DELAY OF ALZHEIMER-LIKE PATHOLOGY IN MICE WITH 7,8-DIHYDROXYFLAVONE

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Alzheimer's disease (AD), a devastating age-related neurodegenerative disorder, is the most common form of dementia, currently afflicting 35.6 million people worldwide and with an incidence rate that doubles every 20 years. Global increases in lifespan make the challenge to prevent and treat Alzheimer's disease (AD) urgent. At present, there is no known cure for the disease, owing to poor mechanistic understanding of its pathogenesis. Synaptic degeneration is a major pathophysiological hallmark in AD. Thus, promoting synaptic transmission, synaptic plasticity or synaptic growth through BDNF-based "synaptic repair" strategies might provide a novel therapeutic approach for AD. The neurotrophic function of BDNF is mainly mediated by TrkB receptor which is widely expressed in the CNS and small molecules that can modulate TrkB receptors would be valuable candidates for disease-modifying therapies. Among several ligands targeting the TrkB receptor, 7,8-Dihydroxyflavone (7,8DHF) was recently shown to be specific and to be able to penetrate the blood-brain barrier when administered peripherally. By using an *APP* (amyloid precursor protein) transgenic model of AD (arcAbeta mice) we examined whether sub-chronic oral treatment with 7,8DHF, during the asymptomatic phase, would delay or prevent the overproduction of Abeta and cognitive decline. Our data show that transient oral treatment with the small molecule prevents age-associated decline in hippocampus- and prefrontal-dependent learning and memory. Importantly, drug effects were observable for at least 5 months after discontinuation of the treatment. Importantly, those effects were accompanied by a reduction of Abeta levels in Hipp and PFC and restructuring of dendritic arborization that provide a potential explanation for the cognition-improving effects of the drug. The treatment also normalized expression levels of BACE1, a key proteolytic enzyme involved in the generation of pathogenic derivatives of APP. Ongoing analysis of the BACE1 promoter suggests that epigenetic alterations may be responsible for the long-term effects of 7,8DHF. To our knowledge, this is the first demonstration that a transient, systemically-administered compound can prevent the appearance of AD-like behavioral impairments.

8
EVALUATION EXPERIENCE IN RESEARCH PROJECTS

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The Department of Pharmacology at the UPV/EHU has a nearly 20 years experience in developing a practical activity within the teaching of *General Pharmacology* in the third year of the former *Degree in Medicine and Surgery*. This practical activity consists of the performing by the students of a small research project and it was taken as an active method of getting knowledge, skills and attitudes. Students, in small groups of 5 and with the guidance of a teacher, select a topic; propose a research project (background, hypothesis, objectives, methodology, working plan and economical estimate), perform the experiments, data analysis and interpretation of the results and prepare the presentation of their work for its exhibition and defense in the "*Congress of Pharmacology Students*" at the end of the academic year. Following the regulations that govern the development of curricula for adaptation to the European Higher Education Area (EHEA), and that establish the requirements for official University Medical Degrees, the UPV/EHU considered the value of this teaching methodology and included the subject "*Research Project*" into the Module 2 of the new *Degree in Medicine.* Nowadays*, "Research Project" is* an interdepartmental annual subject of 6 ECTS in the third year of this Degree. Its overall objective is the acquisition of basic training for research activities by the Medical student. This new subject represents a challenge for the objective individual student assessment for several reasons: 1) it basically consists of practical work with few theoretical content; 2) it is mainly a teamwork which makes difficult to distinguish the individual contribution of each student; 3) the evaluation is by continuous assessment, without exams; 4) the high number of students lead to many groups of work and to the requirement of a quite large number of tutors for their supervision that, in addition, belong to different departments. This last factor is an important additional difficulty that reinforces the need to standardize as much as possible the method for student assessment in order to avoid inequalities. Among the aspects evaluated in this subject there are four basic points: 1) Attendance at theoretical and practical sessions (80% minimal attendance, 20% of final mark); 2) The student involvement in teamwork and the aptitudes displayed (some different items evaluated, 25% of the final mark); 3) Each student must prepare a report about the activities conducted throughout the course (questionnaires, bibliography search, laboratory notebook, etc.; some different items evaluated; 30% of the final mark) and 4) The communication skills and command of techniques in knowledge dissemination are evaluated by some specific documents prepared by the research group such as the research project application

form and the congress abstract, poster, and oral communication (some different items evaluated, 25% of the final mark). The periodical revision of this evaluation system is necessary for the identification of its gaps and for its improvement.

9
EXPERIENCES OF EVALUATION IN CLINICAL PHARMACOLOGY: OUR MODEL IN THE JOINT DEGREE OF MEDICINE UAB-UPF

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Teaching of clinical pharmacology has some singularities as a consequence of being a *bridge discipline* between basic and clinical subjects, and it has an intrinsic value in showing medical students which is the better use of drugs in their future patients. The picture is still complicated by the continuous change in marketed drugs. In consequence, the evaluation should consider the acquisition of the basic knowledge as well as the ability to maximize the effectiveness of pharmacological treatments. Our clinical pharmacology course is based in three pedagogical methods: lectures, practical activities and seminars. Lectures are used to introduce the basic clinical pharmacology concepts and this is the most important objective of this activity. Some lectures introduced mini-cases. These consist of a five-line text with two questions on the subject of the lecture. They were delivered just before the lecture, and students were given five minutes to answer the questions at the end. Practical activities include the basic skills that can be learned in pharmacology and simulation laboratories. Seminars allow the work in small groups to discussing specific aspects which are of special importance for the rational prescribing as, for example, the critical reading of medical literature, the preparation of therapeutic plans or beliefs of general population on drug treatments.

We follow the model based in the formative and evaluative assessments using a polynomial approach. Each term of the polynomial consider the relative weight of teaching activities to the total number of ECTS credits. Formative assessments are optional and allow information about the reaching of educational objectives to students and teachers. Lecture content is evaluated using the traditional multiple choice question approach and a short essay discussing a clinical case. Practical activities are evaluated using a case report form designed for each activity and also in a specific session in the final evaluative assessment. Finally, seminars are assessed by short essays prepared by each group of students and delivered a week later of the end of the activity. In the evaluative assessment, several scenarios with questions are given to the students.

In summary, we consider that the structure of the assessment may allow to establish if the students have acquired the basic concepts, are able to perform the basic procedures and get the abilities to practice a rational therapeutics and not only a *spinal reflex-based* prescription.

10
FACING VARIABILITY IN PHARMACOLOGY: USES AND CHALLENGES OF MODELLING AND SIMULATION

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Nowadays, biology is seen as a complex interaction of nonlinear systems, thus leading to an important source of interindividual variability. Hence, as biological systems, our way of falling ill and of responding to drugs, is continuously challenging clinical practice as each one of us can be considered as a unique case.

Recent advances in modelling and simulation (M&S) techniques in biomedicine integrate clinical and pharmacokinetic data, as a way to foresee interindividual variation in treatment response in the real life population. With the addition of a Bayesian perspective, robust models can be achieved by integrating prior knowledge with the data in hand.

M&S are sometimes used synonymously because they both transform a real system in mathematical equations for its control and prediction. In fact, they are distinct in that a model is any mathematical abstraction built from data relating inputs to outputs, whilst simulation builds upon these models by incorporating random variability as a way to understand its long-term impact. In other words, modelling looks back in time, whereas simulation looks forward in time allowing us to make better predictions of future outcomes.

Many in the biomedical community are embracing M&S for different purposes; therapeutic drug monitoring (TDM) is a clear example. This clinical discipline has a strong theoretical framework for improving patient outcomes by assuming that circulating drug concentrations better predict the effect of pharmaceutical agents and clinical outcome than doses.

However, just measuring drug concentrations may not be enough to estimate the optimal systemic exposure for a given patient. Pharmacodynamics (PD) variability proves to be as relevant or even more so than PK variability. In this sense, applying M&S to TDM can contribute to the process of selecting an initial dosage regimen, estimate dosage adaptation from drug concentrations and establish relationships between patient characteristics and PK and PD parameters.

As TDM is a core activity of clinical pharmacology departments in many medical facilities, we decided to learn more about how it is approached. First of all, Dr. A. Terleira will address TDM at a large public hospital, where many patients of many specialties though with scarce clinical data have to be attended to. On the other hand, Dr. B. Valenzuela will comment on her practice in TDM from a M&S approach at a private in-patient facility specialized in oncology. Both lectures are meant to provide us with an overview of TDM as it is practiced currently and may serve to explore the suitability of M&S in different settings such as those with a very demanding workload.

By managing variability, M&S techniques are offering pharmaceutical companies to reduce the number of required studies and to efficiently design them, thus minimizing failure rates and drug development costs significantly. Moreover, M&S is being used as part of regulatory submissions relating to a wide range of areas such as dose non-linearity, dosage adjustment in pediatrics, genotypic differences and propensity for drug-drug or drug-disease interactions.

In this sense, Dr. J.J. Pérez-Ruixo will highlight the challenges, progress, and future of model based drug development (MBDD) in the decision making processes during drug development.

11
GENETIC SCREENS AS A POWERFUL TOOL TO UNCOVER PHARMACEUTICAL TARGETS

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Cancer arises when a set of biological capabilities that include evading growth suppressors, resisting cell death or enabling replicative immortality are acquired. Underlying all the main cancer hallmarks is the generation of genomic instability, which can have multiple sources, including loss of telomere integrity and inactivation of some genes involved in DNA repair (e.g. BRCA1 and BRCA2).

Telomere integrity is crucial to prevent cells from entering senescence, apoptosis or tumorigenesis. When telomeres become dysfunctional, for e.g. due to loss of telomeric proteins, end-to-end fusions occur. These fusions promote genomic instability either through repetitive breakage-fusion-bridge cycles or through tetraploidization and consequent aneuploidy. Importantly, there is a thin line between telomere homeostasis and dysfunctional telomeres, known to cause disease. Understanding the mechanisms by which telomeres become uncapped and how

dysfunctional telomeres escape senescence or apoptosis and trigger tumorigenesis is a crucial quest that is still far from being completed.
Inactivation of DNA repair proteins also greatly impacts in the loss of genomic stability and directly contributes to telomere dysfunction. In the last years several studies have shown genetic interactions between specific proteins involved in DNA damage response. Uncovering more of these interactions is extremely important in order not only to prevent tumorigenesis, but also to contribute for a patient-specific treatment related to the specific mutations or telomere dysfunction.
Here I will present a state of the art strategy to unravel new factors that trigger telomere dysfunction and key molecular mechanisms behind this process. In addition, I will show how the use of genetic screens can be a powerful tool to identify new targets for drug development. Altogether the aim of my work is to develop new tools and strategies and to identify new factors directly involved with telomere dysfunction and ultimately with tumorigenesis.

12
INTERACTION OF SEROTONIN 5-HT2A RECEPTOR AND METABOTROPIC GLUTAMATE RECEPTOR MGLUR2 IN THE PHARMACOLOGICAL ACTIONS OF HALLUCINOGENIC AND ANTIPSYCHOTIC DRUGS

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LSD-like hallucinogenic drugs are agonists of serotonin 5-HT2A receptors (2AR) and can reproduce some of the symptoms associated with schizophrenia. Moreover, atypical antipsychotics display high affinity for 2AR. Drugs that interact with metabotropic glutamate receptors (mGluR) also have potential for the treatment of schizophrenia. Previous work has shown that mGluR2 interacts through specific amino acids with 2AR, to form functional complexes in cortical pyramidal neurons of mouse brain cortex [1]. In postmortem human prefrontal cortex (PFC) of schizophrenic subjects, the described upregulation of 2AR and downregulation of mGluR2 represents a pattern that could predispose to psychosis [2]. However, information about the functionality of 2AR–mGluR2 complex is still scarce and its role and modulation in human brain, further unknown.
Experiments evaluating functional coupling of 2AR to different G-proteins in membranes of human PFC have revealed that hallucinogenic drug (±)DOI signalling through 2AR activates Gαi1/2/3/0/z as well as canonical pathway Gαq showing clear evidence of agonist-directed signalling [3]. In the present study, we observed that (±)DOI (10 μM)-exerted activation of Gαi1 and Gαz proteins was abolished in cortical membranes of mGlu2R KO mice (but unaffected in KO mice of related mGluR3) revealing that 2AR activation by (±)DOI might involve the specific interaction with mGluR2. In membranes of human PFC, co-incubation of (±)DOI (10 μM) with mGluR2/3 agonist LY379268 (10 μM) specifically decreased LY379268 signalling towards Gαi1/z subunits, the pharmacological interaction of (±)DOI+LY379268 being blocked by 2AR antagonist ketanserin (10 μM). These experiments suggest the existence of a 2AR/mGluR2 heteromeric complex in cortical neurons that would enable: first, (±)DOI signalling to inhibitory G proteins and second, the modulation exerted by (±)DOI on mGluR2 agonist signalling through Gαi1/z.
Microdyalisis experiments in mouse cortex showed that local (±)DOI (300 μM)-induced increases in extracellular dopamine concentration were reduced in mGluR2 KO animals, showing the relevance of the 2AR-mGluR2 complex in vivo. Evaluation of prepulse inhibition (PPI) in mice showed that (±)DOI (0.5 mg/kg)-induced PPI disruption was absent in mGluR2 KO mice. Also, clozapine did not revert MK801 (0.35 mg/kg)-disrupted PPI in mGluR2 KO mice opposing the effect observed in wild type animals. In line with this, previous work demonstrated that head-twitch response by (±)DOI and LSD required the expression of mGluR2 [4]. Thus, these results suggest that the effect of hallucinogenic (±)DOI and antipsychotic clozapine in vivo is supported by the existence of 2AR–mGluR2 complex.
In human PFC, competition studies of radioligand binding showed that the affinity of LY379268 displacing [^{3}H]LY341495 was reduced in the presence of (±)DOI (10 μM), the effect of (±)DOI being higher in subjects with schizophrenia. Consistent with a dysregulated 2AR–mGluR2 complex in schizophrenia, competition studies of (±)DOI displacing [^{3}H]ketanserin showed a higher fraction of high-affinity binding sites for (±)DOI in subjects with schizophrenia. Interestingly, high-affinity binding fraction of (±)DOI was absent upon co-incubation of membranes with LY379268 (10 μM). Thus, the dysregulated 2AR–mGluR2 complex in schizophrenia can be modulated by drugs acting at the involved receptors with antipsychotic potential as suggested for 2AR antagonists and mGluR2 agonists.
Acknowledgements: Supported by Spanish MINECO SAF2009-08460, SAF2013-48586-R, the Basque Government (IT616/13) and NARSAD.
References:
1. Moreno JL, Muguruza C, Umali A, Mortillo S, Holloway T, Pilar-Cuéllar F, Mocci G, Seto J, Callado LF, Neve RL, Milligan G, Sealfon SC, López-Giménez JF, Meana JJ, Benson DL and González-Maeso J. Identification of three residues essential for 5-hydroxytryptamine 2A-metabotropic glutamate 2 (5-HT2A·mGlu2) receptor heteromerization and its psychoactive behavioral function. The Journal Of Biological Chemistry 2012; 287: 44301–44319.
2. González-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, López-Giménez JF, Zhou M, Okawa Y, Callado L F, Milligan G, Gingrich JA, Filizola M, Meana JJ, and Sealfon SC. Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 2008; 452: 93–97.
3. Meana JJ. Agonist signal trafficking at serotonin 5-HT2A receptor in human brain: implications for schizophrenia and antipsychotic treatment. Basic and Clinical Pharmacology and Toxicology (2013); 113, Issue Supplement s1, 6.
4. Moreno JL, Holloway T, Albizu L, Sealfon SC, and González-Maeso J. Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT2A receptor agonists. Neuroscience Letters 2011; 493: 76–79.

13
LONG-ACTING INJECTABLE ANTIPSYCHOTICS: FROM PHARMACOKINETICS TO THE BENEFITS FOR THE PATIENT

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Medication compliance is a critical issue across all chronic conditions, including schizophrenia, with non-compliance rates ranging from 55% to 75%. However, compliance is not an all-or-nothing phenomenon, with a continuum from taking all medications as prescribed to partial compliance to complete noncompliance. Partial and non-adherence to medication is a common problem in schizophrenia, leading to an increased risk of relapse, increased likelihood of hospitalization and poorer long-term outcomes. On the contrary, continuous medication in the treatment of schizophrenia is associated with positive outcomes, including improved clinical status, improved quality of life and functioning, and reduced risk of relapse and rehospitalization. Long-acting injectable (LAI) antipsychotics are a pharmacologic strategy for treating patients with schizophrenia with partial or non-compliance to antipsychotic medication. Rather than the daily pill-taking required with oral antipsychotics, LAI antipsychotics are administered by injection at two to four week intervals. LAI antipsychotics may minimize the fluctuations in peak and overall plasma levels compared with oral agents, indicating they may allow more consistent and predictable administration.

The absorption rate constant is slower than the elimination rate constant and therefore, the LAI antipsychotics exhibit 'flip-flop' kinetics where the time to steady-state is a function of the absorption rate, and the concentration at steady-state is a function of the elimination rate. LAI antipsychotics should maximize pharmacokinetic coverage and minimize antipsychotic withdrawal symptoms resulting from partial compliance. In addition, LAI antipsychotics are not influenced by first-pass metabolism, decreasing the potential for drug-drug interactions. Finally, because the slow rate of absorption associated with LAI antipsychotics leads to reductions in differences between (peak) and (trough) plasma levels, they should induce less side effects (an important predictor of poor quality of life), relative to oral antipsychotics.

References:

1. Citrome L. New second-generation long-acting injectable antipsychotics for the treatment of schizophrenia. Expert Rev Neurother. 2013 Jul;13(7):767–83.
2. Dold M, Leucht S. Pharmacotherapy of treatment-resistant schizophrenia: a clinical perspective. Evid Based Ment Health. 2014 May;17(2):33–7.
3. Kishimoto T, Nitta M, Borenstein M, Kane JM, Correll CU. Long-acting injectable versus oral antipsychotics in schizophrenia: a systematic review and meta-analysis of mirror-image studies. J Clin Psychiatry. 2013 Oct;74(10):957–65.
4. Sheehan JJ, Reilly KR, Fu DJ, Alphs L. Comparison of the peak-to-trough fluctuation in plasma concentration of long-acting injectable antipsychotics and their oral equivalents. Innov Clin Neurosci. 2012 Jul;9(7–8):17–23.
5. Spanarello S, La Ferla T. The Pharmacokinetics of Long-Acting Antipsychotic Medications. Curr Clin Pharmacol. 2013 Jan 4.

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MECHANISMS OF ACTION OF TAPENTADOL. AN OPIOID AND MORE. . .

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According to a recent Internet-based Europe-centered survey, on 2008, 1 of 5 European citizens was suffering from chronic pain (Langley, 2011). This estimation suppose than more than 49 millions of persons have pain almost every single day. Despite the existence of a considerable amount of drugs to treat different types of pain (from inflammatory to neuropathic), frequently patients do not receive adequate pain alleviation. There are multiple causes for this lack of efficacy, from individual aspects (from genetic background to personal situations or even social environment), to others directly drug-related, as side effects or lack of efficacy due to tolerance development.

Among all available drugs to treat chronic severe pain, opioids are one of the most used and more efficient ones. Unfortunately, these drugs are not free from side effects, some of them so uncomfortable that can even suppose the abandon of the drug treatment. Those effects include peripheral ones (as constipation, almost always present in chronic treatment and that suppose a severe and potentially dangerous side effect) as well as central ones (from tolerance to addiction).

Despite the fact that doctors expert in pain treatment are able to adequately treat these side effects in almost all situations (from adding laxatives or peripheral opioid antagonists to rotate pain drugs to adequately solve the problem of tolerance or addiction), the need for new opioids, with less side effects and a broader profile, to be used in a more general situations, still a quest for basic research.

Tapentadol is a centrally acting analgesic that shows a dual mode of action: this drug is a mu-opioid receptor agonist and is also able to inhibit noradrenaline reuptake (Tzschentke et al., 2009). This double mechanism offers a singular profile, since its analgesic effect is evident, not only in the situations where an opioid drug offers good pain relief, but also in other chronic pain situations where opioids classically are not so useful, as neuropathic pain.

Tapentadol has been tested in different animal models of pain, from pure nociceptive ones, as tail flick, to more complex-clinically relevant as ostheoarthritis or neuropathic pain (spinal nerve ligation) ((Schröder et al., 2010). Its dual mechanism of action permits tapentadol to show analgesic efficacy in all these pain situations, combining the efficacy of opioid agonism with the inhibition of noradrenaline reuptake, the well-known mechanism of action or antidepressants that is supposed to be the reason of the efficacy of antidepressants on neuropathic pain (Vorobeychik et al., 2011).

The clinical trials have confirm that this drug posses analgesic effect in chronic pain situations, as ostheoarthritis, low back pain of cancer pain. Recent results demonstrate its ability not only to show good analgesic profile, but a lesser incidence of side effects, mainly constipation, and other central effects (Afilalo et al., 2010; Baron et al., 2014).

References:

1. Tzschentke TM, Jahnel U, Kogel B, Christoph T, Englberger W, De Vry J, Schiene K, Okamoto A, Upmalis D, Weber H, Lange C, Stegmann JU, Kleinert R. Tapentadol hydrochloride: a next-generation, centrally acting analgesic with two mechanisms of action in a single molecule. Drugs Today (Barc). 2009;45(7):483–96.
2. Schröder W, Vry JD, Tzschentke TM, Jahnel U, Christoph T. Differential contribution of opioid and noradrenergic mechanisms of tapentadol in rat models of nociceptive and neuropathic pain. Eur J Pain. 2010;14(8):814–21.
3. Vorobeychik Y, Gordin V, Mao J, Chen L. Combination therapy for neuropathic pain: a review of current evidence. CNS Drugs. 2011; 25(12):1023–34.
4. Afilalo M, Etropolski MS, Kuperwasser B, Kelly K, Okamoto A, Van Hove I, Steup A, Lange B, Rauschkolb C, Haeussler J. Efficacy and safety of Tapentadol extended release compared with oxycodone controlled release for the management of moderate to severe chronic pain related to osteoarthritis of the knee: a randomized, double-blind, placebo- and active-controlled phase III study. Clin Drug Investig. 2010;30(8):489–505.
5. Baron R, Martin-Mola E, Müller M, Dubois C, Falke D, Steigerwald I. Effectiveness and Safety of Tapentadol Prolonged Release (PR) Versus a Combination of Tapentadol PR and Pregabalin for the Management of Severe, Chronic Low Back Pain With a Neuropathic Component: A Randomized, Double-blind, Phase 3b Study. Pain Pract. 2014 Apr 17. doi: 10.1111/papr.12200.

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MENOPAUSE, SEX HORMONES AND ENDOTHELIAL FUNCTION

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During their fertile stage, women show a lower incidence of cardiovascular diseases than aged matched men, benefit that disappears after menopause. Therefore, age- and sex-related differences point to sexual hormones as possible cardiovascular protective factors. Among them, estrogen has been long studied in basic and clinical studies.

Estrogens, used as contraceptive agents or as principal constituents of hormone replacement therapy formulations in postmenopausal women, have shown a cardiovascular protective effect in a considerable number of observational clinical studies. However it has not been confirmed by randomized placebo-controlled trials designed to study the effects of hormonal therapy in either secondary or primary prevention[1].

Most of the cardiovascular protective effects shown by 17β-estradiol, the most widely used estrogen molecule, regards to the regulation of vascular endothelium functions[2]. Estrogen promotes the release of vasoactive compounds, such as endothelial-derived nitric oxide (NO) through an increased expression and activity of endothelial NO synthase, and prostacyclin and thromboxane A2, through the regulation of cyclooxygenases expression and activity.

The wide range of vascular actions mediated by estrogens are mainly exerted by estrogen receptors (ER) through rapid and/or genomic mechanisms. Vascular cells, both endothelial and smooth muscle,

express the classic ER, α and β[1], but also the newly described GPER, a G-protein coupled membrane ER[3]. Likewise, a number of ER isoforms have been described so far. In addition, estrogens differently interplay with other sex hormones, mainly with, by modifying the expression of ER and progesterone receptors and by regulating the hormone levels.

Therefore, the current cardiovascular approach in menopause is to resolve discrepancies between the cardiovascular beneficial profile exhibited by estrogens in experimental and some clinical studies and the large randomized trials that could be explained, at least, by two, non-exclusive evidences[4]. On the one hand, the "Timing Hypothesis", which states that estrogen-mediated vascular benefits occur only before the detrimental effects of aging are established in the vasculature. On the other hand, changes in the expression of ER, associated to aging and/or hormone status, that could led to a deleterious balance of ERβ over ERα, generally associated with higher oxidative stress and atherosclerotic plaque formation.

Supported by the Spanish Ministerio de Economía y Competitividad, Instituto de Salud Carlos III –FEDER-ERDF (grants FIS PI13/00617 and RD12/0042/0052).

References:

1. Sobrino, A., Laguna-Fernandez, A., Oviedo, P. J., Novella, S., Cano, A. & Hermenegildo, C. (2009). Gene expression profile induced by estradiol in cultured human endothelial cells. *Acta Physiol.* **195 (suppl. 667)**, 117.
2. Novella, S., Dantas, A. P., Segarra, G., Medina, P. & Hermenegildo, C. (2012). Vascular aging in women: is estrogen the fountain of youth? *Frontiers in Physiology* **3**.
3. Han, G., Li, F., Yu, X. & White, R. E. (2013). GPER: a novel target for non-genomic estrogen action in the cardiovascular system. *Pharmacol Res* **71**, 53–60.
4. Novella, S., Heras, M., Hermenegildo, C. & Dantas, A. P. (2012). Effects of Estrogen on Vascular Inflammation. *Arteriosclerosis, thrombosis, and vascular biology* **32**, 2035–2042.

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MODELING & SIMULATION FOR THE DOSE PERSONALIZATION IN ONCOLOGY THERAPIES

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Numerous studies have found a clear relationship between systemic exposure and the toxicity and/or the efficacy of anticancer agents. Moreover, the clearance of most of these drugs differs widely between patients. These findings, combined with the narrow therapeutic index of anticancer drugs, suggest that patient outcome would be improved if doses were individualized to achieve a target systemic exposure. Thus, real tailored treatments for cancer should take into account genetic information of the tumor and drug systemic exposure that can be controlled by therapeutic drug monitoring levels (TDM). TDM is the measurement and interpretation of drug concentrations in biological fluids so as to determine the correct drug dosage for an individual patient. TDM is an important tool in personalized tumor therapy because: 1) after selecting the correct patient and the correct drug, we often fail to confirm the correct exposure; 2) the majority of cancer patients have exposures outside the therapeutic window and approximately 40% of these are under-dosed and 3) failure of phase III trials is, at least in part, a result of low drug efficacy due to underdosing and not necessarily a result of toxicity.

Bayesian maximum *a posteriori* probability forecasting is an efficient and robust method for the optimization of drug therapy, but its use for anticancer drugs is not yet extensive. Bayesian estimation takes into account the pharmacokinetic characteristics of a typical population that represent all the patients included afterwards in a given study. Then, to estimate individual pharmacokinetic parameter values, the Bayesian estimator combines the prior knowledge of the population parameters and the individual patient information from drug plasma concentrations measured after drug administration and, possibly, physiological parameters, such as bodyweight, age, gender, or others. After determining the individual pharmacokinetic parameters, the use of modeling and simulation techniques allows to anticipate the individual patient exposure after several dosing schedules in order to administer the right dose to the individual patient.

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MULTIFUNCTIONAL ANALGESICS

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Worldwide, chronic pain is the main reason for seeking healthcare, and pain relief represents an unmet clinical need. Pain has been classically viewed as a symptom accompanying another disease and, therefore, therapy has been geared towards symptom reduction as opposed to treating the underlying cause. More recently, it was recognized that chronic pain is a disease itself. For many years drug discovery was based on the principle "one molecule-one target-one disease", but many diseases remain inadequately treated by such an approach. It has become clear that single compounds that interact with multiple targets can result in a more effective and safer therapeutic approach for pain management. Compared with drug combinations, multitargeted drugs may have a much improved potency and efficacy, fewer side effects and better compliance. A distinction must be made between bifunctional compounds and bivalent ligands. Bifunctional drugs interact in a monovalent fashion with two different targets. Bivalent compounds, which contain two distinct pharmacophores linked by a spacer, interact simultaneously with two receptor binding sites. The usefulness of multitargeted compounds as promising analgesics is herein illustrated with examples from the field of opioids. Early studies showing that morphine-mediated antinociception could be potentiated by both activation and blockade of the δ-opioid receptor (OR) suggested a functional interaction between μOR and δOR. Bivalent ligands targeting μOR-δOR heteromers, composed of two pharmacophores with μOR agonistic activity and δOR agonistic or antagonistic activity have been synthesized and extensively investigated. Such μOR-δOR–biased ligands exhibit antinociceptive activity similar to morphine but develop lesser antinociceptive tolerance following chronic administration. Opioids have also been combined with non-opioid pharmacophores acting as agonists or antagonists of other signaling pathways involved in pain perception (e.g. receptors for substance P, neurotensin, cholecystokinin, cannabinoids, melanocortin, bradykinin, glutamate, etc.). Such chimeras may interact independently with their respective receptors and potentially result in more effective antinociceptive properties. Multifunctional compounds with μOR agonist activity and monoamine-reuptake inhibitor properties, such as tramadol and tapentadol, are currently available analgesics in the clinic. Both mechanisms of action contribute to the analgesic activity in a synergistic manner. [Dmt]DALDA is a new synthetic peptide with high affinity and selectivity for spinal μOR, which inhibits noradrenaline reuptake, is a mitochondria-targeted antioxidant and promotes the release of endogenous opioid peptides. [Dmt]DALDA is 3000 times more potent than spinal morphine in experimental models of pain. In summary, the multifactorial etiology of chronic pain disorders has determined a shift of paradigm from the "magic bullet", single-mechanism-drugs, to the rationally designed multi-target-directed ligands ("magic shotguns") as a strategy for developing new analgesic drugs.

References:

1. Fujita et al. Mu-Delta opioid receptor heteromers: New pharmacology and novel therapeutic possibilities. Br J Pharmacol. 2014. doi: 10.1111/bph.12663.
2. Kleczkowska et al. Hybrid opioid/non-opioid ligands in pain research. Curr Pharm Des. 2013;19:7435–50.
   Schiller PW. Bi- or multifunctional opioid peptide drugs. Life Sci. 2010;86:598–603.

3. Tzschentke et al. The Mu-Opioid Receptor Agonist/Noradrenaline Reuptake Inhibition (MOR-NRI) Concept in Analgesia: The Case of Tapentadol. CNS Drugs. 2014;28:319–29.

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NALMELFENE BLOCKS THE NEUROINFLAMMATION INDUCED BY ETHANOL

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We have previously reported that ethanol, by activating the innate immune receptor tall-like 4 (TLR4) signalling in glial cells, triggers the production of cytokines and inflammatory mediators in the brain and causes brain damage and behavioural dysfunctions. The *in vivo* relevance of these findings was demonstrated by eliminating TLR4 function (TLR4-KO) in mice, since these mice are protected against neuroinflammation, gliosis, myelin disruptions, neural death and behavioural dysfunctions induced by chronic ethanol treatment. Our results also provide evidence of the role of TLR4 signalling in the alterations of brain plasticity and cognitive/behavioural dysfunctions induced by binge drinking during adolescence.

Recent findings demonstrate that opioids are also capable to activate TLR4 receptor signalling causing neuroinflamation and contributing to drug reinforcement. Interestingly, naloxone has been shown to inhibit TLR4 signalling response suppressing opioid-induced conditioned place preference and morphine-induced elevations of extracellular dopamine in rat nucleus accumbens (NAc). These findings raise the question as to whether nalmelfene by inhibiting TLR4 receptors can block the neuroinflammation and behavioral dysfunctions induced by ethanol consumption.

Our preliminary results demonstrate that both nalmelfene (0.1 μM) and naloxone (150 μM) are capable to inhibit TLR4 signalling response (MAPKs and NFkB) and the induction of inflammatory mediators (iNOS, COX-2) in glial cells in primary culture, although nalmelfene shows greater efficacy that naloxone in the TLR4 inhibition. Furthermore, using an experimental model of binge drinking during adolescence, our results demonstrate that administration of nalmelfene (0.1 gr/kg) prior to ethanol (3 gr/kg) ip injection prevents ethanol-induced elevation of cytokines (IL-β, TNF-α, IL-17A) and chemokines (Fractalkina orCX3CL1, CXCL12, MCP-1, MIP 1α, KC) in the mice cerebral cortex, striatum (containing NAc) and plasma and reduces neural death. Nalmelfene administration also decreases the preference to alcohol (two bottle choice test) and prevents the alterations in the glutamatergic and dopaminergic systems induced by ethanol. These results suggest that nalmelfene by blocking TLR4 response, prevents neuroinflammation and brain damage. The findings also support the role of the central proinflammatory immune signaling in alcohol consumption/addiction (RTA-Network 2013-16).

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PHARMACOLOGICAL MODULATION OF THE ENDOTHELIAL DYSFUNCTION THROUGH NUCLEAR RECEPTOR ACTIVATION

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Endothelial dysfunction is a pro-thrombotic and pro-inflammatory state of the endothelium that contributes to the early stages of atherogenesis[1]. Angiotensin II (Ang-II) is implicated in atherogenesis and mononuclear leukocyte infiltration into the subendothelial space is a key event in this process[2]. Consequently, pharmacological modulation of inflammatory cell infiltration of the subendothelial space may impede the atherogenic process. Statins can exert anti-inflammatory and anti-oxidant activities and, a potential interplay between statins and peroxisome proliferator-activated receptors (PPARs) has been described[3]. Though statins in general are well tolerated, myopathy and acute renal events have been a significant concern with the use of high potency statins, in particular simvastatin and rosuvastatin (Rosu). Since these adverse effects are frequently dose-related, there remains an overt need to identify agents that, when combined with statins, can provide the greatest benefit on cardiovascular disease with the least added risk. In this regard, bexarotene (Bex) is a retinoid X receptor (RXR)-selective agonist that inhibit mononuclear leukocyte attachment to the stimulated arterial endothelium[4] although causes dyslipidemia. However, it is well established that PPARs form permissive RXR heterodimers which synergistically respond to agonists of RXR and the partner receptor. In an attempt to identify more effective strategies to treat and prevent atherosclerosis, coronary heart disease, and co-morbid metabolic disorders characterized by endothelial dysfunction, we have evaluated the effect of combined use of both Rosu and Bex on Ang-II-induced arterial mononuclear cell recruitment.

Results: Vehicle, Rosu (10–30 nM), Bex (0.3–1 μM) or a combination of both, were administered to human umbilical arterial endothelial cells (HUAECs) 20h prior to stimulation with 1 μM Ang-II (4h). Surprisingly, a combination of Rosu (10 nM)+Bex (0.3 μM), which did not influence Ang-II-induced MC recruitment when either stimulus was studied alone, significantly reduced this response. This effect was accompanied by diminished Ang-II-induced ICAM-1, VCAM-1 and CX_3CL1 endothelial expression and CXCL1, CXCL8, CCL2 and CCL5 production. Preincubation of HUAECs with Rosu+Bex inhibited Nox5 expression and Nox5-induced RhoA activation stimulated by Ang-II through increased RXRα, PPARα and PPARγ expression in addition to RXRα/PPARα and RXRα/PPARγ interactions. Chronic Ang-II administration to mice (500 ng/kg/min, 14 days) increased cremasteric arteriolar leukocyte adhesion. While chronic Rosu (1.25 mg/kg/day) or Bex (10 mg/kg/day) treatment did not exert any significant effect on this parameter, their combined administration reduced leukocyte adhesion by 65%.

Conclusion: Combined administration of two clinically available drugs, rosuvastatin and bexarotene, at suboptimal doses may constitute a new alternative and effective therapy in the control of the vascular inflammation associated to cardiometabolic disorders since they synergize in their anti-inflammatory actions and counteract their associated adverse effects.

References:

1. Landmesser U, Hornig B, Drexler H. Endothelial function: a critical determinant in atherosclerosis? *Circulation.* 2004;109:II27–33.
2. Libby P. Inflammation in atherosclerosis. *Nature.* 2002;420:868–874.
3. Balakumar P, Mahadevan N. Interplay between statins and PPARs in improving cardiovascular outcomes: a double-edged sword? *Br J Pharmacol.* 2012;165:373–379.
4. Sanz MJ, Albertos F, Otero E, Juez M, Morcillo EJ, Piqueras L. Retinoid X receptor agonists impair arterial mononuclear cell recruitment through peroxisome proliferator-activated receptor-gamma activation. *J Immunol.* 2012;189:411–424.

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REGULACIÓN NEUROFARMACOLÓGICA DE LA ACTIVIDAD CORTICAL CEREBRAL Y SU INTERÉS EN LA BÚSQUEDA DE NUEVAS DIANAS EN ESQUIZOFRENIA

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Non-competitive NMDA receptor antagonists are widely used as pharmacological tools in schizophrenia research due to their ability to evoke the symptoms of the illness in healthy individuals and to aggravate the clinical state in schizophrenic patients. Likewise, these drugs evoke a series of behavioral alterations in laboratory animals which are

antagonized by antipsychotic drugs. Likewise, serotonergic hallucinogens, acting on 5-HT_{2A} receptors, induce perceptual and behavioral alterations that may underlie some psychotic symptoms. Despite the wide use of these agents in antipsychotic drug development, the neurobiological basis of these alterations is not fully elucidated. Data obtained in recent years revealed that the NMDA receptor antagonist phencyclidine (PCP) and the serotonergic hallucinogens DOI (1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]) and 5-MeO-DMT (5-methoxy-*N,N*-dimethyltryptamine) produce a series of common actions in rodent prefrontal cortex (PFC) that may underlie psychotomimetic effects [1–4]. Hence, these agents markedly disrupt PFC function by altering pyramidal neuron discharge (with an overall increase in activity) and reducing the power of low frequency oscillations (LFO; ~1 Hz). In parallel, PCP increased *c-fos* expression in excitatory neurons of various cortical areas, the thalamus and other subcortical structures, such as the amygdala [5]. Electrophysiological studies revealed that PCP altered similarly the function of the centromedial and mediodorsal nuclei of the thalamus, reciprocally connected with PFC, suggesting that its psychotomimetic properties are mediated by an alteration of thalamocortical activity (the effect of serotonergic hallucinogens was not examined in thalamus). Interestingly, the observed effects were prevented or reversed by the antipsychotic drugs clozapine and haloperidol, supporting that the disruption of PFC activity is intimately related to the psychotomimetic activity of these agents. We recently extended these observations by showing that classical (chlorpromazine, haloperidol, perphenazine) and atypical (clozapine, olanzapine, quetiapine, risperidone, ziprasidone) antipsychotic drugs countered PCP-evoked fall of LFO in rat medial prefrontal cortex (mPFC). Classical antipsychotic action may involve the concurrent blockade of D1-R and D2-R since raclopride and SCH-23390 partially reversed PCP effect. Atypical drug reversal may additionally involve 5-HT_{1A}-R activation (but not 5-HT_{2A}-R blockade) since 8-OH-DPAT and BAYx3702 (but not M100907) fully countered PCP effects. Blockade of histamine H1-R and α_1-adrenoceptors –for which antipsychotic drugs exhibit moderate affinity- appears unlikely to account since pyrilamine and prazosin were ineffective. Recent results indicate that PCP preferentially inhibits GABAergic neurons of the reticular nucleus of the thalamus, which provides feed-forward inhibition to the rest of thalamic neurons. Hence, NMDA receptor blockade by PCP disinhibits thalamocortical networks, thus producing aa uncontrolled increase of nerve activity in the neocortex, an effect likely involved in its psychotomimetic activity.

Overall, the present experimental model can be successfully used to elucidate the neurobiological basis of schizophrenia symptoms and to examine the potential antipsychotic activity of new drugs in development.

References:

1. Kargieman et al., *Proc Natl Acad Sci USA*, 2007; 104:14843–14848.
2. Celada et al., *Biol Psychiatry* 2008; 64:392–400.
3. Riga et al., *Int J Neuropsychopharmacol* 2014 ; Mar 20:1–14. [Epub ahead of print].
4. Kargieman et al., *Neuropsychopharmacology* 2012: 37:723–733.
5. Santana et al. *Biol Psychiatry* 2011; 69:918–27.

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SIMULATION AND THERAPEUTIC DRUG MONITORING NEEDS IN CLINICAL PRACTICE

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Certain drugs exist that, due to their narrow therapeutic range, need a more stringent control of plasma concentration. The reason is that above the upper limit of the therapeutic range, the drug could be toxic and may lead to irreversible toxicity (e.g. aminoglycoside nephrotoxicity) and, on the other hand, below certain concentration the drug can be ineffective. Therefore, the principal objective of therapeutic drug monitoring (TDM) is to give an individualized dosage recommendation, taking into account the drug plasma concentration, so that the drug will be safe and effective. TDM is based on the principle that for some drugs there is a close relationship between the plasma level of the drug and the pharmacological or toxic effects. If such a relationship does not exist or the pharmacological effects can be clinically quantified, TDM is of little value. Another major criteria, among others, is that appropriate analytical techniques are available to determine the drug levels in a reasonable amount of time. That means, in clinical practice, performing a therapeutic recommendation before doctors give the patient's new treatment to nurses.

Moreover, for a correct interpretation of drug plasma concentration, the sample must be obtained in the proper way. Therefore, it is not uncommon that in the daily clinical practice and, due to the huge workload, the timing of sample collection may be incorrect, leading to a mistake in the therapeutic recommendation. An example could be a theoretical "through level" obtained after the drug administration because it has been forgotten that the patient has a request for a drug monitoring. In this case the plasma concentration could be in toxic range without any sign or symptom and it is difficult to verify how the sample was obtained because the nurse shift has changed.

For these reasons it is essential that, together with plasma concentration, information regarding clinical data and timing of sample collection should be recorded. All this information will provide a correct interpretation of plasma levels and an individualization of treatment. However, all this information is not easy to obtain. Sometimes it is difficult to access the patient files, the nurse in charge of the sample collection is not available, there is not a recent analysis test, and so on. Therefore, the interpretation of results and therapeutic recommendation will be more empirical than real and there is no reliable method, unfortunately, but the experience of the personnel in charge of monitoring, in our case, Clinical Pharmacologists.

Taking into account the variety of scenarios in clinical practice, creating pharmacokinetic models for every situation may be unaffordable. Thus only with those patients for whom there is adequate information, could their data be used as much with more or less complex models as with simple methods of adjusting maintenance dose.

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STRATEGIES FOR THE SYNTHESIS OF DUAL ACTING DRUGS

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Drug discovery has traditionally been based on the "one target one disease approach", that is identifying a particular process, either physiological or pathological that can be augmented or reduced by targeting a specific macromolecule (enzyme, receptor or protein). Illustrative examples are the use of inhibitors of DHFR to suppress the growth of bacteria, or the use of statins which interfere with HMG-A Co reductase thus blocking the biosynthesis of cholesterol. However, a recent trend in drug dscovery is to shift from this paradigm to develop agents that address, simultaneously, multiple drug targets that are involved in the complex pathophysiology of certain diseases such as cancer, obesity etc.

From a simple point of view, there are three possibilities to deal with polypharmacology: treating patients with more than one active principle, the cocktails of drugs; the administration of multicomponent drugs in which two or more active principles are coformulated in a single tablet, and a third strategy, the most challenging for a synthetic chemist, which is to design ligands that can act, simultaneously, on multiple targets. These kind of drugs have been referred to by different names such as hybrid molecules, dual-acting drugs, dual ligands etc., but the best established defintion is that of Morphy and Rankovic who have termed them DMLs, designed multiple ligands.

From a synthetic point of view there are three possibilities for joining two pharmacophoric groups: linking, fusing or merging, and so two groups can be separated by a linker which will be metabolized to provide two independent ligands. When the size of the linker is small or even non existent, the two frameworks are very close or fused. The

third possibility is to use some of the common features of the starting compounds and merge them.
Our first example dates from 2002 in which we combined agonism at two G protein coupled receptors, the mu opioid and the I2 imidazoline receptor, in our quest for an oipioid analgesic with reduced side effects. Thus, a guanidinium group from agmatine was incorporated in the opioid fentanyl and the resulting compounds showed activity at both receptors, although they had higher affinity for the opioid receptor.
We have also worked with the other two strategies mainly by fusing/merging PPAR alpha and cannabinoid ligands, because it has been reported that combining cannabinoids with other compounds (other cannabinoids or PPAR agonists) increases their therapeutic potential. For example, the combination of OEA (PPAR alpha agonist) with rimonabant (a CB1 antagonist/inverse agonist) resulted to be more effective suppressing feeding and increasing weight loss. The coadministration of anandamide (CB1 agonist) and GW7647 (PPAR lpha agonist) led to synergistic effects on reducing pain behaviour.
In this presentation, examples will be discussed of the synthesis of DMLs combining cannabinoids and PPAR alpha ligands (structural motif of rimonabant) with the pharmacophore of the fibrates, fenofibrate (PPAR lpha agonist) and, also, cannabinoids and opioids (rimonabant and fentanyl).

23
THE ACADEMIC CAREER: WHICH SHOULD BE THE FORMATION OF A UNIVERSITY TEACHER?

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When a student finishes his or her studies of Degree and considers to continue studying, doing a Master and a Doctorate, already he or she starts sowing a seed that can give place, with time and suitable dedication, to a good university teacher; one of the most complete and exciting career opportunities that one may have.
To increase knowledge, to have doubts and try to solve them, to learn to work in order to obtain a few aims, to have a project and not to spare efforts and spirits to develop it... These are important training milestones to become a university teacher. But, in addition, another important characteristic that one who is going to pursue an academic career must have is the desire to transmit his or her knowledge, doubts and worries to others, specially to those younger students that are joining the research group.
Those who want to devote their lives to teaching at university must have clear that their professional lives need to be developed at three different levels, each one with a different orientation and dedication during the diverse stages: teaching, research and management. They are going to compose a triad that, in some occasions, can be easily reconciled, although in others not so much, creating tensions that force oneself to constantly re-balance and reorganize the professional life.
As I said, it is a complete career opportunity because you will always keep on learning and allows you to develop skills and a variety of capacities; it is exciting because, in any of the 3 pilars on which this career rests, your achievements cause much satisfaction and can impact and improve the lives of others.
Normally, the first thing that attracts us is research, the possibility of a doctoral thesis; but gradually, especially if one is lucky enough to be part of a Department with good teachers, becomes aware of the importance of teaching at University. The realization of how important good teachers have been in your path and how much they contribute to awaken concerns, draws you to see things from another perspective. One begins to discover that imparting knowledge and sharing the learning process with others may be part of his professional life.
The road is long and sometimes uncertain: collaboration scholarship, predoctoral scholarship, postdoctoral fellowship, stays abroad, sometimes extending more than we would like ..., the return!!... But all the effort and dedication is worth it!

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THE ASSESSMENT OF PHARMACOLOGY'S LEARNING UNDER REVIEW

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Nowadays one of the problems that most worries teachers is to ensure appropriate means to establish to what extent learners reach the pre-set educational goals, the famous key or target skills or competencies; in other words, how to reach a fair and valid assessment of learning.
The main objective of the evaluation is the feedback the teaching-learning process. This means that the data obtained in the evaluation will serve to those involved in this process, both teachers and students, in a direct way to improve the deficiencies that arise in the implementation of the teaching learning and have an impact on the improvement of the quality and thus the performance in the teaching-learning process. For this purpose, it is important to differentiate the term measurement of evaluation, as well as the classification of the latter and their didactic function. It is important to defined which type (diagnostic, formative and summary), who (teachers, students, the school or the State), when, how (diagnostic, formative, summary, theoretical, practical) and by which assessment techniques (oral evaluation, written assessment by test or theme development, clinical cases, problem-based learning, simulations, virtual assessment, role-playing games, prior learning assessment, self-assessment, peer to peer evaluation) in each student.
By the evaluation you can learn to what extent students have modified their duct as a result, planned and direct the educational action. The teaching-learning process includes a continuous and interconnected series of decisions concerning the statement that seek to increase the quality of the student learning. However, this effectiveness depends in large middle of the quality of information provided by the assessment on which the decisions that systematize and regulate every stage of this process must be based.
The assessment thus allows to the teacher to: -Know what were the objectives achieved and to what extent was the achievement. -Have an analysis of the causes that could have caused deficiencies in the proposed goals and decisions. -Avoid incurring the same mistakes in subsequent experiences. -Timely strengthening the areas of study in which the learning has been insufficient (detectable with relative ease in the group performance evaluation tools). -Judge the feasibility of schemes in the light of the circumstances and real operating conditions. And it allows to the student to: Have a source of information that will reaffirm the successes and errors will be corrected (to review the exams). Direct their attention toward the central aspects of the study material. -Keep it aware of its stage of completion. -Strengthening the areas of study in which the learning has been insufficient.
Conclusion: Evaluation not as "an act whereby a teacher deems a student, but a process through which the teacher and the student appreciated in what degree achieved the latter learning both chasing" and that "as essential in the educational process activity can provide a clear view of errors to correct them, obstacles to overcome and the successes to improve them" and thus fulfil functions that have the evaluation education in the teaching-learning process which is essential for effective education in pharmacology.

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THE BASIS OF THE TEACHING ASSESSMENT

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Assessment of students' academic learning is a very important step in any educational project since it provides information about the success in the attainment of our specific teaching objectives. It is well known that students adjust their learning processes according to the particular type of assessment used so the choice of the most suitable type of assessment is very important.

The communication summarizes the basis of the learning assessment trying to answer five questions: What, Why, How, When, and by whom.
It will stress in selecting methods depending on the type of educational objectives to evaluate (knowledge, skills or behavior).

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THE EVALUATION IN PROBLEM-BASED LEARNING TEACHING: A PRAGMATIC APPROACH

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The use of problem-based learning (PBL) in teaching students of health sciences has increasingly been used since McMaster University started its implementation in the Bachelor in Medicine in late sixties[1]. Since this initial experience, PBL has spread in universities of around the world and especially in the schools of health sciences. In short, PBL consists in teaching small groups of students under the supervision of a tutor using scenarios (*problems*). Scenarios are prepared after establishing the educative objectives and the available time to discuss and solve them. Real or fictional plots are used to allow that students acquire content knowledge and improve their generic competences, such as oral communication, writing skills, group management or time deadlines.[2]
Implementation of PBL has several limitations. One of the most difficult to overcome is how to organize an adequate assessment in order to assure that educational objectives have been reached. The main paradox is how to evaluate factual content using the same approach that has been used to teach the students. This is especially relevant when PBL is the only pedagogical method used in teaching. We have used PBL in a hybrid model which combines this method with traditional teaching approaches (i.e. lectures and seminars), both at undergraduate and postgraduate courses, since 1998[3–5]. Assessment considered several dimensions, as factual knowledge, writing and oral communication skills, analytical thinking, information search, working group and time management.
Our evaluation included formative and summative evaluations. In the first we considered the work during the full trimester. Students prepared final reports individually or in group after each problem was solved. Their tutor reviewed them and gave feedback on its weaknesses and strengths, both in formal and content aspects. Before the summative assessment, a wrap-up session was scheduled to assure that students had reached the educative objectives of each problem. This also allowed them to clarify any aspect that remained difficult to understand. In the summative assessment, we used a multiple choice question to assess the factual knowledge. A text with a new scenario was also included and students should to identify the relevant questions and answer one of them. In some courses, a questionnaire of ten questions about the content of an article, previously delivered to students, was also included.
In conclusion, a multiple and hybrid assessment was used to evaluate PBL courses. This approach allowed considering the different dimensions of this teaching method and a better understanding of how students learn both factual knowledge and generic skills.

References:

1. Neufeld VR, Barrows HS. The 'McMaster Philosophy': an approach to medical education. *J Med Educ* 1974; 49:1040–50.
2. Branda LA. El *abc* del ABP – Lo esencial del aprendizaje basado en problemas. En Carrió M, Branda L, Baños JE (coor.) *El aprendizaje basado en problemas en sus textos. Ejemplos de su empleo en biomedicina*. Cuadernos de la Fundación Dr. Antonio Esteve. Barcelona: Fundación Dr. Antonio Esteve, 203; pp. 1–16.
3. Vivas NM, Badia A, Vila E, Baños JE. El aprendizaje basado en problemas como método docente en Farmacología: la opinión de los estudiantes de Medicina. *Educ Med* 2001; 4:194–201.
4. Aramburu J, Bosch F, Sentí M, Baños JE. Los itinerarios profesionales en Biología: un ejemplo de formación académica orientada a la inserción profesional. *Educ Med* 2006; 9 (4B):23–30.
5. Bosch F, Baños JE. Tendiendo puentes: la utilidad de la historia de la ciencia para comprender el proceso de investigación y desarrollo de medicamentos. *Educ Med* 2010; 13:255–62.
6. Carrió M, Larramona P, Baños JE, Pérez J. The effectiveness of hybrid problem-based learning approach in the teaching of biology: a comparison with lecture-based learning. *J Biol Educ* 2011; i:1–7.

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THE TRANSCRIPTION FACTOR T-BET IN INNATE LYMPHOID CELLS: A POTENTIAL THERAPEUTIC TARGET FOR INTESTINAL INFLAMMATORY DISEASES

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Innate lymphoid cells (ILCs) are a recently recognized group of lymphocytes commonly located at mucosal surfaces, where they play a key role in epithelial homeostasis and responses to intestinal infections. Additionally, ILCs have been proposed to be potentially pathogenic in autoimmune and inflammatory diseases. T-bet is an immune cell-specific member of the T-box family of transcription factors. Originally described as a master regulator of commitment to the T helper 1 cell lineage, T-bet is now recognized as having an important role in many cells of the adaptive and innate immune system.
We have shown that T-bet regulates the effector function of ILCs and modulates immunopathology *in vivo*. Mice lacking the transcription factor T-bet in the innate immune system develop microbiota-dependent colitis. We have found that colitis in the in Tbx21(−/−)Rag2(−/−) ulcerative colitis (TRUC) mice is dependent on interleukin- (IL) 17A-producing ILCs, and it is abrogated by genetic or antibody-mediated depletion of ILCs. In this model, T-bet controls the plasticity of RORγt+ ILCs, by inducing IFNγ expression and repressing IL-17A production. However, in non-colitis mice (Tbx21(−/−)Rag2(−/−) non-ulcerative colitis or TRnUC), deletion of T-bet in ILCs leads to increased numbers of IL-13- and IL-5-producing ILCs (termed ILC2s), not only in mucosal surfaces (colon lamina propria), but also in the spleen and mesenteric lymph nodes.
These data identify T-bet as a critical regulator of ILC lineage relationships during inflammatory processes, which has significant implications for the understanding of mucosal pathology and the development of new therapies for inflammatory bowel diseases.

ORAL ABSTRACTS
CARDIOVASCULAR PHARMACOLOGY

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5-HT_2 RECEPTOR BLOCKADE ENHANCES SYMPATHO-INHIBITORY SEROTONERGIC ACTIONS AT CARDIOVASCULAR LEVEL IN RAT

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Introduction/Objectives: 5-HT evokes a variety of responses influencing vascular tone. 5-HT_2 receptors are associated with platelet aggregation, vasoconstriction, adrenaline release and tachycardia[1,2,3]. The aim of this study was to evaluate whether 5-HT_2 receptor blockade changes serotonergic modulation of sympathetic neurotransmission in pithed rats.
Material/Methods: Wistar rats were orally treated with sarpogrelate (a 5-HT_2 receptor antagonist) during 14 days (30 mg/kg/day). After CNS destruction[4], electrical stimulation of sympathetic outflow (monophasic pulses, 1 ms duration, 15 ± 3 V at increasing frequencies 0.1, 0.5, 1 and 5 Hz) or administration of exogenous noradrenaline (0.01, 0.05, 0.1 and 0.5 µg/kg) were performed. Western blotting for 5-$HT_{1A/1B/1D/7}$ receptors was also carried out.
Results: Electrical stimulation or administration of noradrenaline resulted in frequency- or dose-dependent increases in mean blood pressure. Continuous infusion of 5-carboxamidotryptamine (5-CT, 5-$HT_{1/7}$ receptor agonist; 0.005, 0.1 and 5 µg/kg/min) exerted a dose-dependent inhibition of sympathetic outflow, significantly greater in sarpogrelate-treated rats than in non-treated rats[4]. This effect was mimicked by L-694,247 and AS-19, 5-HT_{1D} and 5-HT_7 receptor agonists respectively (0.1, 5 and 10 µg/kg/min each). However, 1-Phenylbiguanide, CGS-12066B and 8-OH-DPAT, (5-HT_3, 5-HT_{1B} and 5-HT_{1A} receptor agonists, respectively; 5 µg/kg/min each) failed to reproduce 5-CT inhibitory action. A mixture of LY310762 and SB258719 (5-HT_{1D} and 5-HT_7 receptor antagonists, respectively; 1 mg/kg each) completely abolished 5-CT inhibitory effect. None of the agonists modified the pressor responses induced by exogenous noradrenaline. Western blot showed increased expression of 5-HT_{1D} receptors in sarpogrelate-treated animals.
Conclusions: In conclusion, 5-HT_2 receptor blockade potentiates sympatho-inhibitory 5-carboxamidotryptamine actions at cardiovascular level, mainly mediated by prejunctional 5-HT_{1D} and 5-HT_7 receptors.
References:
1. Saini et al., 2004. Cardiovasc. Drug Rev. 22:27-54.
2. Restrepo et al., 2011. Eur. J. Pharmacol. 659:37-44.
3. Watts et al., 2012. Pharmacol. Rev. 64:359-388.
4. Morán et al., 1998. Br. J. Pharmacol. 113:1358-1362.

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RICE BRAN ENZYMATIC EXTRACT REDUCES ATHEROSCLEROTIC LESIONS IN APO E (−/−) MICE

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Background and aims: rice bran is a byproduct of rice milling rich in antioxidants, sterols and γ-oryzanol that is underused mainly due to its low water solubility. We aimed to evaluate the effect of a novel water soluble rice bran enzymatic extract (RBEE) supplemented diet on atherosclerosis.
Methods: ApoE$^{-/-}$ mice were fed standard (ST) or high fat (HF) diet supplemented or not with 1% or 5% RBEE for 23 weeks. Serum lipids, oxLDL and nitrites were measured using spectrophotometry, ELISA and Griess reaction, respectively. Atherosclerotic lesions were assessed in the aortic sinus and brachiocephalic artery by Mac-3 inmunostaining and Oil Red O staining. ICAM-1 and VCAM-1 expression were measured by WB in aorta homogenates.
Results: 1% and 5% RBEE supplementation reduced the elevation of nitrites observed in HF fed animals ($P < 0.05$ and $P < 0.01$, respectively) and augmented HDL cholesterol ($P < 0.05$) while total cholesterol and triglycerides decreased only in HF 5% ($P < 0.05$). ApoE$^{-/-}$ showed increased serum and aorta oxLDL that was reduced by 1% and 5% RBEE supplementation in serum in ST diet ($P < 0.05$) and in aorta in HF fed mice ($P < 0.001$). Macrophage infiltration and lipid deposition in the aortic sinus was reduced by 1% and 5% RBEE supplementation in HF fed mice. However, in the brachiocephalic artery, only ST 5% showed reduced lipid deposition ($P < 0.01$) while macrophage infiltration was also reduced in HF 1% and HF 5% ($P < 0.01$). VCAM-1 and ICAM-1 expression was reduced in HF 5% ($P < 0.01$ and $P < 0.05$, respectively).
Conclusions: RBEE supplemented diet improved lipid profile and prevented atherosclerotic lesions development showing its interest as functional food in atherosclerosis disease.

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PHARMACOPHORE OF DRUGS THAT INCREASE $I_{KIR2.1}$

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Introduction: Drug-induced increase of the inward rectifier current (I_{K1}) generated by Kir2.1 homotetramers ($I_{Kir2.1}$) is a mechanism of drug-induced proarrhythmic effects that we recently identified. Here we analyzed whether propafenone, similarly to flecainide, also increases $I_{Kir2.1}$. Furthermore, by testing the effects of timolol, atenolol, and dronedarone we identified the chemical determinants of drug affinity to the receptor within Kir2.1 channels.
Material and Methods: Currents were recorded with the patch-clamp technique using whole-cell, inside-out, and cell-attached configurations in transfected Chinese hamster ovary CHO cells and cardiac myocytes.
Results: Propafenone (0.1 nM–1 µM) did not modify either I_{K1} recorded in human right atrial myocytes or the current generated by homo- or heterotetramers of Kir2.2 and Kir2.3 channels recorded in CHO cells. Conversely, propafenone increased $I_{Kir2.1}$ (EC_{50} = 12.0 ± 3.0 nM) as a consequence of its interaction with Cys311, an effect which decreased inward rectification of the current. Propafenone significantly increased mean open time and opening frequency at all the voltages tested which resulted in a significant increase of the mean open probability of the channel. Timolol, which interacted with Cys311, was also able to increase $I_{Kir2.1}$. On the contrary, neither atenolol nor dronedarone modified $I_{Kir2.1}$. Molecular modeling of the Kir2.1-drugs interaction allowed to the proposal of the pharmacophore of drugs that increase $I_{Kir2.1}$.
Conclusions: The results demonstrated that those drugs that are able to increase $I_{Kir2.1}$ exhibit an L-like structure with electronegative and

hydrophobic groups in their short and long arms, respectively. Both arms must be linked by an aromatic ring.

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ROLE OF RGS5 IN THE ANTIHYPERTENSIVE EFFECTS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)-β ACTIVATION IN ANGIOTENSIN II-INFUSED MICE

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Introduction and Objective: Activation of nuclear hormone receptor peroxisome proliferator-activated receptor-β (PPARβ) lowers blood pressure in genetic and mineralcorticoid-induced hypertension. These effects may be mediated by overexpression of key regulators of G-protein-coupled receptor signaling (RGSs) proteins through the vasculature. Whether PPARβ activation prevents angiotensin (Ang)II-induced hypertension regulating resistance artery tone is not clear.
The aim of this study was to analyze the effects of the PPARβ agonist GW0742 on blood pressure, vascular remodeling and function in AngII-infused mice by modulating of RGS proteins expression.
Methods: C57BL/6J male mice were divided into 5 groups, control-mice, GW0742-treated-mice, AngII-infused-mice, GW0742-treated-AngII-infused-mice, and AngII-infused-mice-treated with GW0742 plus PPARβ antagonist GSK0660, and were followed for 3 weeks.
Results: GW0742 prevented the increase in arterial blood pressure, reduced the mesenteric arterial remodeling and improved the endothelial dysfunction, and the hyperresponse to vasoconstrictors (AngII, and endothelin-1) in AngII-infused mice. These effects were accompanied by an increase on NO bioactivity through an inhibition of NADPH oxidase-driven vascular superoxide production. Gene expression profiling revealed a marked loss of vascular RGS4 and RGS5 in AngII-infused mice, which was restored by GW0742. GW0742-induced effects were abolished by GSK0660. Small interfering RNA targeting RGS5 caused augmented contractile response to AngII in resistance mesenteric artery, but blunted both, the upregulation of RGS5 and the inhibition of the contractile response to AngII induced by GW0742.
Conclusions: PPARβ activation exerted antihypertensive effects, and restored the vascular structure and function in AngII-infused mice. These effects are associated with interference with the AngII signaling as a result of RGS5 upregulation.

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MITOCHONDRIAL DYSFUNCTION AFFECTS THE CAPACITATIVE CALCIUM ENTRY AND INDUCE CALCIUM OVERLOAD IN HYPERTENSIVE RATS

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Introduction: Mitochondria located in close proximity to calcium microdomains are exposed to high Ca^{2+} concentrations, and therefore they are able to buffer it. We hypothesized that mitochondria Ca^{2+} buffering are impaired in vascular cells and induce adaptive changes that affect capacitative calcium entry (CCE) and cytosolic calcium overload, in spontaneously hypertensive rats (SHR) compared with normotensive Wistar Kyoto rats (WKY).

Material and Methods: All procedures in animals were conducted in accordance with the European Communities Council Directive 2010/63/UE, and Royal Decree RD 53/2013. Changes in isometric tension were recorded by force transducers FT202 connected to an amplifier ETH 400 (CB Sciences-U.S.A.) and to an analogical/digital converter PowerLab (AD Instruments-U.S.A.) linked to a computer. The setup for fluorescence recordings was composed of a Leica DMI 4000 B inverted light microscope. Fura-2 was excited alternatively at 340 and 387 nm using a Küber CODIX xenon 8 lamp (Leica). Emitted fluorescence was collected through a 540 nm emission filter. Rhod2 and TMRE were excited at 480 and collected through a 660 nm emission filter.
Results: In the present work, we have observed that SHR shows an organelle and cytosolic calcium overload which may be due to mitochondrial damage and a consequent Ca^{2+} homeostasis dysfunction mediated by CCE-mitochondria interaction in aorta rings. This conclusion is supported by the following findings in SHR, compared with WKY rats: 1) augmented contraction of vascular tone after depletion of intracellular Ca^{2+} stores with successive stimulation with caffeine, ryanodine or thapsigargin in 0 Ca^{2+} solutions; 2) reintroduction of 2.5 Ca^{2+} in store-depleted vessels at 0 Ca^{2+}, induce more potent contractions; 3) contractions of aortic rings induced by Ca^{2+} reintroduction were antagonized by Gd^{3+}, La^{3+} and SKF-96365 (a Stromal Interaction Molecules inhibitor; STIM inhibitor); 4) FCCP (a mitochondrial protonophore) induced greater contractions; 5) western-blot shows that STIM-1 expression is up-regulated in vascular tissue of SHR; 6) This result is also supported by immunohistochemistry studies in aortic rings and cell cultures of myocytes.
Conclusion: These results support the hypothesis that mitochondrial Ca^{2+} imbalance affect the regulation of CCE which is an important factor in calcium overload and cell damage. Thus, mitochondria and STIM could be new targets for the treatment of arterial hypertension.
This work was supported by the following grants to AGG: (1) SAF 2010-21795, Ministerio de Economía y Competitividad, Spain; (2) Fundación Teófilo Hernando, Madrid, Spain.

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ROLE OF ET_A AND ET_B RECEPTORS IN THE ENDOTHELIN-1-INDUCED AUGMENTED REACTIVE OXYGEN SPECIES (ROS) PRODUCTION AND ENDOTHELIAL DYSFUNCTION IN OBESITY

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Enhanced endogenous activity of the vasoconstrictor, proinflammatory and mitogenic endothelial peptide endothelin 1 (ET-1) has been reported in human overweight, obesity and type 2 diabetes (Mather *et al, Diabetes* 53: 2060, 2004; Rafnsson et al., *Diabetologia* 55: 600, 2012).
In the present study we assessed whether ET-1 may restrain endothelial NO bioavailability and contribute to endothelial dysfunction through its ability to stimulate ROS generation in insulin resistant obese Zucker rats (OZR). Penile arteries from OZR and lean Zucker rats (LZR) were mounted in microvascular myographs to assess function.
Changes in basal and stimulated levels of superoxide (O^2-) were detected by lucigenin-enhanced chemiluminescence and ET receptors expression was determined by immunohistochemistry. ET-1 stimulated acute O_2^- production that was reduced by tempol and by the NADPH oxidase inhibitor, apocynin and markedly enhanced in obese animals. ET-1 (0.3 nM) blunted the vasorelaxant effects of acetylcholine and of NO donors in arteries from both LZR and OZR. Both ET_A (BQ-123) and ET_B receptor (BQ-788) antagonists reduced ET-1-stimulated ROS generation and restored NO-mediated relaxations blunted by ET-1- in OZR. ET-1-induced vasoconstriction was markedly enhanced by NO

synthase blockade and reduced by endothelium removal and apocynin treatment to a larger extent in OZR arteries. Both ET_A and ET_B receptors were expressed in both smooth muscle and endothelial layer and up-regulated in arteries from OZR.
Up-regulation of vascular ET_A and ET_B receptors is involved in the ET-1 stimulated NADPH oxidase-dependent ROS generation that restrains endothelial NO and induces endothelial dysfunction and enhanced vasoconstriction in insulin resistant obese rats.
This work was supported by grant SAF 2012-31631 from MINECO, Spain.

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CHRONIC CARVEDILOL TREATMENT DECREASES THE EXPRESSION OF β_1-ADRENOCEPTORS AND GRKS IN LEFT VENTRICLE FROM HEART FAILURE PATIENTS AND WISTAR RATS

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Background: Carvedilol antagonizes α_1, β_1, and β_2 adrenoceptors (AR) and is recognized as a biased agonist that induces β-AR-mediated activation of the GRK/ β-arrestin pathway but inhibits Gαs-coupled/cAMP pathway [1, 2]. This peculiar pharmacological profile, unique among β-blockers, could justify the better survival profile of carvedilol in heart failure patients, *vs* the older β-blockers.
Objective: To analyze changes in the expression of β-ARs and GRKs induced by chronic carvedilol treatment.
Methods: Heart and circulating lymphocytes were obtained from heart failure patients treated (n = 13) or not (n = 18) with carvedilol. Heart and aorta were obtained from Wistar rats orally treated (n = 5) or not (n = 5) with carvedilol (20 mg/kg) for 4 weeks. HEK293 cells were incubated with carvedilol 10^{-6} M for 48 h. mRNA levels for each gene was obtained and quantified as previously published [3].
Results: Table 1. mRNA levels of β-ARs and GRKs

			β1-AR	β2-AR	GRK2	GRK3	GRK5
HF Patients	LV	*control*	78.75 ± 11.3	77.45 ± 8.5	36.31 ± 4.3	17.08 ± 3.4	83.33 ± 10.3
		CVD	**36.49 ± 8.4 ****	59.91 ± 6.5	**21.45 ± 4.6 ***	**7.91 ± 0.8 ***	**56.36 ± 6.3 ***
	RV	*control*	51.59 ± 8.8	79.45 ± 12.0	20.24 ± 4.2	10.49 ± 2,0	67.15 ± 12,0
		CVD	70.77 ± 22.4	86.18 ± 14.2	21.76 ± 6.3	11.78 ± 1.1	68.50 ± 7.4
	Lymph	*control*	47.14 ± 20.7	289.8 ± 94.1	261.2 ± 69.3	281.1 ± 50.3	635.6 ± 164.5
		CVD	70.55 ± 42.7	437.5 ± 94.7	358.7 ± 78.5	275.9 ± 44.3	912.5 ± 130.7
Rats	LV	*control*	63.29 ± 5.6	16.59 ± 1.8	56.23 ± 8.3	2.32 ± 0.4	20.56 ± 1.4
		CVD	**44.11 ± 4.0 ***	11.01 ± 1.3	**31.91 ± 1.6 ***	1.24 ± 0.3	**12.55 ± 1.1 ****
	Aorta	*control*	49.45 ± 1.6	41.73 ± 2.1	296.3 ± 27.1	2.19 ± 0.5	354.4 ± 57.1
		CVD	57.94 ± 10.4	43.45 ± 7.6	**155.7 ± 34.8 ***	2.82 ± 0.3	**166.1 ± 35.4 ****
HEK 293		*control*	1.39 ± 0.2	10.81 ± 0.8	23.94 ± 1.4	21.76 ± 1.9	10.04 ± 0.3
		CVD	1.27 ± 0.2	11.87 ± 0.8	23.53 ± 1.1	23.38 ± 1.4	10.48 ± 0.4

HF = Heart Failure; LV = left ventricle; RV = right ventricle; Lymph = circulating lymphocytes
*Values are means ± SE. *P < 0.05; **P < 0.01 vs control (Student's t test)*
Conclusion: Opposite to classic β-blockers [4], *in vivo* chronic CVD treatment induces a significant decrease in the β_1-AR expression in left ventricle. A decrease in GRKs expression, common to other β-blockers, was also observed.
References:
1. Erickson CE, *et al*. (2013) *PLoS ONE,* 8(8): e71980.
2. Reiter E et al. (2012). *Annu Rev Pharmacol Toxicol*, 52:179–97
3. Monto F *et al*. (2012) *Am J Physiol Heart Circ Physiol,* 303:H368-H376
4. Ahmed A. (2003) *Eur J Heart Fail*, 5 (2003) 709–715.

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TYROSINE HYDROXYLASE PHOSPHORYLATION IS AUGMENTED AFTER COMBINATION OF BINGE ETHANOL AND MDMA IN MICE

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In the last years it is very common the combined abuse of ethanol and 3,4-methylendioxymethamphetamine (MDMA) but the cardiac consequences of such association have not been yet elucidated. Several studies have demonstrated that drinking at least 5 units of ethanol on a single occasion could produce negative behavioural consequences. MDMA ingestion in humans can also induce endocrine alterations similar to those produced by exposure to acute stress. One of those alterations could be over Tyrosine Hydroxylase (TH). TH catalyzes the limiting step in synthesis of catecholamines like noradrenaline which is involved in the Brain Stress System. The aim of this study was to evaluate the cardiac effects of ethanol and MDMA combination through the measure of TH phosphorylation on serine31, 48 h, 72 h and 7 days later.
Adolescent naive male CD-1 mice weighing 25–30 g at the beginning of the experiments were used in this study. Ethyl alcohol diluted in water (20% (v/v)) was administered during two weeks. Last day, MDMA hydrochloride (2 mg/ml; i.p.) was injected 48 h, 72 h and 7 days later, mice were sacrificed and TH phosphorylation on serine31 and TH expression were quantified by Western-Blot in the right ventricle.
Our results showed that combination of ethanol and MDMA produced significative changes in phosphorylation of TH in the right ventricle at 48 h and 72 h compared to control group. These changes are also seen in TH expression at 48 h. These results demonstrate that consumption of ethanol and MDMA in combination by adolescents, can produce stressful effects at cardiac level.
Supported by SAF/FEDER2010/17907 and RETICS-RD12/0028/0003.

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AGEING AND LACK OF ESTROGENS INCREASE VASCULAR CONTRACTION TO THROMBOXANE A_2 THROUGH RHO KINASE IN MOUSE AORTA

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Introduction: Both ageing and lack of estrogens are well-known cardiovascular risk factors converging at menopause. Rho signaling pathways in vascular wall are highly activated in a variety of vascular diseases. Our aim was to check the involvement of Rho kinase (ROCK) on the impaired vascular responses induced by ageing and estrogen deprivation.
Material/Methods: Six-month-old female, senescence-accelerated mice (SAMP8, n = 21) or the control, SAM-resistant (SAMR1, n = 21), were divided into sham-operated, ovariectomized and ovariectomized plus estradiol groups. After 28 days, aortas were mounted for isometric recording of tension. Concentration-response curves to the thromboxane A_2 analogue U46619 (10^{-9}–3×10^{-7} M) were performed in the absence and in the presence of the ROCK inhibitor, hydroxyfasudil (10^{-5} M).
Results: Contractions to U46619 were greater ($P < 0.05$) in aorta from SAMP8 compared with SAMR1 in all groups studied. In

SAMP8, ovariectomy increased the maximal contraction to U46619 (Sham 16.2 ± 0.6 *vs.* ovariectomized 19.2 ± 0.4 mN, $P < 0.05$), an effect that was reversed by estrogen treatment. The experiments performed with hydroxyfasudil demonstrated a higher activity of ROCK in senescent mice, which was further increased in ovariectomized SAMP8. Estradiol supplementation reverted the effect of ovariectomy on ROCK.

Conclusions: ROCK activity is involved in the increased response to thromboxane A_2 induced by ageing and estrogen deprivation in aged female mouse. Accordingly, Rho kinase inhibitors may have therapeutic potential for preventing vascular disease induced by ageing and menopause.

This work was supported by Ministerio de Economía y Competitividad, Instituto de Salud Carlos III-FEDER-ERDF, Red de Investigación Cardiovascular RD12/0042/0052, FIS PI13/00617 and Universitat València (UV-INV-AE13-141529).

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HYDROGEN PEROXIDE-MEDIATED ENDOTHELIUM DEPENDENT RELAXATIONS OF RENAL ARTERIES ARE ENHANCED IN OBESITY

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Obesity and metabolic syndrome are associated with an increased risk for diabetic complications such as diabetic nephropathy and chronic kidney disease (Abrass, *J Am Soc Nephrol* 15: 2768, 2004), although obesity independently increases the risk for vascular dysfunction and renal disease (Kramer et al, *Am J Kidney Dis* 46: 587, 2005).

Oxidative stress has been proposed as a key pathogenic mechanism in the altered function of several organs in obesity including kidney. In the present study, we aimed to determine whether reactive oxygen species (ROS) may alter endothelial function of renal arteries from insulin resistant obese Zucker rats (OZR).

Intrarenal arteries isolated from the kidney of OZR and lean Zucker rats (LZR) were mounted in microvascular myographs to assess function, superoxide production was measured by chemiluminescence and antioxidant enzymes were detected by Western blotting. Both basal and stimulated superoxide production were increased in arteries of OZR and reduced by the ROS scavenger tempol. Relaxations to ACh were significantly reduced in renal arteries from OZR compared to LZR, suggesting endothelial dysfunction.

However, under conditions of cyclooxygenase and nitric oxide (NO) synthase blockade, endothelium-dependent relaxations were enhanced in OZR and blunted by catalase and by inhibition of CYP450 enzyme indicating an enhanced production of vasodilator hydrogen peroxide (H_2O_2) in obese animals. In addition, antioxidant enzymes including catalase and both cytosolic and mitochondrial SOD were up-regulated in renal arteries from OZR. These results demonstrate oxidative stress and renal endothelial dysfunction in obesity, along with an upregulation of antioxidant enzymatic defenses associated to enhanced production of protective vasodilator H_2O_2.

This work was supported by grant SAF 2012-31631 from MINECO, Spain.

PAIN AND INFLAMMATION

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EXTRA VIRGEN OLIVE OIL POLYPHENOL DERIVATIVES DOWNREGULATE LPS-INDUCED INFLAMMATORY RESPONSE IN MURINE PERITONEAL MACROPHAGES SUPPRESSING NFKB SIGNALLING PATHWAY

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Introduction: Extra virgin olive oil (EVOO), has demonstrated significant anti-inflammatory properties. Nowadays, it is clear that its minor components have an important role in these beneficial effects. EVOO is rich in different phenolic compounds such as hydroxytyrosol (HT), hydroxytyrosyl acetate (AC) and 3,4-dihydroxyphenylglycol (GLY), among others.

Aims: In comparison with HT, we tested the *in vitro* effects of AC and GLY as well as two new acyl derivatives of GLY on lipopolysaccharide (LPS)-stimulated inflammatory response in murine peritoneal macrophages and deep insight into the intracellular mechanisms underlying the redox modulation involved in its anti-inflammatory effects.

Methods: The reactive oxygen species (ROS)-scavenging activity was identified by the DPPH radical assay. Isolated murine peritoneal macrophages were treated with HT,AC,GLY and its acyl derivatives: 4-(1,2-di(butanoyloxy)ethyl)benzene-1,2-diol and 4-(1,2-di(lauroyloxy) ethyl)benzene-1,2-diol in the presence or absence of LPS (5 μg/ml) for 18 h. Cell viability was determined using sulforhodamine B (SRB) assay and nitric oxide (NO) production was measured using the Griess reaction. Pro-inflammatory enzymes and transcription factors expression were detected by western blotting. Results: AC, GLY and its acyl derivatives showed a strong ROS-scavenging activity Moreover, these compounds reduced significantly nitrites levels and induced a significant decrease on inducible nitric oxide synthase (iNOS) expression. However, only AC and GLY but not the acyl-derivatives down-regulated cyclooxigenase (COX)-2 protein expression and prevented the nuclear transcription factor kappa B (NFκB) translocation.

Conclusion: This study establishes for the first time that both HT polyphenolic derivatives (AC and GLY) could improve LPS-induced oxidative stress and inflammatory response reducing NO generation by down-regulation of iNOS and COX-2 protein expression via inhibition of NFκB signalling pathway.

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SQUALENE MODULATES MYELOID CELLS ACTIVATION THROUGH MAPK, NRF2, PPARΓ AND MMP SIGNALLING PATHWAYS SQUALENE MODULATES MYELOID CELLS ACTIVATION THROUGH MAPK, NRF2, PPARΓ AND MMP SIGNALLING PATHWAYS

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Introduction: Squalene (2,6,10,14,18,22-tetracosahexaene) is a natural lipid widely present in nature, especially in wheat germ, rice bran and

olive oils. Up to date, has demonstrated important pharmacological properties including anti-oxidant, cardioprotective and anti-carcinogenic activities. Nevertheless, its anti-inflammatory effects remain unclear. The present study was designed to characterize the cellular mechanisms underlying the redox modulation on lipopolysaccharide (LPS)-stimulated inflammatory response in murine peritoneal macrophages and human monocytes and neutrophils by squalene and deep insight into the intracellular mechanisms involved.
Material/Methods: Murine peritoneal macrophages, human blood monocytes and neutrophils were treated with squalene in the presence or absence of LPS. Cell viability was determined using sulforhodamine B (SRB) assay and nitric oxide (NO) production was measured using Griess reaction. Pro-inflammatory enzymes, cytokines and transcription factors were detected by western blotting or real-time reverse transcription PCR.
Results: Without affecting cell viability, squalene reduced the level of nitrites, inflammatory cytokines (IL-1β, IL-6, TNF-α and IFN-γ), inducible NOS (iNOS) and COX-2 inflammatory proteins while enhanced the expression of heme oxygenase (HO-1) via NFκB, JNK, Nrf2, PPARγ and MMPs (MMP-1, MMP-3 and MMP-9) signalling pathways both in murine peritoneal macrophages and human leukocytes.
Conclusions: This study establishes that squalene inhibits LPS-induced inflammatory response by modulating oxidative stress-sensitive signalling pathways in murine peritoneal macrophages and human blood monocytes and neutrophils. Thus squalene might be a new promising target for diseases associated with overactivation of myeloid cells.

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OLEOYLETHANOLAMIDE AND PALMITOYLETHANOLAMIDE SHOW ANTI-INFLAMMATORY AND NEUROPROTECTIVE PROPERTIES BUT ONLY OEA DISRUPTS ANHEDONIA AFTER LPS SYSTEMIC ADMINISTRATION IN RATS

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Introduction: The acylethanolamides oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) are endogenous lipid mediators with proposed neuroprotectant properties in some neurological disorders. The precise mechanisms remain partly unknown, but growing evidence shows an anti-inflammatory/antioxidant profile.
Methods: We tested whether OEA/PEA (10 mg/kg, i.p) attenuate both neuroinflammation in frontal cortex and acute phase responses (HPA stress axis activation, thermoregulation and anhedonia) induced by lipopolysaccharide (LPS, 0.5 mg/kg, i.p.) administration.
Results: LPS increased mRNA levels of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6, the nuclear expression of NF-κB in cell and its inhibitory protein IκBα in citoplasm, the inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, microsomal prostaglandin E_2 synthase (m-PGES-1) mRNA, and pro-inflammatory prostaglandin E_2 (PGE_2) content in frontal cortex 150 min after administration. As a result, the markers of nitrosative/oxidative stress nitrites (NO_2^-) and malondialdehyde (MDA) were increased. Pretreatment with OEA/PEA prevented LPS-induced increase in TNF-α. OEA and PEA prevented LPS-induced NF-κB/IκBα up-regulation in nuclear and cytosolic extracts, respectively, the expression of iNOS, COX-2 and m-PGES-1 and the levels of PGE_2. Additionally, both acylethanolamides reduced LPS-induced oxidative/nitrosative stress. Interestingly, OEA and PEA had selective effects in LPS-induced acute phase responses: neither OEA nor PEA modified plasma corticosterone levels after LPS, but both acylethanolamides reduced the expression of hypothalamic markers of thermoregulation. Finally, only OEA potently disrupted LPS-induced anhedonia in a saccharine preference test.
Conclusion: Our results indicate that OEA and PEA have anti-inflammatory/neuroprotective properties and suggest a role for these acylethanolamides as modulators of psychopathologies with a neuroinflammatory component.

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THE ALPHA2-ADRENOCEPTOR OF ADRENOMEDULLARY CHROMAFFIN CELLS: A NEW TARGET FOR THE TREATMENT OF NEUROPATHIC PAIN?

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We are interested in identifying novel molecular targets for drugs potentially useful in the treatment of neuropathic pain. To this aim, we have implemented the chronic constriction of the sciatic nerve as a rat model of neuropathic pain. In this model, both tactile (as determined with the Ugo Basile aesthesiometer) and cold (acetone test) allodynia were fully sensitive to the alpha2-adrenoceptor agonist, dexmedetomidine (DE_{50} 10 and 12 μg/kg i.p., for the tactile and cold allodynia, respectively). Likewise, the fact that atipamezol (1 mg/kg, i.p.) reverted the effect of dexmedetomidine confirmed the involvement of alpha2-adrenoceptors in such antiallodynic effect.
Interestingly, we have observed that the adrenal glands from injured animals had a greater content of epinephrine and exhibited an increased frequency of spontaneous excitatory synaptic currents (sEPSCs) at the splanchnic nerve-chromaffin cell junction as detected by patch-clamp recordings in tissue slices. While these results are suggestive of a stress response to the pain syndrome, we wonder whether such a response (specifically, epinephrine release from chromaffin cells of the adrenal medulla) could in turn contribute to the alterations underlying neuropathic pain. In this regard, it is worth mentioning the following observations:
i) Dexmedetomidine 0.1 μM inhibited calcium entry through Ca_v channels and the associated membrane capacitance increase (an electrophysiologial correlate of epinephrine exocytosis) in chromaffin cells, this effect being prevented by 1 μM atipamezol.
ii) At concentrations above 1μM, dexmedetomidine blocked currents mediated by nicotinic cholinergic receptors (sEPSCs and those activated by exogenous acetylcholine) and Na_v channels from chromaffin cells, albeit these effects were not sensitive to atipamezol.
As a conclusion, it is suggested that adrenomedullary alpha2-adrenoceptors might contribute to the antiallodynic effect of dexmedetomidine by reducing the sympatho-adrenal response associated to chronic neuropathic pain.
Funded in part by CSD2008-00005, BFU2011-26253 and Instituto Teófilo Hernando grants.

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DEVELOPMENT OF PHENOTYPICAL *IN VITRO* MODELS IN PAIN FOR EARLY DRUG DISCOVERY

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Pain is one of the most prevalent pathologies and also one of the most common reasons for primary care consultations. New drugs do not

show the expected efficacy. One problem to solve is the lack of translationality of the primary assays for specific pain indications in early drug discovery. In this joint academic-industry PhD, our aim is to develop translational assays for the discovery of new neuropathic pain analgesics. We hypothesize that cell lines derived from the fusion of mouse neuroblastoma and DRG neuron would be appropriate for neuropathic pain translational phenotypic models. Our objective is to develop a HTS (high-throughput screening) for new neuropathic pain analgesics discovery.
We functionally evaluated the response of immortalized dorsal root ganglion (DRG) cell lines F11 and ND7/23 to seventeen nociceptive stimuli by means of label-free measurements using dynamic mass redistribution assays employing a Epic wavelength interrogation system (Perkin-Elmer).
We found that F11 cell line responded to both P substance and nerve growth factor; we built the concentration-response curves and obtained a EC_{50} values of 6×10^{-6} M and 5×10^{-7} M, respectively. ND7/23 cells only responded to P substance and we obtained an $EC_{50} = 1 \times 10^{-4}$ M in the concentration-response curve. These results highlight the importance of the cell selection because the response profiles are different depending on the cell line employed.
Future work will allow us to identify the response profiles in neuron differentiated cells to be used in HTS assays for the discovery of new analgesic drugs for neuropathic pain.

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RILPIVIRINE INDUCES LEUKOCYTE-ENDOTHELIAL CELL INTERACTIONS

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Introduction: Combined antiretroviral therapy (cART) has been linked to the development of cardiovascular diseases; however, given that this therapy consists of a combination of different drugs, it is difficult to exclude any one of them from being the causal agents. Leukocyte-endothelial cell interactions in the vessel wall are a hallmark of cardiovascular diseases, and we have previously demonstrated that Abacavir, Efavirenz and Nevirapine, but not Lamivudine, Tenofovir, Emtricitabine and Lopinavir induce such interactions *in vivo/in vitro*. The aim of the present study was to analyse the effects of Rilpivirine (RPV), Atazanavir, Darunavir, Raltegravir and Maraviroc on leukocyte accumulation.
Matherial/Methods: Four hours after treatment with clinically relevant doses of the abovementioned drugs, leukocyte parameters were monitored *in vivo* in rat mesentery vessels using intravital microscopy and *in vitro* using a parallel plate flow chamber system in which leukocytes flow over endothelial cells. Data were expressed as mean ±SEM. A one-way ANOVA-Newman-Keuls analysis was performed, and statistical significance was set at * $P < 0.01$ (vs. vehicle), $n \geq 4$.
Results: RPV promoted a significant increase in leukocyte rolling flux (RPV 0.5 μM: 82.5 ± 2.5* vs. vehicle: 38.0 ± 3.1), adhesion (RPV 0.5μM: 8.8 ± 1.1* vs. vehicle: 2.3±0.3) and emigration (RPV 0.5 μM: 1.0 ± 0.4 vs. vehicle: 0.5±0.3) *in vivo*, and a significant increase in leukocyte rolling flux (RPV 0.5 μM: 305.5 ± 5.1* vs. vehicle: 74.3 ± 5.0) and adhesion (RPV 0.5 μM: 24.9 ± 4.8* vs. vehicle: 3.9 ± 0.8) *in vitro*. No effect was produced by any of the other antiretrovirals evaluated.
Conclusion: RPV induces leukocyte accumulation *in vivo/in vitro*. These results point to the involvement of RPV in the genesis of cardiovascular diseases observed in cART-treated patients.

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INTERFERENCE OF ABACAVIR WITH THE PURINERGIC SIGNALLING PATHWAY

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Introduction: Abacavir, one of the most widely used antiretroviral agents, has recently been linked to cardiovascular diseases. Leukocyte accumulation at the vessel wall, especially adhesion to arteries, is a characteristic feature of these vascular diseases and is mediated by the interaction between adhesion molecules expressed on white blood cells and/or endothelial cells. We have recently demonstrated, both *in vitro* and *in vivo*, that cyclic purine analogues (abacavir and didanosine) induce leukocyte accumulation in the arterial endothelium through Mac-1/ICAM-1 interaction, and that this effect is not produced by pyrimidine analogues (lamivudine, zidovudine, emtricitabine) or the acyclic nucleotide tenofovir. However, the molecular mechanism underlying these interactions remains elusive. Given the chemical structure of abacavir, we have explored the effects of abacavir on the purine signalling pathway.
Material and Methods: Human umbilical arterial endothelial cells (HUAEC) and human peripheral blood mononuclear cells (PBMC) were treated with abacavir (0.5-15 μmol/L, 4h) to determine: 1) intracellular ATP levels by a luciferase bioluminescence assay; and 2) expression of CD73 and CD39 (enzymes responsible for ATP degradation) by western blotting. Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA and a Newman-Keuls post-hoc test, with significance set at ** $P < 0.01$ (vs. control), $n \geq 4$.
Results: Clinical concentrations of abacavir promoted a significant increase in intracellular ATP levels on HUAEC (abacavir 10 μmol/L: 173.4 ± 9.4** vs. 100% control) and on PBMC (abacavir 10 μmol/L: 196.51 ± 24.7** vs. 100% control) and a significant and dose-dependent decrease in CD73 expression on HUAEC (abacavir 10 μmol/L: 54.5 ± 11.1** vs. 100% control) and on PBMC (abacavir 10 μmol/L: 37.1 ± 6.1** vs. 100% control). Expression of CD39 was not modified.
Conclusion: Our results demonstrate a structure-activity relationship in the effects of abacavir on ATP levels and CD73 expression. Interference of abacavir with the purine signalling pathway could lead to a proinflammatory imbalance in homeostatic levels of ATP/ADP/AMP and Adenosine molecules that may promote the leukocyte-arterial endothelium accumulation characteristic of the cardiovascular pathologies observed in abacavir-treated patients.

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SELECTIVE ACTIVATION OF α7 NICOTINIC ACETYLCHOLINE RECEPTOR (NACHRα7) INHIBITS MUSCULAR DEGENERATION IN MDX DYSTROPHIC MICE

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Introduction: Amount evidence indicates that α7 nicotinic acetylcholine receptor (nAChRα7) activation reduces production of inflammatory mediators. This work aimed to verify the influence of endogenous nAChRα7 activation on the regulation of full-blown muscular inflammation in *mdx* mouse with Duchenne muscular dystrophy.
Material/Methods: We used *mdx* mice with 3 weeks-old at the height of myonecrosis, and C57 nAChRα7$^{+/+}$ wild-type (α7WT) and

nAChRα7$^{-/-}$ knockout (α7KO) mice with muscular injury induced with 60 μL 0.5% bupivacaine (bp) injected into the gastrocnemius muscle. Pharmacological treatment included selective nAChRα7 agonist PNU282987 (0.3 mg/kg and 1.0 mg/kg) and the antagonist methyllycaconitine (MLA) at 1.0 mg/kg injected intraperitoneally for 7 days.
Results: Selective nAChRα7 activation of *mdx* mice with PNU282987 reduced circulating levels of lactate dehydrogenase (LDH, a marker of cell death by necrosis), the area of perivascular inflammatory infiltrate and production of the inflammatory mediators TNFα and metalloprotease MMP-9 activity. Conversely, PNU282987 treatment increased MMP-2 activity, an indication of muscular tissue remodeling associated with regeneration, in both *mdx* mice and α7WT mice with bp-induced muscular lesion. Treatment with PNU282987 had no effect on α7KO, and MLA abolished the nAChRα7 agonist anti-inflammatory effect in both *mdx* and α7WT.
Conclusions: nAChRα7 activation inhibits muscular inflammation and activates tissue remodeling by increasing muscular regeneration. These effects were not accompanied with fibrosis and/or non-functional collagen. The nAChRα7 activation may be considered as a potential target for pharmacological strategies to reduce inflammation and activate mechanisms of muscular regeneration.

NEUROPSYCHOPHARMACOLOGY

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COLONIC BACTERIAL TRANSLOCATION IS INVOLVED IN THE NEUROINFLAMMATION DETECTED IN A DEPRESSION-LIKE MODEL THROUGH ACTIVATION OF MAPK PATHWAYS

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Introduction: One promising development relevant to depression is the emergence of inflammation and increased intestinal permeability as a mechanism that may play a role in neuropsychiatric diseases. Thus, we aim to evaluate if the exposure to an experimental model of depression induces bacterial translocation and the subsequence activation of the TLR-4 in the brain results in stimulation of the MAPK pathways.
Material and Methods: Experimental groups of male Wistar rats (n = 10/group): control and chronic mild stress (CMS). Forced Swim Test, Sucrose Intake Test, body weight and plasma costicosterone levels were performed to evaluate depressive behavior. The presence of bacteria in different organs and plasma LPS levels was studied. Colonic mRNA or expression levels of iNOS/NOS-2, COX-2, ZO-1, occludin, IgA and CCL28 were measured to evaluate intestinal dysfunction. In the brain, the expression and/or the mRNA levels of TLR-4, p-ERK 1/2, p-p38, p-JNK and AP-1 (c-Fos/c-Jun) were measured.
Results: There is a presence of bacteria in the mesenteric lymph nodes, liver and spleen in the CMS experimental group. The CMS induces an intestinal dysfunction (colonic expression of pro-inflammatory enzymes is increased and IgA levels are decreased). In the brain, there is an induction of the TLR-4 and an increase of the activated (phosphorylated) forms of ERK 1/2 and p38 resulting in an increased nuclear expression of AP-1.
Conclusion: our data suggest a role of the intestinal bacteria in the pathophysiology of depression through the MAPK pathways and could activate innate immune receptors aggravating the neuroinflammation and the oxidative/nitrosative damage.

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THE ATYPICAL ANTIPSYCHOTIC PALIPERIDONE PREVENTS BRAIN INFLAMMATION THROUGHOUT INNATE IMMUNE MECHANISMS

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Alterations on the innate immune/inflammatory system have been proposed in the pathophysiology of psychotic disease, but the mechanisms implicated remain elusive. The main agents of the innate immunity are the family of toll-like receptors (TLRs), which detect circulating pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPS). Current antipsychotics are capable to modulate pro/antiinflammatory pathways, but their actions on TLRs signalling pathways remain unexplored.
Methods: This study was conducted to elucidate the effects of paliperidone (1 mg/kg i.p.) on acute (6 hrs) and chronic (6 hrs/day during 21 consecutive days) restraint stress-induced TLR-4 pathway activation and neuroinflammation, and the possible mechanism/s related (bacterial translocation and/or DAMPs activation) in adult male Wistar rats. The expression of the elements of TLR-4-dependent neuroinflammatory pathway was analyzed at mRNA and protein level in prefrontal cortex samples.
Results: Paliperidone pretreatment prevented TLR-4 activation and neuroinflammation in the prefrontal cortex stress. Regarding the possible mechanisms implicated, paliperidone regulated stress-induced increased intestinal inflammation and plasma lipopolysaccharide (LPS) levels. In addition, paliperidone also prevented the expression of the endogenous activators of TLR-4, HSP70 and HGMB1.
Conclusions: Our results showed a regulatory role of paliperidone on brain TLR-4, which could explain the therapeutical benefits of its use for the treatment of psychotic diseases beyond its effects on dopamine and serotonin neurotransmission. The study of the mechanisms implicated suggest that gut increased permeability, inflammation and bacterial traslocation of Gram negative microflora and HSP70 and HGMB1 expression/activity could be new therapeutic targets for the treatment of psychotic and other stress-related psychiatric pathologies.

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FUSION PORE KINETICS IN C57 MOUSE CHROMAFFIN CELLS CHALLENGED WITH DIFFERENT SECRETAGOGUES

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The release of catecholamine elicited by the physiological neurotransmitter acetylcholine (ACh) is triggered by Ca^{2+} ions that enter adrenal medullary chromaffin cells (CCs) through voltage-activated calcium channels (VACCs). However, other secretagogues that mobilise Ca^{2+} from the endoplasmatic reticulum store (ER) also causes a secretory response (García et al., 2006, Physiol Rev. 2006 Oct;86:1093-131; García et al., 2012, Cell Calcium 2012;51:309–20). The last steps of this response are best analysed by the kinetic of the fusion pore through which adrenaline is released to the extracellular space.
It is known that the fusion pore kinetics may change with the concentrations of Ca^{2+} (Alés et al., Nat Cell Biol. 1999 May; 1:40-4.) and these parameters vary with different secretagogues. Here we have investigated the kinetics of the fusion pore in C57 mouse chromaffin cells challenged with ACh, DMPP, muscarine, caffeine, histamine or K^+, that cause exocytosis through $[Ca^{2+}]_c$ elevations elicited by Ca^{2+} mobilisation from different sources.
Material/Methods: Amperometric secretory events in single C57 mouse chromaffin cells challenged with the different secretagogues were monitored with a carbon fibre microelectrode. During 1 minute the number of secretory spikes, the cumulative secretion and the kinetics of single-spike events were analysed as previously described; (Fernández- Morales et al., Am J Physiol Cell Physiol. 2009 Aug; 297: C407-18).
Results: We have studied the number of spikes and some kinetic parameters. First, spike number with ACh and K^+ was similar. DMPP and caffeine elicited an initial burst of spikes that was not sustained with time. When using muscarine and histamine, the initial spike burst was absent and a slow-rate secretion was sustained along the 1 minute time period.
Kinetic parameters of amperometric spikes analysed were: t½, amplitude, Q, raise rate, decay time and the presence of foot. We saw remarkable differences among the average spikes evoked by all of the secretagogues we used, except for Q which was very similar for all stimuli. Interestingly, we noticed the spikes produced by ACh are faster than the spikes produced by the other secretagogues: they had shorter t1/2, faster rise and shorter decay time.
Conclusions: The common requirement for all secretagogues here studied in eliciting the exocytotic release of catecholamine from C57 mouse chromaffin cells, is the $[Ca^{2+}]$ elevation. However, the mode and kinetics of the exocytotic fusion pore deeply differ. This is particulary relevant in the context of studies of synaptic release of neurotransmitters in transgenic C57 mouse models of neurodegenerative diseases (de Diego AM et al, Biochem Biophys Res Commun, 2012 Nov 30; 428:482-6). It will be of interest to discern the role of Ca^{2+} transporters of mitochondria in controlling the secretory responses to the different secretagogues.

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CHONDROITIN SULFATE ELICITS DIFFERENT $[CA^{2+}]_C$ SIGNALS AT THE VARIOUS SUBTYPES OF HIPPOCAMPAL CELLS

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Introduction: We have recently observed that glucosaminoglican chondroitin sulphate (CS) elicits transient elevations of cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_c$) in rat embryo hippocampal cell cultures (Maroto et al., J Neurochem 2013). Because these cultures contain glial and various neuronal types, we have investigated here whether they responded equally or differently to a CS and high-K^+ challenges.
Methods: Hippocampi from E20 rat embryo were disaggregated and cultured for 10 days, to allow the establishment of a neuronal net mixed with glia cells were loaded with fura-2 and $[Ca^{2+}]_c$ changes were measured with microfluorimetry.
Results: Glial and neuronal cell types were morphologically identified; they responded differently to CS and K^+ as summarised in the following points: (1) Glia responded to CS but not K^+; $[Ca^{2+}]_c$ transients had a slow kinetics and clearance (τ_{cl}). This observation suggests a response mediated by metabotropic glutamate receptor. (2) Granule cells generated responses that were higher for CS, compared with K^+; τ_{cl} was faster in compared with glia. (3) Conversely, pyramidal cells produced greater responses to K^+, compared with CS; responses to CS were blocked by CNQX. (4) Both hilar spiny cells ("mossy cells") and aspiny cells ("fast-spiking") responds to CS and high-K^+, but depending on GABAergic subpopulations into each group, these cells can show differences in their τ_{Cl} values. (5) CS-elicited neuronal $[Ca^{2+}]_c$ transients were mediated by Ca^{2+} influx through voltage-activated Ca^{2+} channels (VACCs) of the N-subtype (α_{1B}, Cav 2.2) and P/Q subtype (α_{1A}, Cav 2.1), as revealed by ω-conotoxin GVIA and ω-agatoxin IVA.
Discussion: In neurons, the CS-elicited $[Ca^{2+}]_c$ elevation are due to Ca^{2+} entry through AMPA/kainite receptor channels; this is consistent with the observation CS causes neuronal depolarisation by activating those receptors. In glial cells, the CS-elicited Ca^{2+} signals seem to be mediated by metabotropic receptors and Ca^{2+} mobilisation from intracellular stores.
Conclusions: Two conclusions emerge from this study: (i) different patterns of responses to CS and K^+ may be a fine functional tool to identify the subtypes of cells present in hippocampal cultures; (ii) being a major component of extracellular matrix and perineuronal nets, CS may play relevant functional roles in the context of neuroplasticity and neurorreparation after a lesion.Supported by CABICYC Bioibérica/UAM, SAF2010, and Fundación Teófilo Hernando.

NATURAL PRODUCTS

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ANTI-INFLAMMATORY MECHANISMS OF ACTION OF OXYLIPINS ISOLATED FROM MICROALGAE

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Introduction: Oxylipins are PUFA-derived mediators involved in the resolution of many inflammatory disorders. A recent study in our group has revealed the interesting anti-inflammatory activity of an oxy-

lipin-containing lyophilized from *Chlamydomonas debaryana* and its major oxylipin constituent, 13-S-HOTE, in an acute ulcerative colitis model in rats.
Objective: The aim of this study was to investigate the mechanisms of action of several oxylipins isolated from the microalgae *Chlamydomonas debaryana* and *Nannochloropsis gaditana* in order to clarify their potential value in the prevention of IBD.
Materials/methods: *In vitro* anti-inflammatory activity was performed using two human cell lines, THP-1-macrophages and HT-29 colon cells. Several pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8) were measured by ELISA in both cell lines. The effect of the oxylipins (13-HOTE, 13-HODE and 15-HEPE 100μM) on the cellular localization of NFκB and PPAR-γ was studied by using confocal microscopy.
Results: Oxylipins decreased the pro-inflammatory cytokines production in these cells in a dose-dependent manner. In addition, confocal microscopy study showed that the treatment of THP-1 and HT-29 cells with the oxylipins decreased the nuclear location of NF-kB and increased the presence of PPAR-γ in the nucleus of these cell lines, which is known to down-modulate the expression of inflammatory cytokines.
Conclusion: These results suggest that these microalgae-derived-products could reduce the production of inflammatory cytokines by means of PPAR-γ activation, by mechanisms that could be independent or dependent on NFκB. Therefore, these oxylipins might be an emerging therapeutic strategy for the treatment of colitis active phase.
Grant from MINECO, Spain, ALGALIMENTO-Project-IPT-2011-1370-060000.

PHARMACOKINETICS

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EXPOSURE-RESPONSE ANALYSIS OF PERIPHERAL NEUROPATHY IN PATIENTS WITH SOLID TUMORS TREATED WITH PM060184

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Introduction: Antitubulin agents (AT) produce apoptosis of tumoral cells by binding to tubulin of microtubules during mitosis, but also produce peripheral neuropathy (PN) as main adverse event, by binding to tubulin of neuronal axons. PM060184 is a novel AT; after treating 70 patients, 6 cases of Grade 3 (G3) PN have been reported. The aim of this study was to evaluate the relationship between pharmacokinetics (PK) of PM060184 and the probability of developing G3 PN, as a way to characterize the safety profile of PM060184.
Material/Methods: PK data from the first two phase 1 clinical trials with PM060184 were used. Individual PK parameters were calculated by means of the population PK model of PM060184. A logistic regression model was used to assess the effect of exposition to PM060184 on the probability of developing G3 PN.
Results: A tricompartmental model with linear elimination from the central compartment adequately described PK of PM060184, with population values of total clearance of 58 L/h, volumes V1, V2 and V3 of 3.6, 16 and 189 L respectively and a fast terminal half-life of 4.5 h. The probability of developing G3 PN is related with exposition to PM060184 (95% IC de la OR: 1.005-1.024; $P < 0.01$). The regression model predicts a 33% incidence of G3 PN in patients exposed to AUC of 470 mg*hr/L, corresponding to a total dose of 27 mg/m².
Conclusion: Considering these results together with the preliminary antitumoral activity of PM060184 observed in treated patients so far, further clinical development of PM060184 is warranted.

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PHARMACOKINETICS/PHARMACODYNAMICS POPULATION MODELING OF TRANSAMINITIS IN CANCER PATIENTS TREATED WITH KAHALALIDE F

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Objectives: Transaminitis was the dose limiting toxicity in the phase I studies and the major causes of kahalalide F (KF) dose reduction and treatment discontinuations. We aimed to characterize the transient elevation of the alanine aminotransferase transaminase (ALT) after the intravenous (i.v.) administration of KF in cancer patients.
Methods: A total of 250 patients from 3 Phase 1 and 3 Phase 2 (Ph2) studies, receiving i.v. infusion of KF as monotherapy at doses ranging 266–6600 μg/m² were included. An open linear two-compartmental pharmacokinetic (PK) model described the time course of KF plasma concentrations using NONMEM® VII. A precursor-dependent indirect pharmacodynamic (PD) response model, where the transfer process of ALT from hepatocytes to plasma was stimulated by KF plasma concentrations, was used to characterize the ALT time course. The effect of patient demographics and/or pathophysiological factors on PK and PD parameters were evaluated. Model evaluation was conducted using bootstrap and predictive check. Model-based simulations were conducted to explore the role of dose, schedule and infusion duration on the incidence of severe transaminitis.
Results: Liver metastasis was associated with a 63% decreased in serum ALT. The Model captures the tolerance phenomena after multiple doses and distinguishes patients with high sensitivity to KF-induced ALT elevation (15%). Model-based simulations show that time course of ALT elevation depends on dose and schedule but not on infusion duration. Bootstrap and predictive check evidenced the model was deemed appropriate to describe KF PK and the incidence and severity of transaminitis. Among the dosing regimens evaluated, the dosing regimen selected for Ph2 provide less incidence of ALT increase.
Conclusions: The developed model adequately describes PK and PD after KF i.v infusion in cancer patients. The interpatient variability is moderate and similar to other anticancer drugs. Other dosing schedules than the one tested in Ph2, in terms of ALT increase are not warranted.

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PHARMACOKINETIC AND PHARMACODYNAMIC MODELING OF NEUTROPENIA FOR LURBINECTEDIN

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Introduction: Lurbinectedin is an antineoplastic agent in clinical development. Plasma samples have been collected to characterize concentration-time profiles of lurbinectedin in 8 clinical trials. The main toxicity observed during the treatment with lurbinectedin has been hematological with marked decreases of absolute neutrophils count (ANC).

The objective of the current work has been to develop a PK model for lurbinectedin as single agent, to characterize the sources of variability of drug disposition and finally to build a PK/PD model to describe the time course of ANC.
Methods: The concentration-time profiles of lurbinectedin as single agent (3 phase 1 trials and 3 phase 2 trials) were pooled with the most relevant covariates. Once the PK model was established the profiles of time course of ANC were included. The PK/PD model for neutropenia was based on the work done by Friberg (1).
Results: The results obtained allowed a better knowledge on the clinical development of lurbinectedin and to explain part of the wide variability which affects the time course of ANC. The simulations were useful to optimize the schedules of administration and to assess the strategies used to minimize neutropenia: dose delays, dose reductions and the use of GCSF.
Conclusions: The use of modeling tools has allowed integrating the relevant information to explain the time course of ANC, to improve the administration schedules and to understand the pharmacokinetics of lurbinectedin.
Bibliography:
1. Friberg LE, Henningsson A, Maas H, et al. (2002) Model of chemotherapy-induced myelosuppression with parameter consistency across drugs. J Clin Oncol 20:4713–4721.

MOLECULAR PHARMACOLOGY

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NOVEL NEGATIVE GATING MODULATORS OF KCA2/3 POTASSIUM CHANNELS AS PHARMACOLOGICAL TOOLS FOR NOVEL TRATMENTS

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Introduction: Calcium/calmodulin-regulated K^+ channels, KCa2/3, are present in many organs and tissues such as vascular endothelia, intestinal and bronchial epithelia, neurons or red and white blood cell lineages. Therefore, they play important roles in cardiovascular, immunological and neurological functions and emerged as therapeutic targets. Here, we generated innovative small-molecules with a good "drug-like" profile (Lipinski's-rule-of-five) for pan-negative-gating modulation of KCa2/3 channels.
Materials/Methods: We performed organic synthesis of a series of small-molecule entities and performed patch-clamp experiments to evaluate their potency. In ex-vivo model systems, we evaluated their functional activity by myography on porcine coronary arteries and we determined cardiovascular safety and efficacy by telemetric cardiovascular monitoring on mice.
Results: Our novel small-molecules met Lipinski's rule and we identified one di-benzoate compound 815 as a KCa2/3 pan-negative-gating modulator, with potencies in the nanomolar range (KCa3.1:IC50≈30 nM, KCa2.3IC50≈100 nM). The compound had no considerable effects on distantly related human K^+ channels. Isometric myography revealed its capacity to block endothelium-derived hyperpolarization-induced relaxation to 100 nM bradykinin by ≈90% and increased contraction to 5-HT to ≈145% of controls. Telemetric cardiovascular monitoring showed that single injection of 100 mg/kg 815 was safe and moderately reduced heart rate by ≈10%.
Conclusions: We synthesized and identified new chemical entities that could serve as pharmacophores for specific pan-negative-gating modulation of KCa2/3. Of these, 815 had nanomolar potency, and ex-vivo and in-vivo activity. 815 could be a tool to define physiological and pathomechanistic roles of KCa2/3 in the vasculature, CNS, and during inflammation in-vivo.

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GAQ INDUCES APOPTOSIS THROUGH A NOVEL INTERACTING REGION WITH THE EFFECTOR PKCZ

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Introduction: G proteins play an essential role in the initiation of G-protein coupled receptor (GPCR). Particularly, the Gαq/11 family of G proteins has been classically shown to associate to and activate PLCβ. However, a growing body of evidence points at additional effectors to be responsible for some Gαq functions. We have reported that GPCR activation promotes a direct interaction between Gαq and PKCζ, leading to the stimulation of the ERK5 pathway and to the development of cardiac hypertrophy, independently of the canonical effector PLCβ.
Methods: Biochemical characterization of the complex was performed by mutagenesis and co-immunoprecipitation analysis. Functionality of the complex was determined performing xCELLigence meassurements, propidium iodide incorporation and annexin V/7-AAD binding assays.
Results: Here we present a biochemical characterization of the Gαq/PKCζ interaction and its implication in the promotion of apoptosis. This association involves the basic PB1-type II domain of PKCζ and a novel acidic effector-binding region in Gαq, which was termed *pseudo*-PB1 type I domain and that has been proved to be essential for the association with PKCζ and activation of the ERK5 pathway. Indeed, we have observed that PKCζ acts as a scaffold bringing Gαq and ERK5 together. Additionally, we reveal that Gαq/PKCζ/ERK5 complex link Gαq to an apoptotic cell death pathway.
Conclusions: Overall, this study provides concluding evidence showing that PKCζ acts as a functional effector of Gαq through the engagement of a novel binding region in the alpha subunit leading to ERK5 activation and apoptotic cell death, a process with paramount importance in the progression to heart failure. These results could help to uncover the indentification of new potential pharmacological targets for the treatment of cardiovascular diseases.

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RESIDUE HIS264 IN EXTRACELLULAR LOOP 3 OF ADENOSINE A_{2A} RECEPTOR IS CRITICAL FOR THE KINETICS OF LIGAND BINDING

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Adenosine A_{2A} receptors (A_{2A}AR) are therapeutic targets in asthma, inflammation, pain, Parkinson or Huntington diseases. The crystal structures of A_{2A}AR show a polar interaction between extracellular loops (EL) 2 and 3 through the family conserved residue Glu5.30 in EL2 and residue 7.29 in EL3, occupied by His264 in A_{2A}AR or Asn266 in the closely related A_{2B}AR. Molecular dynamics simulations of both receptors suggested that the interaction between Glu5.30 and His7.29 in A_{2A}AR is key to maintain low mobility of the ELs[1], which act as a lid closing the binding site and stabilizing the A_{2A}AR/ZM-241385 (4-[2-[[7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl] amino]ethyl]phenol) complex.

We hypothesize that the His7.29Asn replacement is responsible for a faster dissociation rate of ligands, which might explain the lower affinity of most ligands for A_{2B}AR. Considering that residence time of drugs on their targets [t_R, the inverse of the dissociation rate constant (k_{off})] may be a critical parameter to predict *in vivo* efficacy[2], we aimed to determine the effect of His264Asn replacement on ligand binding kinetics of [^{3}H]-ZM-241385 at A_{2A}AR.

Kinetic binding dissociation experiments in membranes of HEK293 cells stably expressing the receptors provided values of $k_{off} = 0.01417 \pm 0.00242$ min^{-1} and 0.1516 ± 0.02610 min^{-1} (mean ± SEM, $n = 4$) (**$P < 0.01$, unpaired t test, two-tailed) at wild type and His264Asn mutant A_{2A}AR, respectively (t_R of [^{3}H]-ZM-241385 = 70.57 min and 6.59 min, respectively), being k_{off} value at A_{2A}AR similar to that previously reported (0.01 min^{-1})[3]. These experimental results confirm a role of EL3 mobility in ligand binding kinetics.

References:

1. Rodríguez D *et al.* Biochemistry. 2011, 50:4194-4208.
2. Copeland R. Expert Opin Drug Discov. 2010, 5:305-310
3. Guo D *et al*. Br J Pharmacol. 2012, 166: 1846-1859.

HORMONAL PHARMACOLOGY AND METABOLISM

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TRIGLYCERIDE-RICH LIPOPROTEINS MODULATE THE NIACIN-INDUCED PRODUCTION OF THE VASODILATOR PGD_2 IN HUMAN MACROPHAGES

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Introduction: Niacin is one of the most effective agents for lowering triglycerides, cholesterol, LDL and raising HDL. However, its use is limited due to a severe vasocutaneous flushing reaction mediated by PGD_2. The main PGD_2 producing cells and molecular targets of niacin remain unclear. Herein, the potential of myeloid granulocytes, monocytes and monocyte-derived cells in niacin-flush and the underlying mechanisms by which postprandial triglyceride-rich lipoproteins (TRL) could modulate niacin-induced PGD_2 were investigated.

Material/Methods: Monocytes and neutrophils were obtained from healthy volunteers, and TRL after the ingestion of single meals containing refined olive oil (MUFAs), fat cream (SFAs), or refined olive oil + fish oil (MUFAs + PUFAs omega-3). Monocytes were differentiated and polarized to macrophages (M0, M1 and M2) and dendritic cells. Cells were 30 min pre-incubated with/without TRL (100 μg/mL) or AAS (100 μM) and then treated with niacin (3 mM at different times). PGD_2 release was determined by EIA and gene expression changes by qPCR.

Results: In contrast to neutrophils, monocytes and the myeloid monocyte-differentiated lineage were PGD_2-producing cells. Macrophages (notably M0) were the major cell type involved in niacin-induced PGD_2 release. Pre-incubation of macrophages with AAS or TRL (in a fatty acid-dependent manner) decreased the production of PGD_2 in agreement with gene signatures of the niacin flushing response (GPR109A, PTGDS, PLA2G4D, NOS-2, COX-2, NAMPT, SIRT-1 and PPARG).

Conclusion: These findings provide evidence that macrophages could play a role in niacin-flush and that fatty acids in TRL could help to buffer such a side effect of niacin.

GASTROINTESTINAL/RESPIRATORY PHARMACOLOGY

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NOVEL MARKERS TO EVALUATE IMMUNOMODULATORY EFFECTS OF ANTIBIOTICS IN DSS-COLITIS: MICRO-RNA EXPRESSION AND MICROBIOTA POPULATIONS

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Introduction: Minocycline and doxycycline exert immunomodulatory effects that could be beneficial in IBD. Micro-RNAs have been recently reported to play a key role in intestinal homeostasis that can be influenced by microbiota populations. The aim of the study was to evaluate the effect of these antibiotics in the DSS-colitis model in mice, characterizing the modifications induced in the micro-RNA expression and bacterial diversity.
Methods: Female C57BL/6J mice were assigned into non-colitic and DSS-colitic groups. Colitis was induced by dextran sodium sulfate (DSS) in the drinking water (3%) for 6 days. Once the colitis process was established, colitic mice were divided in three groups: DSS-control (without treatment), MNC (receiving minocycline 50 mg/kg/day) and DXC (receiving doxycycline 10 mg/kg/day). After six days of treatment, all mice were sacrified. The inflammatory status was evaluated by a disease activity index (DAI), qPCR of inflammatory markers, including micro-RNAs. Also, changes in microbiota populations were characterized by pyrosequencing.
Results: According to the DAI values, minocycline and doxycycline treatment improved the recovery of colitic mice, ameliorating some of the inflammatory markers, including IL-1β, IL-17, TGFβ, MMP-2, MUC-2, MUC-3, ZO-1 and occludin. A micro-RNA expression profile was established for this model of colitis showing an increased expression of miR-155, miR-223 and miR-150 while miR-143 and miR-375 were decreased. Both antibiotics partially restored the expression of some of these markers. Pyrosequence characterization of microbiota showed that minocycline and doxycycine treatments increased the bacterial diversity, reverting the dysbiosis produced by DSS-colitis.
Conclusion: Minocycline and doxycycline are able to modify the expression of different inflammatory markers and microRNAs, as well as to increase the intestinal bacterial diversity. These observations confirm the combined contribution of antibiotic and immunomodulatory properties ascribed to these compounds that could be of great interest to control the complex pathogenesis of IBD.

PHARMACOGENETICS AND PHARMACOGENOMICS

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EFFECT OF *CYP2C9* AND *CYP2C8* POLYMORPHISMS ON PHARMACOKINETICS OF IBUPROFEN ENANTIOMERS

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Introduction: Ibuprofen is a widely used chiral nonsteroidal anti-inflammatory drug, metabolized by CYP2C9 and CYP2C8, which are polymorphic. The aim of this study was to evaluate the involvement of *CYP2C9* and *CYP2C8* genetic polymorphisms on the pharmacokinetics of ibuprofen enantiomeric forms.
Methods: 122 healthy volunteers (63 men and 59 women) participating in six ibuprofen bioequivalence clinical trials were genotyped for CY2C8*2, CYP2C8*3, CYP2C8*4, CYP2C9*2 and CYP2C9*3 polymorphisms by real time PCR. *R*- and *S*-ibuprofen plasma levels were measured using high-performance liquid chromatography combined with tandem mass spectrometry. Pharmacokinetic parameters were calculated from the plasma concentration–time data using noncompartmental methods.
Results: Allelic frequencies for *CYP2C8* were 81.97% *1, 1.64% *2, 10.65% *3, and 5.74% *4, and for *CYP2C9* were 81.97% *1, 10.24% *2, and 7.79% *3. Linkage disequilibrium was observed between CYP2C8*3 and CYP2C9*2. *CYP2C8* polymorphisms did affect neither *R*- nor *S*-ibuprofen pharmacokinetics. Compared with *1/*1 subjects, CYP2C9*3 carriers showed lower *R*- and *S*-ibuprofen clearance, and higher *S*-ibuprofen AUC and half life. Allele CYP2C9*2 carriers showed higher *S*-ibuprofen AUC and half life and lower clearance than *1/*1. Compared with wild type subjects, *S*-ibuprofen clearance diminished 16.4% in *1/*2, 27.1% in *1/*3, 38.7% in *2/*2 and *3/*3, and 44.6% in *2/*3. R-ibuprofen clearance diminished 6.4% in *1/*2, 9% in *1/*3, 28.3% in *2/*2 and *2/*3, and 32.3% in *3/*3.
Conclusions: The pharmacokinetics of *S*-ibuprofen and *R*-ibuprofen is affected mainly by *CYP2C9* polymorphisms, but *CYP2C8* polymorphisms have not a significant role.

RECEPTORS

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PHARMACOLOGY OF NATIVE α6β4 NICOTINIC ACETYLCHOLINE RECEPTORS IN HUMAN ADRENAL CHROMAFFIN CELLS

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Introduction: Human adrenal chromaffin cells have been shown to express nicotinic acetylcholine receptors (nAChR) of the α6β4 subtype which facilitate the release of catecholamines from chromaffin cells when activated. This release is excessive in diseases such as hypertension or pheochromocytoma. In addition, a related subtype, α6β2, is expressed by catecholamine releasing neurons in the brain and has been implicated in several neurological and neuropsychiatric conditions including Parkinson's disease and nicotine reward and dependence.
Material and Methods: Perforated-patch whole-cell electrophysiology was performed on isolated human chromaffin cells obtained from organ donors.
Results: Ligands known to activate α6β2 nAChRs, nicotine, varenicline, and the experimental compound 5-I-A-85380, were also found to activate α6β4 receptors at concentrations in the nano to low micromolar range. Experiments with acetylcholine and nicotine revealed that chromaffin cell α6β4 receptors were relatively rapidly desensitizing. The effects of these compounds were not due to the presence of α6β2 receptors because the β2-seletive antagonist α-conotoxin LvIA(N9R, V10A) had little effect on acetylcholine-evoked currents.
Conclusions: Our results have broad implications in the development of compounds with potential use in the therapeutics of hypertension and pheochromocytoma and nicotine addiction. They suggest that the cardiovascular side effects observed may be the result of activity on α6β4 receptors expressed by adrenal chromaffin cells.

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NEUROTROPHIN-3 MODULATES THE FUNCTIONALITY OF ENDOTHELIAL β_3-ADRENOCEPTORS IN MOUSE AORTA

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Background: Neurotrophin-3 (NT-3) has been studied as a regulator of the survival, development and function of sympathetic neurons during embryonic development (1). In the adult cardiovascular system, NT-3 modulates the sympathetic innervation (1) and increases NO production in endothelial cells (2).
Objective: To analyze the role of NT-3 on vascular α_1- and β-adrenoceptor (AR) mediated responses.
Methods: Responses to α_1-AR agonist (phenylephrine), β-AR agonists (isoprenaline, non-selective; salbutamol, β_2-selective and SR58611A, β_3-selective), acetylcholine and sodium nitroprusside (NO donor) were analysed in isolated aorta from 8-10 month old male *Ntf3*$^{+/lacZneo}$ mice that express reduced levels of NT-3 (3) and wild type (WT) with (E+) or without (E-) endothelium, as previously described (4). Two consecutive concentration-response curves (CCR) of phenylephrine were obtained to test the reproducibility of contraction after incubation with β-AR agonists.
Results: Phenylephrine-induced contraction and acetylcholine, nitroprusside, salbutamol and isoprenaline vasodilatations were similar in both groups of animals and in (E+) or (E-) rings. In WT (E+) mice, SR58611A showed biphasic relaxation curves ($pEC_{50\ high}$: 7.54 ± 0.57, pEC_{50low}: 5.07 ± 0.15, β_3-AR high affinity sites: 20.2 ± 5.8%, $n = 6$). In *Ntf3*$^{+/lacZneo}$ mice, SR58611A relaxed monophasically with low pEC_{50} (5.27 ± 0.12, $n = 6$), suggesting a lack of β_3-AR participation. The maximal phenylephrine response was significantly reduced after SR58611A treatment in (E+) WT (36.4 ± 6.9% vs. 79.6 ± 7.6% of KCl 80 mM, $n = 7$, $P < 0.01$) but not in NT3$^{+/-}$ mice.
Conclusions: Neurotrophin-3 exhibits an endothelium-dependent activity which decreases β_3-AR mediated vasodilatation as well as the β_3-AR-mediated "desensitization" of the contractile response to α_1-AR in mouse aorta.
References:
1. Caporali A and Emanueli C, Physiol Rev, 89: 279-308, 2009
2. Meuchel et al., Cardiovas Res 91, 668-676, 2011
3. Fariñas et al., Nature, 369(6482):658-61, 1996.
4. Flacco et al., Br J Pharmacol, 169:413-2, 2013.

TEACHING IN PHARMACOLOGY

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REPLACEMENT OF LABORATORY WORK BY AUDIOVISUAL SIMULATIONS IN PHARMACOLOGY TEACHING: THE STUDENTS' POINT OF VIEW

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In the last decades a progressive replacement of traditional laboratory practices by alternative teaching methods has been applied in most pharmacology departments, mainly to attend ethical issues concerning animal experimentation. The actual effectiveness of such replacement has been discussed, the opinion of the students being often ignored in this debate. Recently, the remaining laboratory practices in our area of Pharmacology were totally replaced by workshops where students worked on audiovisual simulations, clinical case studies and bibliography to virtually reproduce experiments, discuss clinical problems and prepare oral presentations.
An 11-item questionnaire was anonymously filled out by 13 students of "General Pharmacology" (degree of Pharmacy) and 35 students of

"Basic Pharmacology" (degree of Medicine) to evaluate different aspects of these workshops. One item specifically addressed animal experimentation by raising this question: "In the case that experiments with animals could have been conducted, would you have preferred to run them instead of working on audiovisual materials?" Generally speaking, the evaluation of workshops was highly positive in both groups of students. Concerning the former question, 77% of Pharmacy students and 46% of Medicine students expressed an unconditional preference for real experiments in the lab; 8% and 25% transmitted mixed thoughts and 15% and 23% overtly preferred audiovisuals.
Many students of the three categories remarked that laboratory work cannot be fully replaced by simulations. If the expectations of the students are to be taken into account, some representative and ethically acceptable laboratory practices should be considered at least as an option for motivated individuals.

OTHERS | OTROS

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MULTICENTRIC OSTEOARTHRITIS INTERVENTION STUDY WITH SYSADOA (MOVES): EFFECTS OF COMBINED GLUCOSAMINE HYDROCHLORIDE AND CHONDROITIN SULFATE VS CELECOXIB FOR PAINFUL KNEE OSTEOARTHRITIS

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Introduction: The purpose was to assess whether combination of glucosamine hydrochloride and chondroitin sulfate (2 capsules GH 250 mg + CS 200 mg TID) has comparable efficacy to Celecoxib (CE 200 mg QD) to reduce pain in knee OA patients with severe pain after 6 months of treatment.
Material/Methods: MOVES was an international multicentric, phase IV, double-blind, non-inferiority trial to compare efficacy and safety of GH+CS (Droglican®, Bioiberica SA) and CE. Patients were eligible if ≤40 years, fulfilled ACR criteria for knee OA, had Kellgren-Lawrence grade 2 or 3 and WOMAC pain>30 (0-500). Patients with gastrointestinal or cardiovascular risk were excluded. Primary outcome was mean decrease in WOMAC Pain.
Results: 763 patients were screened and 606 randomized (GH+CS $N = 304$ or CE $N = 302$). 522 (86.1%) completed trial and were included in PP non-inferiority analysis. Mean (SD) age was 62.7 (8.9) years, 438 (83.9%) were women; KL grade 2 changes were in 327 (62.6%). Basal WOMAC pain score was 372.0 (41.8) and 370.6 (41.4) in GH+CS and CE respectively; at 180 days was 185.8 (7.4) and 184.7 (7.6), corresponding to a mean (SEM) difference of 1.11 (10.63) units (CI95%-21.99, 19.76) ($P = 0.917$) that respects non-inferiority margin. There was no significant difference between groups in the secondary outcomes and proportion of patients with treatment-emergent adverse events between the groups (50.7% overall); no deaths occurred in this 6-month study.
Conclusions: These results demonstrated comparable efficacy of GH+CS (Droglican) to celecoxib for relief of severe knee pain in patients with knee OA and a similar safety profile.

POSTER ABSTRACTS
CARDIOVASCULAR PHARMACOLOGY

1
CARDIOPROTECTION EXERTED BY LABDANE DITERPENES IS RELATED TO ACTIVATION OF AKT PATHWAYS IN ISOLATED RAT HEARTS SUBJECTED TO ISCHEMIA/REPERFUSION

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Introduction and objectives: Diterpenes are bioactive natural products with great therapeutic potential. Labdane-type diterpenes exert anti-inflammatory and cytoprotective activities, although their therapeutic potential in cardioprotection has not been evaluated. Development of new compounds targeting different pathways and acting as multitarget inhibitors may provide new strategies for cardiovascular therapy, in particular, for ischemia/reperfusion (I/R) injury, a primary cause of cardiac failure. This study was designed to investigate the cardioprotective effects of a labdane diterpene (DT1) against cardiac I/R injury in isolated rat hearts and the molecular mechanisms involved.
Materials and Methods: Hearts from 21 adult male Wistar rats were randomly assigned to 1 of 3 experimental groups: non-ischemia group, I/R group and treatment group (DT1 + I/R). Isolated rat hearts were mounted in a Langendorff device underwent ischemia (I) for 30 min and 60 min of reperfusion (R). DT1 (20 μM) was added when reperfusion started.
Results: We found that I/R resulted in a loss of cardiomyocytes, while treatment with DT1 significantly decreased the number of cardiac apoptotic cells detected as TUNEL-positive nuclei, compared with untreated animals following global I/R (20.0 ± 3.0% *vs.* 59.6 ± 3.6%; $P < 0.001$). Increases in the expression of anti-apoptotic proteins and a significant reduction of caspasa-3 activity were also observed. Analysis of survival pathways showed that treatment with DT1 promotes a significant increase in the levels of relevant proteins involved in cytoprotection (pAKT, pERK1/2 and pPDK1). Moreover, the labdane-induced cardioprotection involves activation of AMPK, suggesting a role for energy homeostasis in its mechanism of action.
Conclusions: The results obtained demonstrate an efficient cardioprotection by DT1 against myocardial I/R, mediated by activation of cell-survival signalling pathways as AKT and AMPK and reduction of apoptosis. These compounds could provide new therapeutic strategies for the pharmacological treatment of ischemic injury.
This work was supported by Grant PR6/13-18857 from Santander-UCM to B.H.

2
5-HYDROXYTRYPTAMINE MODULATION OF VAGALLY-INDUCED BRADYCARDIA IN FLUOXETINE-TREATED RAT

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Introduction/Objectives: Selective serotonin reuptake inhibitors, as fluoxetine, are the usual treatment in psychiatric disorders, such as depression[(1)], where 5-HT concentrations are altered. The aim of this study was to evaluate 5-hydroxytryptamine modulation of vagally-induced bradycardia in fluoxetine-treated rat.
Material/Methods: Animals were orally treated with fluoxetine (10 mg/kg) for 14 days. After CNS destruction[(2,3,4)], vagal stimulation (monophasic pulses, 1 ms duration, 15 ± 3V at 3, 6 and 9 Hz frequencies) or administration of exogenous acetylcholine (1, 5 and 10 μg/kg) were performed[(2)].
Results: Vagal stimulation or administration of acetylcholine resulted in frequency- or dose- dependent bradycardia. Intravenous administration of 5-HT (10, 50, 100 and 200 μg/kg) had a dual effect on the bradycardia. 5-carboxamidotryptamine (5-CT; 10 μg/kg), 5-HT1/7 agonist, and L-694,247 (50 μg/kg), 5-HT1D agonist, reproduced low doses serotonin inhibitory action; meanwhile, 1-phenylbiguanide (10 μg/kg), 5-HT3 agonist, mimicked 5-HT high doses potentiating effect. Alpha-methyl-5-HT (5-HT2 agonist; 10 μg/kg), 8-OH-DPAT (5-HT1A agonist; 50 μg/kg), or CGS-12066B (5-HT1B agonist; 50 μg/kg) did not modify the electrically-induced bradycardia. Pretreatment with SB269970 (5-HT7 antagonist; 0.5 mg/kg), did not modify 5-CT effect. Inhibition of the bradycardia induced by L-694,247 was completely blocked by BRL-15572 (5-HT1D antagonist; 1 mg/kg). Enhancement of the bradycardia by 1-phenylbiguanide was completely abolished by pretreatment with MDL-72222 (5-HT3 antagonist; 1 mg/kg). Neither 5-HT (10 μg/kg) nor 5-CT (10 μg/kg) modified the bradycardia induced by exogenous acetylcholine; however, 5-HT (200 μg/kg) and 1- phenylbiguanide (10 μg/kg) potentiated the exogenous acetylcholine-induced bradycardia.
Conclusions: Serotonin exerts a dual effect on the vagally-induced bradycardia. Low doses mediate prejuntional inhibition (5-HT1D receptors) and high doses potentiate this effect (pre and postsynaptic 5-HT3 receptor activation) in fluoxetine-treated rats.
References:
1. Marcus et al., WHO, 2012.
2. Morán et al., 1994. Eur. J. Pharmacol. 252(2):161–166.
3. García et al., 2005. Br. J. Pharmacol. 145(5):593–601.
4. Restrepo et al., 2010. Exp Diabetes Res. doi: 10.1155/2010/686734.

3
SEVOFLURANE INDUCED LOWER POSTOPERATIVE RELEASE OF TROPONIN 1 AND NT-PROBNP THAN PROPOFOL IN PATIENT SUBJECTED TO OFF-PUMP CORONARY ARTERY BYPASS GRAFT SURGERY

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The effect of general anaesthesia with sevoflurane vs. propofol in perioperative cardiac function of patients undergoing elective off-pump coronary artery bypass graft surgery (CAGB) was evaluated by NT-proBNP quantification.
Methods: A prospective, longitudinal, double-blind, randomized and controlled clinical trial was done in ASA class II-IV patients undergoing elective off-pump CAGB surgery under general anaesthesia. Exclusion criteria were Euroscore scale value >7, combined surgery, hemodynamic instability, heart failure and need for vasoactive drugs before surgery. Patients were randomized in three groups: SEV intraoperative+ postoperative (SEV), SEV intraoperative+ PRO postoperative (SP), or PRO intraoperative+ postoperative (PRO). Cardiopulmonary function, cardiac troponin 1, NT-proBNP and inotropic drugs were recorded perioperatively during 48 h of follow up.

Results: Sixty patients, 48.3% male, aged 61–74 years old, $n = 20$/group, have been followed. The groups were comparables at the initial evaluation. Anaesthesia with SEV induced lower postoperative release of troponin 1 and NT-proBNP than PRO. At 24 h of follow up the troponin 1 ranking order was: i): SEV 0.5 ± 0.4 ng/mL* <<SP 1.61 ± 1.30 ng/mL <PRO 2.27 ± 1.5 ng/mL ($P < 0.05$). At 24 h of following the NT-proBNP ranking order was: SEV 501 ± 280 pg/mL*<<SP 1270 ± 498 pg/mL =PRO 1775 ± 527 pg/mL ($P < 0.05$). Lesser inotropic drug requirements were associated to SEV with respect to PRO, patients treated with inotropic were (%): i) At 24 h SEV 10%* =SP 19% <<PRO 51%; and ii) At 48 h: SEV 5%* <<SP 34% <PRO 47% ($P < 0.05$).
Conclusion: Sevoflurane exert higher cardioprotective effect than propofol perioperatively in patients undergoing elective off-pump coronary artery bypass graft.

4
ZOFENOPRIL AVOIDS CARDIOVASCULAR ALTERATIONS IN ANGIOTENSIN II INFUSED MICE

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The renin-angiotensin system is a well-recognized contributor to the pathogenesis of cardiovascular disease. Angiotensin II (Ang II) regulates vascular tone and maintains normal vessel structure and function, although elevated levels have been implicated in the pathophysiological processes in hypertension. We examined the effect of zofenopril, an ACE inhibitor (ACEI), on blood pressure, oxidative stress and changes in the cardiovascular system in a model of hypertension induced by Ang II.
Methods: CD_1 mice were infused with Ang II (1.4 mg/kg/day) via osmotic minipumps implanted subcutaneously for 14 days (Ang). Sham-operated mice were used as control (Sham). A group of mice infused with Ang II was treated with 10 mg/kg/day of zofenopril p.o. (Ang Z-10). At the end of treatment mean blood pressure (MBP) was measured from the left carotid artery. Aorta and heart were isolated. Cardiac hypertrophy index was calculated as ventricles weight/body weight ratio. An estimate of the ventricular collagen concentration, the hydroxyproline concentration, was evaluated in heart samples. Also superoxide (O_2^-) production was measured by lucigenin-enhanced chemiluminescence in aorta.
Results: MBP was increased in Ang II-infused mice (Ang, 64 ± 2 mmHg vs Sham, 52 ± 2 mmHg, $P < 0.05$). Zofenopril prevented the Ang II-dependent increased in MBP (45 ± 3 mmHg). Ang II infusion caused cardiac hypertrophy and increased hydroxyproline concentration in heart and the O_2^- production in aorta. The treatment significantly attenuated these effects.
Conclusion: Antihypertensive effect of zofenopril was accompanied by prevention of cardiac remodeling and improvement of redox status. Antioxidant activity of this ACEI could be involved in these effects.

5
ROLE OF OXIDATIVE STRESS IN HYPERTENSION AND VASCULAR DYSFUNCTION ASSOCIATED TO ACTIVATION OF TLR4 BY ANGIOTENSIN II

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Hypertension is considered a low-grade inflammatory disease, with the adaptive immunity being an important mediator of this pathology. TLR4 seems to contribute to the development of several cardiovascular diseases; however, little is known about its participation in hypertension. We aimed to investigate whether TLR4 activation due to the increased renin-angiotensin system (RAS) contributes to hypertension and the associated endothelial dysfunction.
For this, we used aortic segments from WKY and SHR untreated and treated with losartan (15 mg/kg day) or the neutralizing antibody anti-TLR4 (1 μg/day) and cultured vascular smooth muscle cells (VSMC) from SHR.
TLR4 mRNA levels were greater in VSMC and aorta from SHR compared with WKY; losartan treatment reduced those levels in SHR. Anti-TLR4 treatment of SHR: 1) reduced blood pressure, heart rate and the phenylephrine-induced contraction while it increased acetylcholine relaxation; 2) increased the potentiation of phenylephrine contraction after endothelium removal; and 3) abolished the inhibitory effect of tiron, apocynin and catalase on phenylephrine-induced response as well as its potentiatory effect of acetylcholine-induced relaxation. In VSMC from SHR, angiotensin II increased TLR4 mRNA levels and losartan reduced that increase. CLI-095, a TLR4 inhibitor, reduced the increase in NADPH oxidase activity, superoxide anion production, migration and proliferation induced by angiotensin II in these cells.
In conclusion, TLR4 pathway activation by the increased RAS activity is involved in hypertension and by inducing oxidative stress contributes to the endothelial dysfunction associated to this pathology. These results point to the role of innate immunity in hypertension and the associated end-organ damage.
Supported by: MINECO (SAF2012-36400), ISCIII (RD12/0042/0024), MEC (PHB2011-0001-PC), URJC (PRIN13_CS12), CAPES and CNPq.

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NATRIURETIC EFFICIENCY OF TORASEMIDE-PR (10 MG) COMPARED WITH FUROSEMIDE-IR (40 MG) IN PATIENTS WITH CHF

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Objective: To explore whether torasemide-PR 10 mg is more natriuretically efficient than furosemide-IR 40 mg after a single oral dose in patients with compensated heart failure (CHF). To compare the pharmacodynamic profiles of study drugs.
Methods: The efficiency of sodium excretion was assessed as the ratio between the average drug-induced natriuresis and the average drug recovered in urine over 24 hours.
Patients with NYHA functional class II and III CHF, selected by the Cardiology Department of HSCSP and Primary Care Centers, were included in this randomized, open-label, blinded-endpoint, 3 way crossover, single dose clinical trial. The baseline intake of diuretics was stopped and the intake of electrolytes and proteins was controlled for 3 days prior study period. Patients were randomized to receive either torasemide-PR or furosemide-IR in the first two periods and torasemide-IR in the third period. Blood was extracted at 0, +0.25, +0.5, +0.75, +1, +1.25, +1.5, +1.75, +2, +2.5, +3.5, +4.5, +5.5, +7, +10, +18 and +24 h after dosing. Urine was collected at: 12 h before and between different intervals after drug administration. Mictional urgency was recorded using Visual Analogue Scale (VAS). Tolerability and all adverse events were registered. The statistical analysis was performed using SPSS V.19.
Results: It was planned to include 30 patients but due to low recruitment rate, nine (7 males, 2 females) were included. Mean natriuretic efficiency of torasemide-PR (83.2 mmol/h) and torasemide-IR (82.6 mmol/h) was significantly higher ($P < 0.0001$) than furosemide-IR

(81.6 mmol/h). Volumes of urine collected during 0–24 h were similar between three drugs. Maximum mictional urgency was lower (VAS = 34 mm vs. 42 mm) and occurred later (3–4 h vs. 1–1.5 h) with torasemide-PR than with furosemide-IR. Seven adverse events (mild and moderate) were reported.

Conclusions:

1. Torasemide-PR 10 mg is more natriuretically efficient than furosemide-IR 40 mg.
2. The urine volumes collected were similar for all formulations, while the mictional urgency profile was more favourable for torasemide-PR.
3. All three drugs were well tolerated.
4. The results suggest more efficient and convenient diuretic profile of torasemide-PR as compared to furosemide-IR in patients with CHF.

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VASCULAR SMOOTH MUSCLE CONTRACTILE MECHANISMS IN HUMAN MESENTERIC ARTERIES AND THEIR AGEING ALTERATION

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Background: Ageing is associated to a high variability of vascular alterations, which are part of the beginning and progression of CV diseases in elderly people. From middle age arterial dilation starts to diminish and arterial stiffness strongly increases with ageing. We aimed to study the changes produced by ageing in the arterial contractile mechanisms.

Methods: Human mesenteric arteries from patients undergoing abdominal surgery were used. The effects of Nifedipine, voltage gated calcium channel (VGCC) blocker, SKF96365, store and receptor operated calcium channel (SOCC/ROCC) blocker, KB-R7943, inhibitor of the Na^+/Ca^{2+} exchanger (NCX) in the reversal mode, HA-1077, Rho-kinase inhibitor, 2-APB, IP_3Rc inhibitor, and CPA, sarco/endoplasmic reticulum calcium ATPase (SERCA) blocker, were observed by myography on a phenylephrine induced contraction.

Results: The samples were divided in 3 age groups, 14–40, 41–65, 66–83. Nifedipine, KB-R7943 and 2-APB induced a higher vasodilation in young patients compared to middle aged ones and a statistically significant higher vasodilation compared to the elderly ones. CPA showed a huge variability effect among the patients; meanwhile SKF96365 and HA-1077 appeared to be the most potent vasodilators in the three groups of age.

Conclusions: Human mesenteric arteries contraction is dependent on the extracellular calcium and on the calcium released from the SR. Calcium entry through VGCC, the release of calcium through IP_3 channels and the entry of calcium through the reversal mode of the NCX induced contraction are affected by ageing. SOCC/ROCC calcium entry and the calcium sensitization pathway induced contraction do not suffer changes during ageing.

Acknowledgements: FIS-PI080920/PI1200590, RECAVA-RD06/0014/1007, Convocatoria CONACYT-Gobierno del Estado de San Luis Potosí-México 2012.

8
PIOGLITAZONE IMPROVES CEREBRAL VASCULAR FUNCTION IN OBESE AND LEAN ZUCKER RAT

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Introduction: The escalation of obesity and the surge in the prevalence of diabetes fuels the current epidemic of cardiovascular diseases (CVD). The PPARγ agonists, as Pioglitazone, represent a class of synthetic insulin-sensitizing agents that are widely prescribed for the treatment of type 2 diabetes mellitus. Some studies support additional benefits of these drugs including improved endothelial reactivity in both, diabetic and non-diabetic patients. In the present study, we assessed whether Pioglitazone may improve vascular dysfunctions observed in cerebral and carotid artery from obese Zucker rat (OZR), an experimental model of obesity/prediabetes.

Material and Methods: Middle cerebral artery (MCA) and carotid artery (CA) from 17 to 18-wk-old OZR and from their control counterparts, lean Zucker rat (LZR), were mounted in vascular myographs to evaluate vascular function.

Results: Isoproterenol evoked relaxations that were significantly reduced in MCA and CA of OZR compared with those of LZR. Endothelial dependent vasodilation induced by acetylcholine was impaired in CA from OZR, while relaxations independent of endothelial function, such as nitroprusside and forskolin, were preserved in both arterial segments. Contractions to norepinephrine were similar in CA from both LZR and OZR. However, 5-HT vasoconstrictions were potentiated by obesity conditions only in CA. Pretreatment with Pioglitazone enhanced the β-adrenergic relaxant effect in MCA and CA, and the acetylcholine vasodilation response in CA, and reduced the contractile effect to 5-HT in CA.

Conclusions: Pioglitazone may be protective of CVD in cerebral vascular bed since increased vasodilations in CA and MCA and reduced vasoconstrictions in CA.

This study was supported by grant no SAF2009-10448 and no SAF2012-31631.

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DIETARY INTAKE OF LYCOPENE DECREASES THE CARDIOVASCULAR RISK IN SPONTANEOUSLY HYPERTENSIVE RATS

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Increased reactive oxygen species production, especially superoxide anion ($O^{2.-}$), contributes significantly to the functional and structural alterations linked to hypertension. Antioxidant-rich diets have been reported to exert beneficial effects in preventing cardiovascular diseases. The aim of this study was to investigate the effect of lycopene, a natural carotenoid found in several fruits and vegetables, on hypertension, cardiac and renal hypertrophy and oxidative stress in spontaneously hypertensive rats (SHR).

Methods: SHR rats 22–26 weeks old were treated for 4 weeks with lycopene supplementation (10 mg/kg/day, diet) (SHR-LYC) and a group without treatment are used as control (SHR-C). Systolic blood pressure was monitored weekly by the tail-cuff method. At the end of study the animals were sacrificed and aorta, heart and right kidney were removed. Left ventricular hypertrophy (LVH) and renal hypertrophy (RH) index were calculated as left ventricle weight and kidney weight/body weight ratio respectively. Cardiac and renal fibrosis was evaluated in tissues homogenates by spectrophotometric determination of hydroxyproline. Lipid peroxidation was measured in plasma using TBARS method and $O^{2.-}$ production by lucigenin-enhanced chemiluminescence in aorta.

Results: Lycopene decreased blood pressure from the first week of treatment. Both, cardiac and renal hypertrophy were significantly reduced (28% and 14% respectively) and also decreased collagen deposition. MDA levels (SHR-C, 1.96 ± 0.10 vs SHR-LYC, 1.63 ± 0.06 nmol/mL, $P < 0.05$) and $O^{2.-}$ production (SHR-C, 4792 ± 251 vs SHR-LYC, 3504 ± 246 RLU/min/mg, $P < 0.05$) showed an improvement of the oxidative status by lycopene treatment.

Conclusion: The antioxidant activity of lycopene exerts beneficial effects in hypertension carrying an improvement in cardiac and renal remodeling parameters.

Lycopene was kindly supply by DMS NUTRICIONAL PRODUCTS.

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REACTIVE HYPEREMIA AFTER RAT FOCAL CEREBRAL ISCHEMIA: MIDDLE CEREBRAL ARTERY CHANGES AND BENEFITS OF URIC ACID TREATMENT

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Introduction: Reactive hyperemia (RH) is considered an increase in blood flow above basal levels at reperfusion associated with augmented brain damage. Uric acid (UA) treatment protects rat brain from ischemic injury. We hypothesized that RH exacerbates vascular alterations after ischemia/reperfusion and exogenous UA could exert beneficial effects.
Methods: Right middle cerebral artery (MCA) occlusion (90 min)/reperfusion (24 h) was performed in Sprague-Dawley rats. Animals were divided into 6 groups: sham-operated vehicle (n = 14); ischemic vehicle (IV, n = 11); ischemic hyperemic vehicle (IHV, n = 12); sham-operated UA (n = 5); ischemic UA (IUA, n = 7); and ischemic hyperemic UA (IHUA, n = 8). UA (16 mg/kg; i.v.) or vehicle (Locke's buffer; i.v.) was infused at 45 min reperfusion. MCA was mounted in a pressure myograph under physiological conditions. UA concentration was assessed in plasma and brain by HPLC.
Results: Mean arterial blood pressure (IV: 94.2 ± 1.9 mmHg; IHV: 93 ± 3 mmHg; IUA: 91.5 ± 1.8 mmHg; IHUA: 92.5 ± 3.7 mmHg) was similar in all groups. The presence of RH was associated with an increase of MCA wall thickness ($P < 0.001$) and cross-sectional area ($P < 0.001$), while wall stress ($P < 0.05$), but not wall stiffness, decreased compared to sham-operated. Myogenic tone decreased ($P < 0.05$) irrespective of the presence of HR. UA was detected in plasma but was unable to cross the blood–brain barrier. A single dose of UA prevented structural alterations in MCA properties induced by RH.
Conclusions: Our results show that MCA structural but no myogenic alterations after ischemia/reperfusion are linked to RH. We suggest that neuroprotective effects of UA may involve prevention of RH-induced cerebrovascular remodelling.

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CHRONIC ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)-B PREVENTS ENDOTHELIAL DYSFUNCTION INDUCED BY HIGH-FAT DIET IN MICE

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Introduction and Objective: The improvement of both lipid and glucose metabolism induced by PPARβ/δ activation is likely to be associated with vascular protection in obesity. However, a direct action of PPARβ/δ agonists in vascular wall has also been described. The aim of this study was to analyze the effects of PPARβ/δ activation on endothelial dysfunction induced by high fat diet (HFD) in mice, independent of changes in plasma metabolic variables.
Methods: Mice were divided into 4 groups (n = 10): control, HFD, HFD-GW0742 (3 mg kg^{-1} day^{-1}, po), and HFD-GW0742 plus the PPARβ/δ antagonist GSK0660 (1 mg kg^{-1} day^{-1} ip). GW0742 treatment was followed for 3 weeks.
Results: GW0742 treatment was unable to prevent body weight gain, energy intake, heart and kidney hypertrophy, and the higher plasmatic fasting glucose, cholesterol, triglycerides, HDL, and glucose intolerance induced by HFD. However, GW0742 prevented the raise in blood pressure, the impaired endothelium-dependent vasodilator responses to acetylcholine, the increased vascular NADPH oxidase-driven superoxide generation and mRNA levels of IL-6 and IL-1β, and the reduced mRNA levels of gluthatione peroxidase-1 and Mn-superoxide dismutase found in mice fed HFD. Gene expression profiling revealed a marked increase of vascular carnitine palmitoyltransferase 1 (CPT-1) and uncoupling protein-2 (UCP2) in HFD-GW0742 group. GW0742-induced effects were abolished by GSK0660.
Conclusions: PPARβ activation exerted antihypertensive effects, and improved endothelial dysfunction induced by HFD. These effects seem to be associated with reduction of vascular oxidative stress via reduced lipid accumulation.

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ROLE OF COX-2/MPGES-1 AXIS ON VASCULAR REMODELING IN HYPERTENSION

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Introduction: Prostanoids from cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase (mPGES)-1 contribute to vascular damage in different cardiovascular pathologies. The aim was to explore the contribution of COX-2/mPGES-1 to the vascular alterations associated to hypertension.
Material and Methods: Spontaneously hypertensive rats (SHR) and Angiotensin II (AngII) infused mice were treated with the COX-2 inhibitor celecoxib or with the EP1 receptor antagonist SC19220. COX-2 and mPGES-1 deficient mice (COX-2−/−, mPGES-1−/−) infused or not with AngII were also used.
Results: Celecoxib treatment did not modify the altered structural parameters observed in mesenteric resistance arteries (MRA) from SHR or AngII-infused animals (decreased lumen diameter and increased wall:lumen ratio). However, Celecoxib treatment and/or COX-2 deletion improved the increased vascular stiffness and the diminished wall distensibility observed in MRA from SHR and/or AngII-infused mice. This was accompanied with decreased vascular fibrosis since diminished collagen deposition, normalization of altered elastin structure and decreased connective tissue growth factor, tenascin-C and plasminogen activator inhibitor-1 gene expression were observed after COX-2 inhibition. Hypertensive animals showed increased vascular PGE2 production and mPGES-1 expression that were normalized by celecoxib. Ang II-infused mPGES-1-/- mice were protected against the deleterious effects of Ang II in vascular structure, mechanics and fibrosis. SC19220 treatment did not modify vessel structure, but normalized vascular stiffness, wall distensibility and fibrosis after AngII infusion. COX-2 and mPGES-1 deletion and SC19220 treatment also improved the increased vasoconstrictor responses and the endothelial dysfunction induced by AngII.
Conclusion: The COX-2/mPGES-1 axis modulates extracellular matrix deposition, mechanical properties and vascular function in hypertension.

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ENDOTHELIAL F-ACTIN DISRUPTION PREVENTS LOSS OF EDHF DILATION FOLLOWING THROMBOXANE STIMULATION IN RAT CEREBRAL ARTERIES

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Introduction: The small-conductance calcium-activated potassium channel (SKCa) component of EDHF hyperpolarization is lost following thromboxane exposure. Thromboxane is involved in the pathophysiology of cerebral ischemia-reperfusion (CIR). However, EDHF-mediated middle cerebral artery (MCA) dilations are preserved after CIR. Here, we explored whether EDHF-mediated MCA dilations after CIR are modified following thromboxane stimulation.
Methods: Right MCA intraluminal occlusion (90 min)/reperfusion (24 h) was performed in Sprague-Dawley rats. MCAs were mounted in a wire or pressure myograph. Endothelium-dependent relaxation to SLIGRL (20 μM; PAR2 agonist) was evaluated. MCA KCa channel expression was assessed by qRT-PCR and immunofluorescence. Protein tyrosine nitrosylation and F-actin was determined by immunofluorescence and phalloidin staining, respectively.
Results: SLIGRL relaxation was impaired in U46619 (thromboxane receptor agonist)-preconstricted MCAs from ischemic compared to nonischemic hemisphere or sham. In contrast, in L-NAME (300 μM) plus indomethacin (10 μM)-treated arteries, EDHF-mediated dilation was higher in U46619-preconstricted MCAs from the ischemic hemisphere compared to sham. The SKCa component of EDHF dilation was impaired in U46619-preconstricted MCAs from sham but not CIR. Although KCa channel mRNA levels were diminished after CIR, protein expression was unaltered. CIR was associated with endothelial nitrosylation and F-actin disruption. In control rats, peroxynitrite (5 μM) caused CIR-like endothelial nitrosylation. Either peroxynitrite or cytochalasin D (10 nM; F-actin disruptor) caused endothelial F-actin disruption and restored SKCa and EDHF-mediated dilation in the presence of U46619.
Conclusions: Loss of EDHF-mediated dilation following thromboxane stimulation requires intact endothelial F-actin. We suggest that peroxynitrite-induced endothelial F-actin disruption after CIR preserves EDHF-mediated dilation during thromboxane stimulation.

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SIRT3 BLOCKS LIPID-INDUCED INFLAMMATION IN THE HEART

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Introduction: The classical Western diet, owing to its high fat content, has a range of adverse effects on the heart, including enhanced inflammation and hypertrophy. Pro-inflammatory factors secreted by cardiac cells, which are under the transcriptional control of nuclear factor-κB (NF-κB), may contribute to cardiac disease. Since Sirt3, a member of the sirtuin family of protein deacetylases, is an endogenous negative regulator of cardiac hypertrophy, we examined its effects on lipid-induced cardiac inflammation.
Materials and methods: Sirt3 knockout mice were fed a standard diet or a high-fat diet for four months. For in vitro studies, a cardiomyocyte cell line of human origin, AC16, was treated with the saturated fatty acid palmitate after adenovirus-mediated Sirt3 overexpression.
Results: The high-fat diet stimulated the expression of intercellular adhesion molecule 1 (ICAM-1), interleukin-6 (IL-6), suppressor of cytokine signaling 3 (SOCS3) and superoxide dismutase 2 (SOD2), and enhanced the activity of NF-κB in the heart of mice. Deletion of Sirt3 in vivo exacerbated the pro-inflammatory profile induced by a high-fat diet. In agreement with the in vivo data, Sirt3 overexpression in human cardiac cells partially inhibited the enhanced pro-inflammatory profile induced by palmitate.
Conclusions: Sirt3 can attenuate the inflammatory response induced in human cardiac cells exposed to palmitate and in mice fed a high-fat diet. This is relevant, especially taking into account the potential link between chronic low-grade inflammation and metabolic disorders, and also that Sirt3 activity targets enzymes involved in cell metabolism regulation.
Acknowledgements: SAF2012-30708 and CIBERDEM (ISCIII and *Ministerio de Economía y Competitividad*).

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STUDY OF CILOSTAZOL IN PATIENTS WITH INTERMITTENT CLAUDICATION. RELATIONSHIP BETWEEN CLINICAL PARAMETERS, OXIDATIVE STRESS AND NUMBER OF ENDOTHELIAL PROGENITOR CELLS

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Introduction: Cilostazol is a vasodilator drug for intermittent claudication (IC). However, it is not known whether other mechanism may be mediating its clinical benefit observed in patients with distal limb ischemia. We aimed to determine the role of oxidative stress (OS), the number of endothelial progenitor cells (EPCs) and the relationship of these parameters with the clinical effect in patients with IC.
Methods: Patients with IC were evaluated as baseline and after 6 months of treatment with Cilostazol. The following parameters were evaluated: maximum walking distance and ankle-brachial index, parameters of oxidative stress [malondialdehyde (MDA), total glutathione (GT), oxidized glutathione (GSSG) and nitric oxide (NO)] and EPCs number (by flow cytometry, mononuclear cells double positive: CD34 + /KDR+ or CD34 + /CD144 +).
Results: Patients treated with Cilostazol (100 mg/day) presented an increase in to maximum walking distance (414 m vs 282 m, $P = 0.0411$) and also, the ankle-brachial index was increased (0.67 vs 0.60, $P = 0.0186$). Besides, we observed an improved in the oxidative stress [decreased of MDA (0.1470 vs 0.1262, $P = 0.009$) and NO (10.4822 vs 8.8978, $P = 0.044$), and an increase of GSSG (3.3292 vs 10.9544, $P = 0.0001$)]. The study of the EPCs biomarkers revealed an increase in their number: CD34 + /KDR+ ($0.053 \pm 0.009\%$ vs $0.128 \pm 0.029\%$, $P = 0.0161$), and CD34 + /CD144 + ($0.178 \pm 0.032\%$ vs $0.228 \pm 0.060\%$, $P = 0.47$).
Conclusion: Treatment with Cilostazol for 6 months was associated to a better clinical outcome. Cilostazol have showed a benefit for the patients with CI, through the increased number of EPCs and an improvement of the oxidative stress status.
Acknowledgments: FIS-PI080920/PI1200590, RECAVA-RD06/0014/1007, Convocatoria CONACYT-Gobierno del Estado de San Luis Potosí-México 2012.

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PHARMACOLOGICAL GATING MODULATION OF ENDOTHELIAL KCA3.1 CHANNELS AND EDH-TYPE RELAXATION

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Introduction: The calcium/calmodulin-regulated K^+ channel, KCa3.1, causes membrane hyperpolarization to calcium-mobilizing-agonists in the vascular endothelium. Here, we aimed to define the mechanistic role of KCa3.1 for endothelium-dependent hyperpolarization-induced (EDH) relaxation in porcine coronary arteries, as reference model for humans, using novel specific and potent positive-gating modulators.
Materials and methods: The compounds SKA-111 and SKA-121 were tested on KCa3.1 expressed in primary porcine coronary artery endothelial cells (PCAEC) by patch-clamp electrophysiology. We performed isometric myography on PCA rings to study SKAs effects in endothelium-dependent EDH-regulation of arterial tone. We monitored systemic effects by cardiovascular telemetry on mice.
Results: Whole-cell patch-clamp experiments showed that SKA-111 and SKA-121 potentiated KCa3.1-currents. Current-clamp measurements in PCAEC clusters showed that SKA-121 strongly potentiated 5-HT and bradykinin(BK)-induced membrane hyperpolarization by ≈-20 mV. Responses were fully reversed by the KCa2/3 blockers. Myography in the presence of L-NNA and indomethacin showed that SKA-111 and SKA-121 had no effect on either spontaneous tone or 5-HT-induced contractions. SKA-111- or SKA-121 potentiated EDH-dependent relaxation to BK. The KCa3.1-blocker, TRAM-34, blocked the SKA-111- and SKA-121-potentiated responses. I.p. injections of 100 mg/kg SKA-121 reduced blood pressure by ≈20 mmHg over ≈2 h in mice.
Conclusions: Our study suggested that positive-gating modulation of KCa3.1 channels was capable to selectively potentiate BK-induced EDH-type relaxation in PCA and to reduce blood pressure in mice. Positive-gating modulators of KCa3.1/KCa2 have potential utility for pharmacological manipulation of arterial blood flow in the coronary circulation and blood pressure.

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GLUCOCORTICOIDS AS MODULATORS OF EXPRESSION AND ACTIVITY OF ANTITHROMBIN III: CLINICAL RELEVANCE

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Introduction: An inverse relationship has been reported between decreased postoperative Antithrombin (AT) plasmatic levels and the incidence of complications. We hypothesized that Nuclear Hormone Receptors (NHR) could modulate AT expression through a Hormone Regulatory Element present in its promoter, and could be a pharmacological complement in surgical procedures to activate endogenous AT synthesis.
Methods: The expression of *SERPINC1*, encoding AT, was analyzed in HepG2 cells by quantitative RT-PCR and Western Blot. Two studies were conducted with (a) patients submitted to cardiac surgery with cardiopulmonary bypass receiving (n = 17) or not (n = 16) GC as part of their pharmacological treatment, or (b) patients who received (n = 20) or not (n = 16) GC as part of their surgery (exodontia or knee arthroscopia, respectively). AT activity in plasma was determined by Innovance AT Test on a BCS XP System hemostasis analyzer.
Results: Thirteen NHR ligands were assayed, being GW4064 (FXR ligand) the most potent activator. Retinoids, activating RXR, and Glucocorticoids (dexamethasone and methylprednisolone) also resulted in increased AT expression. Chronic GC treatment avoids the decreased AT activity observed after cardiac surgery. In patients who receive two acute GC doses, pre-operative and post-operative AT activity was similar, whereas a significant decrease was observed after surgery in non GC-treated patients.
Conclusions and implication: Whereas retinoids and FXR ligands are investigational compounds, regulation of AT by glucocorticoids has a higher potential for translation to clinical practice, pre-conditioning the patient against complications related to reduced AT levels.

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EVALUATION OF SDF1 ON THE FUNCTIONALITY OF ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH DIABETES MELLITUS TYPE 2 OR HYPERCHOLESTEROLEMIA

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Background: It is well-known that endothelial progenitor cells (EPCs) are important modulators of vascular repair and maintenance of proper cardiovascular function. Several conditions, such as atherosclerosis and diabetes mellitus, have been linked to depletion and poor functionality of EPCs and subsequent vascular impairment. The normal progression of atherosclerosis is related to alterations on the levels of several endothelial cell adhesion and differentiation molecules, which expression is highly dependent on the signaling axis SDF1a/CXCR4 and the transcription factor Ets-2. Our aim is to investigate the potential role of SDF1a, CXCR4 and Ets-2 in pathologies such as diabetes mellitus type 2 (DM2) and hypercholesterolemia, as well as the impact that the modulation of these molecules by DPP4 inhibitors may have on patient outcomes.
Methods: Sixty patients with DM2 or hypercholesterolemia from the cardiac surgery service were recruited. Peripheral mononuclear cells were isolated with Ficoll-Hypaque density gradient centrifugation. Expression of SDF1α, CXCR4, and Ets-2 was measured by Western blot.
Results: Patients with DM2 showed a statistically significant lower expression of SDF1α, Ets-2 and CXCR-4 compared with non-diabetic. Similarly, when the comparison was made between a group of patients with hypercholesterolemia and without hypercholesterolemia, we observed a significant trend towards a lower expression of SDF1α (1.869 ± 0.3027 vs 3.251 ± 0.9495, $P = 0.0739$) and Ets-2 (0.6893 ± 0.07980 vs 1.007 ± 0.1917, $P = 0.0759$).
Conclusions: SDF1α, CXCR- 4 and Ets-2 are involved on coronary artery disease and lower expression in patients with diabetes mellitus type II or hypercholesterolemia could be used as a biomarker of decreased adhesion of EPCs and with an early restenosis.
Acknowledgments: FIS-PI080920/PI1200590, RECAVA-RD06/0014/1007, Convocatoria CONACYT-Gobierno del Estado de San Luis Potosí-México 2012.

	SDF1	*CXCR4*	*Ets-2*
DM2 patients	1.750 ± 0.4269	0.8951 ± 0.1031	0.5490 ± 0.06163
No DM2 patients	3.242 ± 0.5366	1.440 ± 0.1659	1.124 ± 0.1536
P	***P* = 0.0144**	***P* = 0.0123**	***P* = 0.0020**

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VASCULAR REPAIR AND THE INVOLVEMENT OF ADHESION MOLECULES IN PATIENTS WITH HYPERTENSION AND HYPERCHOLESTEROLEMIA

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Background: The endothelium plays a central role on the evolution of the atherosclerosis. Endoglin (CD105) expression has been related to the regulation of vascular repair. The role of different receptors such as ERG in vascular repair has been investigated, as has the involvement of adhesion molecules such as SDF1, and its CXCR4 co-receptor, which could be impaired. Our specific objective was to explore the expression of CD105, ERG, CXCR4 and SDF1 and their implication in pathologies such as hypertension or hypercholesterolemia.
Methods: Sixty patients from the cardiac surgery service were recruited to the study. Peripheral mononuclear cells were isolated with Ficoll-Hypaque density gradient centrifugation. Proteins were assessed by the bicinchoninic acid assay. Expression of CD105, ERG, CXCR4 and SDF1 was measured by Western blot.
Results: Patients with hypertension showed a statistically significant higher expression of CD105 and lower expression of SDF1 compared with non-hypertensive patients. CXCR4 expression and ERG expression were not statistically significantly different between the patient groups. However, in the group of patients with hypercholesterolemia compared with the group without hypercholesterolemia, we found no differences in the expression of CD105, a significant trend towards lower expression of CXCR4 and SDF1 and a statistically significant lower expression of ERG.
Conclusions: The lower expression of SDF1 in patients with hypertension or lower expression of ERG and SDF1 patients with hypercholesterolemia could be associated with an inadequate functionality of endothelial progenitor cells and consequently slow the process of vascular repair and SDF1 and ERG may be considered a potential pharmacological target.
Acknowledgments: FIS-PI080920/PI1200590, RECAVA-RD06/0014/1007, Convocatoria CONACYT-Gobierno del Estado de San Luis Potosí-México 2012.

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PERIVASCULAR FAT ADIPOKINES OF PROSTANOID NATURE EXERT VASOACTIVE EFFECTS ON MESENTERIC RESISTANCE ARTERIES OF WKY AND SHROB RATS

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Adipocytes, including those from perivascular adipose tissue (PVAT) release myriads of adipokines. Prostaglandins are among them, but the possible role of adipose-derived prostaglandins as vasoactive substances has never been studied in the past. Our first aim is to determine whether this is the case. Our second purpose is to determine if prostaglandins released by PVAT account for the endothelial dysfunction that occurs in resistance arteries within the mesenteric fat depot (MRA) of metabolic syndrome rats (SHROB).
MRA from SHROB and control rats (WKY) were mounted on wire myographs: a) together with a sphere of naturally occurring adiposity (with-PVAT group), or b) dissecting all the adventitial tissue (without-PVAT group). Endothelial function, tested by acetylcholine reactivity of SHROB arteries with PVAT, was markedly lower than that of WKY. With-PVAT arteries, especially the SHROB, showed lower responses than those without PVAT. NO synthase inhibition diminished the acetylcholine responses in every group except the with-PVAT SHROB. Blockade of cyclooxygenase II, PGI2-IP, TXA2-TP, or TXA2 synthase increased to different extents the responses of the SHROB with-PVAT group. Immunoassay confirmed the release of PGE2, PGI2 and TXA2 and PVAT of both strains revealed cyclooxygenase II activity and expression. We conclude: a) PVAT is a source of vasoactive prostaglandins in WKY and SHROB; b) the presence of visceral PVAT causes endothelial NO dysfunction of resistance arteries in the SHROB; and c) the pattern of PVAT release of vasoactive prostaglandins and vascular response to these partly underlies the endothelial dysfunction of SHROB arteries.

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EFFECTS OF SPHINGOMYELINASE INHIBITORS AND QUERCETIN IN A RAT MODEL OF HEMORRAGHIC TRAUMATIC SHOCK AND REPERFUSION

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Introduction: We hypothesized that inhibition of acid sphingomyelinase (aSMase) by imipramine or D-609 or HMGB1 by quercetin could result in improved hemodynamics, lung inflammatory parameters and mortality in a rat model of hemorrahagic shock.
Methods: Rats were anesthetized (80 mg/kg ketamine plus 8 mg/kg xylacine i.p.). The protocol included laparatomy for 15 minutes (trauma), hemorrhagic shock (blood withdrawal to reduce the mean arterial pressure to 35 mmHg) for 75 minutes and resuscitation by

	CD105	*SDF1*	*CXCR4*	*ERG*
HTA patients	0.7947 ± 0.09280	2.278 ± 0.3901	1.296 ± 0.1389	0.4686 ± 0.09736
No HTA patients	0.3946 ± 0.08713	4.005 ± 0.8484	0.8853 ± 0.1317	0.4076 ± 0.1741
P	***P* = 0.0280**	*P* = 0.0367	NS	NS

	ERG	*CXCR4*	*SDF1*
Hypercholesterolemic patients	0.3464 ± 0.08170	0.9663 ± 0.09275	1.869 ± 0.3027
No Hypercholesterolemic patients	0.9085 ± 0.2544	1.233 ± 0.1876	3.251 ± 0.9495
P	***P* = 0.0212**	NS	NS

re-infusion of all the shed blood plus lactate Ringer for 90 min. Intravenous drugs (20 mg/kg D-609, 1 mg/kg imipramine or 50 mg/kg quercetin) or vehicle were administered during resuscitation.
Results: There was a trend for increased survival 75.0% (9/12), 76.9% (10/13) and 84.6% (11/13) in the treated groups vs the vehicle group 68.4% (13/19, $P > 0.05$ Kaplan Meier). At the end of reperfusion D609 and imipramine partially prevented the hypotension while none of the drugs prevented the hypoxemia. The three drugs fully prevented the development of lung edema but none of them prevented the increase in proteins in bronchoalveolar fluid (BALF). The activity of aSMase was increased in the vehicle compared to the sham group and the three drugs prevented this effect. However, other inflammatory markers such as myeloperoxidase activity, interleukin-6 in plasma or bronchoalveolar fluid were similar in the sham and shock groups. We found no bacterial DNA in plasma in these animals.
Conclusions: Inhibitors of aSMase and quercetin partially prevented the hemodynamic changes and lung injury in shock associated to hemorrhage and reperfusion.
Funding: Fundación Mutua Madrileña and SAF2011-28150.

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KV7 CHANNELS DETERMINE REGIONAL DIFFERENCES IN THE SENSITIVITY TO CAMP, HYPOXIA AND HIGH GLUCOSE IN CORONARY ARTERIES

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Introduction: Voltage-gated potassium channels encoded by *KCNQ* genes (Kv7 channels) are emerging as important regulators of vascular tone but their regional distribution and pathophysiological role in the coronary circulation remains incompletely characterized. In this study we compared the functional significance of Kv7 channels in left (LCA) and right coronary arteries (RCA) and their modulation by vasoactive factors and elevated glucose.
Methods and Results: We examined the effects of Kv7 channel modulators on K^+ currents and vascular reactivity in rat LCA and RCA. Currents from LCA were more sensitive to Kv7 channels inhibitors (XE991 and linopirdine) and activators (flupirtine and retigabine) than those from RCA. Accordingly, LCA were more sensitive than RCA to the relaxant effects induced by Kv7 channel enhancers. Likewise, relaxation induced by the adenylyl cyclase activator forskolin and hypoxia, which were mediated though Kv7 channel activation, were greater in LCA than in RCA. *KCNQ1* and *KCNQ5* expression was markedly higher in LCA than in RCA. After incubation with high glucose (HG, 30 mmol/l), myocytes from LCA, but not from RCA, were more depolarized and showed reduced Kv7 currents. In HG-incubated LCA, the effects of Kv7 channels modulators and forskolin were diminished and the expression of *KCNQ1* and *KCNQ5* was reduced. Finally, vascular responses induced by Kv7 channel modulators were impaired in LCA, but not RCA, from type 1 diabetic rats.
Conclusions: Our results reveal a greater Kv7 channel expression/activity in LCA than in RCA, which determines regional differences in the sensitivity to important pathophysiological factors regulating coronary blood flow.
Funding: SAF2010-22066-C02-01 and -02, and SAF2011-28150.

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MICROVASCULAR ENDOTHELIAL DYSFUNCTION BY SOLUBLE DIPEPTIDYL PEPTIDASE-4: RELEASE OF THROMBOXANE-A_2

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Introduction: Soluble dipeptidyl peptidase (sDPP)-4 is as a novel adipokine whose release is increased in patients with the metabolic syndrome. This study aimed to explore whether sDPP-4 may impair vascular reactivity considered as an early hallmark of endothelial dysfunction and, if so, to identify the mediators involved as well as the potential preventive effect of DPP-4 inhibitors.
Methods: Vascular reactivity was studied in isolated second-branch mesenteric arteries from 3 month-old female C56/BL6 mice using a small vessel myograph. Thromboxane-A_2 (TXA_2) release by cultured human coronary artery endothelial cells (HCAEC) was determined by ELISA.
Results: sDPP-4 (20–500 ng/ml) did not affect the contractility to noradrenaline (3 nmol/L to 30 μmol/l) in isolated murine mesenteric microvessels. However, sDPP-4 impaired endothelium-dependent relaxations elicited by acetylcholine (ACh; 1 nmol/l to 10 μmol/l) in a concentration-dependent manner, without affecting endothelium independent relaxations induced by sodium nitroprusside. The inhibition of cyclooxygenase (COX) and the blockade of thromboxane TP receptors with indomethacin (10 μmol/l) and SQ29548 (100 nmol/l), respectively, prevented the impaired relaxation to ACh evoked by sDPP-4. Indeed, sDPP-4 evoked the release of TXA_2 by HCAEC. TXA_2 release and impaired relaxation caused by sDPP-4 were both prevented by DPP-4 inhibitors such as K579 (100 nmol/l) or linagliptin (1 nmol/l to 100 nmol/l).
Conclusions: The present study identifies sDPP-4 as a causative agent of endothelial dysfunction through the release of TXA2. By interfering with such deleterious action of sDPP-4, pharmacological DPP-4 inhibitors may help preventing impaired endothelial function associated with type 2 diabetes mellitus.

PAIN AND INFLAMMATION

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MONOSODIUM URATE CRYSTAL INDUCED MACROPHAGE INFLAMMATION IS ATTENUATED BY CHONDROITIN SULFATE: PRE-CLINICAL MODEL FOR GOUT PROPHYLAXIS?

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Introduction/Purpose: Gout is one of the most common forms of inflammatory arthritis and is characterized by acute episodes of joint pain. Chondroitin Sulfate (CS), a natural glycosaminoglycan of the extracellular matrix, has clinical benefit in symptomatic osteoarthritis but has never been tested in gout. *In vitro*, CS has anti-inflammatory and positive effects on osteoarthrititic chondrocytes, synoviocytes and subchondral bone osteoblasts, but its effect on macrophages in unknown. The purpose of our study was to evaluate the *in vitro* effects of CS on monosodium urate (MSU)-stimulated cytokine production by macrophages.
Material/Methods: THP-1 monocytes were differentiated into mature macrophages using a phorbol ester, pretreated for 4 hours with CS in a physiologically achievable range of concentrations (10–200 μg/ml) followed by MSU crystal stimulation for 24 hours. Cell culture media were analyzed by immunoassay for factors known to be upregulated during gouty inflammation including IL1β, IL8 and TNFα. The specificity of inflammasome activation by MSU crystals was tested with a caspase-1 inhibitor (0.01 μM-10 μM).
Results: MSU crystals ≥10 mg/dl increased macrophage production of IL1β, IL8 and TNFα a mean 7-, 3- and 4-fold, respectively. Induction of IL1β by MSU was fully inhibited by a caspase-1 inhibitor confirming inflammasome activation as the mechanism by generating this cytokine. In a dose-dependent manner, CS significantly inhibited IL1β ($P = 0.003$), and TNFα ($P = 0.02$) production from macrophages in response to MSU. A similar trend was observed for IL-8 but was not statistically significant.
Conclusions: CS attenuated MSU crystal induced macrophage inflammation, suggesting a possible role for CS in gout prophylaxis.

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CHONDROPROTECTIVE ACTIVITY OF A COMBINATION OF CHONDROITIN SULFATE, GLUCOSAMINE AND A NATURAL INGREDIENT RICH IN HYALURONIC ACID

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Introduction/Purpose: Osteoarthritis (OA) is a chronic degenerative disorder that represents one of the main causes of disability within the elderly population. The objective is to evaluate the potential anti-arthritic activity of BIS052, a formulation that includes chondroitin sulfate bioactive (100%-purity), glucosamine and hyal-joint, a natural ingredient rich in sodium hyaluronate in an *in vitro* chondrocyte model and in the collagen-induced arthritis (CIA) model in rats.
Material/Methods: An *in vitro* study was performed using human osteoarthritic chondrocytes to evaluate the potential chondroprotective activity of BIS052. The anti-arthritic activity was investigated in the CIA model. Female rats were treated orally once daily with distilled water or BIS052 (160 mg/kg) starting 10 days prior to disease induction through the end of the study (day 35). The arthritic clinical score was examined for each animal.
Results: BIS052 leads to a significant reduction of MMP-1 and MMP-13 activities ($P < 0.05$) and also a stimulation of the synthesis of GAGs compared to the Control. BIS052 showed better efficacy than a standard combination glucosamine (GLU) + chondroitin sulfate (CS), being CS of a lower quality and uncertain traceability. In the experimental conditions of the *in vivo* study, we found that this formulation reduced about 10% the clinical score after 3 weeks of the arthritis induction. This was accompanied by a significant reduction of IL-6 (74%) and TNFα (70%) levels compared to Vehicle group.
Conclusions: The present study indicates that BIS052 has chondroprotective and anti-inflammatory activities. Thus, this natural combination may be useful as a nutritional supplement for osteoarthritis treatment.

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ANTI-INFLAMMATORY AND JOINT PROTECTIVE EFFECTS OF A POLYPHENOLIC EXTRACT FROM EXTRA VIRGIN OLIVE OIL IN TYPE II COLLAGEN-INDUCED ARTHRITIS

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Introduction: In the search of potential agents with relative safety and efficacy in rheumatoid arthritis, natural compounds have been considered as new therapeutic strategies. Polyphenolic compounds from extra virgin olive oil (EVOO) have demonstrated important anti-inflammatory and antioxidant effects. Our previous *in vivo* findings showed that a dietary EVOO-polyphenol extract (PE) supplementation down-regulated the inflammatory response associated to a murine chronic colitis model. The current study was designed to evaluate the effects of the oral administration of EVOO-PE on type II collagen-induced arthritis (CIA) in mice.
Material and Methods: Arthritis was induced in DBA-1/J mice by type II collagen. Mice were treated orally with EVOO-PE (2.5 and 5 mg/kg/day) from days 29 to 42 after the first immunization. In addition to macroscopic and histological analyses, inflammatory mediators were determined by ELISA. COX-2 localization was examined by immunohistochemistry. The expression of mPGES-1, p38, JNK, STAT-3, IκB-α and p65 NF-κB was studied by western blotting in paw homogenates.
Results: Oral administration of EVOO-PE resulted in therapeutic effects on joint edema, cell migration, cartilage degradation and bone erosion. Also, TNF-α, IL-1β, IL-6 and PGE_2 levels were significantly decreased. EVOO-PE treatment drastically decreased COX-2 and mPGES-1 overexpression as well as the activation of STAT-3, MAPKs and NF-κB pathways.
Conclusion: Our study has demonstrated the anti-inflammatory and joint protective effects of PE from EVOO in CIA, which would be related to the inhibition of relevant signalling pathways such as NF-κB, JAK-STAT and MAPKs controlling the production of inflammatory mediators and the progression of joint destruction.

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DAIDZEIN SUPPRESSES MACROPHAGE LPS-INDUCED INFLAMMATORY RESPONSE VIA DEPRESSING STAT-3 AND P-38 MAPK TRANSCRIPTIONAL ACTIVITY

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Introduction: Current epidemiological and experimental studies support a beneficial role of dietary isoflavones in several inflammatory diseases. The isoflavone daidzein (DZ) (4′,7-dihydroxyisoflavone) is the most important bioactive component of soy. The aim of this study was to investigate the mechanism underlying its anti-inflammatory action in lipopolysaccharide (LPS)-stimulated inflammatory response in murine peritoneal macrophages model.
Material/Methods: Cell viability was determined using sulphorhodamine (SRB) assay and nitric oxide (NO) production was measured using the Griess reaction. Moreover, changes in the protein expression of the proinflammatory enzymes cyclooxygenase-2 (COX-2) and the inducible synthase nitric oxide (iNOS), as well as the role of the mitogen-activated protein kinases (MAPKs) and STAT-3 signaling pathways were determined by western blot.
Results: Pretreatment with DZ did not produce any changes in cell viability. Besides DZ significantly reduced NO generation and induced a significant decrease on iNOS expression ($P < 0.01$). Similarly, DZ markedly down-regulated COX-2 expression and STAT-3 and p-38 MAPK transcriptional activity.
Conclusion: This study indicates that DZ inhibited LPS-induced proinflammatory enzymes and NO generation via down-regulation phosphorylation of STAT-3 and p-38 MAPK. These findings reveal, in part, the molecular basis underlying the anti-inflammatory properties of DZ.

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DIFFERENCES OF PERIPHERAL OPIOID ANTINOCICEPTION TO MECHANICAL AND THERMAL STIMULI

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Introduction: The pain pathways for the transmission of mechanical and thermal stimuli are different, and it is unknown whether opioid drugs induce antinociception to both types of stimuli by the same mechanisms. The main goal of this study was to compare the antinociceptive effect of μ opioid agonists with different intrinsic activity against these two types of stimuli, and to explore the contribution of peripheral opioid receptors to this antinociception.
Material/Methods: Female CD-1 mice were used. Mechanical and thermal antinociception were assessed as the increase in the response latency to paw pressure (blunt mechanical stimulation at 450 g) and contact heat (unilateral hot plate at 55°C).
Results: Morphine, oxycodone and buprenorphine, administered systemically (subcutaneously), produced dose-dependent antinociceptive effects to both types of stimuli. However the thermal antinociception induced by all drugs was higher than the effects on mechanical nociception. The antinociception induced by these opioid agonists was completely reversed, and in both sensory modalities, by the centrally-penetrant opioid antagonist naloxone. However, opioid-induced thermal antinociception was much more antagonized than mechanical antinociception by the peripherally-restricted opioid antagonist naloxone methiodide. These results suggest a higher participation of peripheral opioid receptors in thermal antinociception. In fact, the local (intraplantar) administration of these opioids exhibited marked antinociceptive effects to thermal stimulus without inducing any antinociception to mechanical stimulus.
Conclusions: Peripheral opioid antinociception depends on the type of sensory stimulation, being much more prominent to thermal than to mechanical stimuli.
This study was supported by Junta de Andalucía (CTS-109) and Laboratorios Esteve.

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STUDY OF THE EFFECT OF CHONDROITIN SULFATE ON PAIN IN KNEE OSTEOARTHRITIS PATIENTS ASSESSED BY FUNCTIONAL MRI: A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL

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Introduction: The aim was to identify the effects of chondroitin sulfate (CS) treatment on brain activation to pressure painful stimulation in patients with symptomatic knee osteoarthritis.
Material/Methods: PhaseIV, randomized, double-blind clinical trial in which patients received 800 mg/day CS (Condrosan®) or placebo for 4 months. Two fMRI tests were conducted at baseline and after treatment by applying painful pressure on the knee medial interline and on the patella surface. The main outcome measurement was attenuation of the brain response evoked by knee painful stimulation.
Results: Twenty-two patients received CS and 27 placebo. CS-treated patients showed a tendency to report reduced subjective pain with the patella pressure test ($P = 0.077$). fMRI of patella pain, showed a larger activation reduction in the CS group than placebo in a posterior mesencephalon region including the periaqueductal gray (PAG). The entire PAG cluster with significant interaction showed a pre>post-treatment difference ($P < 0.05$). In this paired analysis, the CS group showed significant activation reduction in the primary somatosensory cortex, the primary motor cortex and posterior supplementary motor area. Group by session interaction consistently revealed a tendency for this cortical change to be larger in the CS than in placebo($P < 0.01$). No effects were observed with the knee interline pressure test.
Conclusions: fMRI was sensitive to objectify CS effects on brain response to knee painful stimulation. The positive treatment effect of CS on brain was identified on pain elicited by pressure on patellofemoral cartilage, where the cartilage component of pain is a relevant factor. This result is consistent with the known CS mechanisms of action.

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PHENOTYPIC AND PROLIFERATIVE DIFFERENCES OF MOUSE MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE

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Introduction: Mesenchymal stem cells (MSCs) have an important immunomodulatory role in the inflammatory response. Adipose tissue is an important source of MSCs. It has been described that adipose tissue provides large numbers of stem cells compared to bone marrow.

However, the phenotype of MSCs isolated from adipose tissue from different parts of the body has not been well characterized. The aim of our research was the characterization of mouse MSCs from gonadal and inguinal adipose tissue.
Material and Methods: Gonadal and inguinal fat pads of CD1 male mice were digested by collagenase. The resulting cell suspension was filtered, washed, resuspended in DMEM/F-12 medium (15% fetal bovine serum, 1% antibiotics) and cultured under standard conditions. Cell morphology was characterized and the phenotype was determined by flow cytometry for negative (anti-CD45-FITC and anti-CD11b-APC) and positive (anti-CD105-PE and anti-CD29PerCP-eFluor710) markers.
Results: Cells isolated from both inguinal and gonadal adipose tissue showed a spindle-like morphology. Cells from gonadal fat pads showed a lower expression of negative CD45 (hematopoietic) and CD11b (macrophage) markers and a higher expression of positive CD105 and CD29 markers, relative to cells from inguinal adipose tissue at P0, P1 and P3. Although the yield of cells isolated from inguinal tissue was significantly higher than from gonadal tissue, gonadal cells had a greater capacity of proliferation.
Conclusions: We observed phenotypic and proliferative differences between gonadal and inguinal mesenchymal stem cells in mouse. This study suggests that the source of the adipose tissue is relevant when designing cell therapies with mesenchymal stem cells.

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TRANSIENT RECEPTOR POTENTIAL CHANNELS PRESYNAPTICALY MODULATE COLON MOTILITY

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Introduction: Transient Receptor Potential (TRP) channels are a superfamily of receptors that can be divided into 6 main subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankiryne), TRPML (mucolipine), and TRPP (polycistin) channels. With the exception of $TRPM_4$ and $TRPM_5$, all TRP channels are Ca^{2+} permeable cation channels. TRP channels are thought to have the ability to detect noxious stimuli and conveying the sensation of pain from peripheral organs to the spinal cord. For this reason, TRP-expressing nerves are known to be involved in diseases associated with hypersensitivity of intestine. In this work, we focus on the study of the role of $TRPV_1$, $TRPV_4$ and $TRPA_1$ receptors. $TRPV_1$ and $TRPV_4$ are proposed to be located in visceral afferent and respond to different noxious stimuli (chemical and mechanical). On the other hand, $TRPA_1$ is activated by noxious cold and it is present in sensory neurons.
Material and Methods: To characterize these receptors, we made two sets of experiments. First, we evaluated the response of longitudinal smooth muscle of the rat colon to selective and non-selective agonists and antagonists, by performing cumulative concentration-response curves (ACRC), after precontraction with acetylcholine. In the other set of experiments, we apply electrical stimuli at different frequencies and length to distinguish pre and postsynaptic components of the relaxation observed.
Results: When challenged with either selective or non-selective agonists of TRP channels (capsaicin, RN-1734 and polygodial), longitudinal smooth muscle exhibited significant relaxation characterized as a biphasic response, comprising a slow phase followed by a steeper phase with ascending concentrations. Furthermore, we used selective antagonists of NK receptors, peptide intestinal vasoactive (VIP) receptors, serotonin receptor, and nitric oxide; in so doing, we could find out how smooth muscle associated neurons presynaptically modulated these phases through the liberation of these specific substances. Electrical stimuli at low frequencies also revealed a full presynaptic component of these relaxant responses.
Conclusion: $TRPV_1$, A_1 and to a minor extent V_4 channels have an important role in the regulation of colon motility. Modulation of these targets with selective drugs could contribute to develop better treatments for diseases such as irritable or inflammatory bowel syndrome, diabetic gastroparesis or visceral pain.

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CHARACTERIZATION OF LATER STAGES OF INFLAMMATION IN THE MOUSE AIR POUCH MODEL IN C57BL/6 MICE

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Introduction: The regulation of the inflammatory response results in either chronification or resolution with important pathophysiological consequences. The aim or this work was to study the later stages of inflammation in the mouse air pouch model (MAP) in order to characterize the resolution process. This model was performed in C57BL/6 mice, the most widely used genetic background for genetically modified mice.
Material and methods: Air pouches were established by injecting sterile air into the dorsal site of the animal on days 0 and 3. On day 6, zymosan was administered into the pouch. The exudates were recovered from the air pouches at 8, 24 and 48 h after zymosan injection. Then, cellular infiltrate, prostaglandin E_2 (PGE_2), nitrite and cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8 and IL-4 were determined. The expression of microsomal heme oxygenase-1 (HO-1) was analyzed by Western blot.
Results: Cellular infiltrate, PGE_2 and IL-8 levels increased significantly at 8 and 24 h after zymosan injection. IL1-β levels were higher at early stages. However, TNF-α levels increased over time and IL-4 levels were undetectable in exudates of C57BL/6 mice. Nitrite levels peaked at 24 h. In addition, zymosan administration induced HO-1 with maximal expression at later stages (24 h-48 h), coincident with the resolution phase of inflammation.
Conclusions: The MAP in C57BL/6 mice provides an experimental model that reproduces relevant aspects of the inflammatory response. This assay can be performed in knockout mice to study the pathophysiology of inflammation and the mechanisms of action of anti-inflammatory drugs.

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HYPERNOCICEPTIVE RESPONSES FOLLOWING THE INTRATIBIAL INOCULATION OF RM-1 PROSTATE CANCER CELLS IN MICE

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Prostate cancer is the most common form of non-skin cancer in men in developed countries being frequently associated to painful symptoms due to bone metastases. Here we present a methodological study focussed on the nociception evoked by the intraosseous inoculation of RM-1 cells, an androgen-independent cell line derived from prostate cancer (donated by Dr. Thompson, MD Anderson Cancer Center, Texas University), able to evoke tumor growth in immunocompetent mice (McCabe et al., Clin Exp Metastasis 25, 581–90, 2008). In C57BL/6 mice, the intratibial inoculation of 103 RM-1 cells induced

bone tumor growth showing combined osteolytic and osteoblastic histological features.
At the spinal cord, the expression of Fos and GFAP, an astroglial marker, were increased whereas the labelling of the microglial marker, Iba-1, remained unchanged. Mice inoculated with RM-1 cells display thermal and mechanical hyperalgesia as well as mechanical allodynia from day 4 after inoculation.
The systemic administration of morphine (0.1–5 mg/kg) or the bisphosphonate zoledronic acid (0.3–3 mg/kg) alleviated hyperalgesia and allodynia. Furthermore, RM-1 cells in culture release the chemokine CCL2 and the systemic administration of RS504393 (0.3–10 mg/kg) an antagonist of CCR2 counteracts tumoral hypernociception, as described in another model of tumoral pain (Pevida et al., Naunyn Schmied Arch Pharmacol. 385, 1053–61, 2012). The intratibial inoculation of RM-1 in C57BL/6 mice can represent a useful model to study analgesic strategies to counteract bone cancer-induced pain from prostatic origin.
Funds came from SAF2012-36271. M.P. is recipient of a grant from the IUOPA. IUOPA is supported by Obra Social Cajastur-Asturias.

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INVOLVEMENT OF THE NO/PKG/KATP CHANNEL PATHWAY IN THE ANTIHYPERALGESIC EFFECT OF THE CB2 RECEPTOR AGONIST, AM1241, IN MICE INOCULATED WITH TUMORAL NCTC 2472 CELLS

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AM1241, a cannabinoid type 2 receptor (CB2R) agonist, counteracts thermal hyperalgesia in tumor-bearing mice through an opioid-dependent mechanism (Curto-Reyes et al., Br J Pharmacol, 2010).
The peripheral antinociceptive effects of several analgesic drugs involves the stimulation of nitric oxide (NO) synthase, the activation of protein kinase G (PKG) and the final opening of ATP-dependent potassium channels (KATP) (Menéndez et al., Neuropharmacology, 2007; Negrete et al., PloS One, 2011). We have studied whether CB2R are up-regulated in tumor-bearing mice and if the NO/PKG/KATP pathway is involved in the local antinociceptive effect of AM1241.
Western blot experiments demonstrated an increase of CB2R at the paw of mice intratibially inoculated with NCTC 2472 cells. Besides, tumoral thermal hyperalgesia was inhibited by i.pl. AM1241 (10 μg), being this antinociceptive effect blocked by its coadministration with the CB2R antagonist SR144528 (10 μg), the non-specific NO synthase antagonist L-NMMA (10 μg), the PKG inhibitor KT-5823 (0.1 μg) and the KATP channel blocker glibenclamide (10 μg).
The antihyperalgesic effect induced by AM1241 was also inhibited with the selective antagonist of neural NO synthase inhibitor L-NIO (0.15–1.5 μg) but not with inhibitors of the endothelial or inducible NO isoforms, N-ω-propyl-L-arginine (10 μg) or 1400W (1 μg), respectively. Thus, the antihyperalgesic effect of AM1241 in mice intratibially inoculated with NCTC 2472 cells seems related to the local up-regulation of CB2R and to the stimulation of the NO/PKG/KATP channel pathway triggered by the activation of neural NO synthase.
Funds came from SAF2012-36271. IUOPA, Obra Social Cajastur-Asturias.

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INHIBITION OF PERIPHERAL SIGMA-1 RECEPTORS REVERSES INFLAMMATORY HYPERALGESIA IN MICE

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Introduction: Sigma-1 (σ_1) receptor inhibition ameliorates neuropathic pain by diminishing central sensitization, but it is unknown whether σ_1 receptors participate on inflammatory hyperalgesia, or whether peripheral σ_1 receptors play a role in this process. Our goal was to test the effects of σ_1 receptor inhibition on carrageenan-induced inflammatory hyperalgesia, especially at the inflamed site.
Methods and Results: Subcutaneous (s.c.) administration of the selective σ_1 antagonists BD–1063 and S1RA dose-dependently and fully reversed inflammatory mechanical (paw pressure) and thermal (radiant heat) hyperalgesia in wild-type mice. These antihyperalgesic effects were reversed not only by the systemic (s.c.) administration of the σ_1 agonist PRE–084, but also by the intraplantar (i.pl.) administration of this compound in the inflamed paw, suggesting that blockade of peripheral σ_1 receptors in the inflamed area is involved in the antihyperalgesic effects observed. Moreover, the i.pl. administration of σ_1 antagonists in the inflamed paw (but not in the contralateral paw) completely abolished inflammatory hyperalgesia. σ_1 knockout (σ_1-KO) mice did not develop mechanical hypersensitivity, but developed thermal hyperalgesia; however, the s.c. administration of BD-1063 or S1RA had no effect on thermal hyperalgesia in σ_1-KO mice, supporting on-target mechanisms for the effects of both compounds. Carrageenan-induced edema was unaffected in σ_1–KO mice or by the systemic σ_1 pharmacological antagonism to wild-type mice. Therefore, antiedematous effects of σ_1 inhibition do not account for the decreased hyperalgesia.
Conclusions: σ_1 receptors in the inflamed tissue play a pivotal role in inflammatory hyperalgesia. Targeting σ_1 receptors may be useful for the treatment of inflammatory pain.
Fundings: MEC (SAF2010-15343), Junta de Andalucía (CTS–109), CEI BioTic Granada, FEDER funds, Esteve and CDTI (Genius Pharma).

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N- AND C-TERMINAL PARATHYROID HORMONE-RELATED PROTEIN DOWN-REGULATE THE PRODUCTION OF INFLAMMATORY MARKERS IN OSTEOARTHRITIC HUMAN OSTEOBLASTS

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Introduction: During the osteoarthritic process, different factors that may influence bone metabolism are released. Parathyroid hormone (PTH) and its bone counterpart, PTH-related protein (PTHrP), increase bone turnover through interaction of their N-terminal domain with the PTH type 1 receptor in osteoblasts. We here assessed and compared the ability of different PTHrP peptides, PTHrP (1–37), and the PTH-unrelated peptides, PTHrP (107–139) and PTHrP (107–111) (osteostatin), on inflammatory markers in osteoarthritic osteoblasts stimulated with interleukin-1β (IL-1β).
Methods: Osteoblasts were obtained from 6 patients undergoing total knee joint replacement. Subchondral bone tissue obtained from tibial plateau was minced into small portions and digested with collagenase under agitation. Collected tissue was seeded in osteogenic medium to obtain osteoblastic cells according to a standard procedure. At first passage, osteoblastic cells were treated with PTHrP (1–37), PTHrP (107–139) and osteostatin (each at 100 nM) with or without IL-1β

(10 ng/ml) for 1, 3 and 6 days. Prostaglandin E_2 (PGE_2) was measured by RIA, tumor necrosis factor-α (TNFα) by ELISA, and cyclooxygenase-2 (COX-2) expression was determined by immunocytochemistry.
Results: IL-1β increased the production of TNFα and PGE_2 in osteoarthritic osteoblasts. PGE_2 production was significantly decreased by PTHrP (1–37) on days 1, 3 and 6, and also by each C-terminal PTHrP peptide on day 6. The three peptides assayed were able to reverse IL-1β-induced COX-2 overexpression on day 3, and reduced TNFα release on day 6.
Conclusions: These findings show that both N- and C-terminal PTHrP peptides may induce down-regulation of important inflammatory markers in osteoarthritic osteoblasts.

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MANGIFERA INDICA LEAF EXTRACT, RICH IN POLYPHENOLIC COMPOUNDS, PROTECTS AGAINST DEXTRAN SULFATE SODIUM-INDUCED COLITIS IN MICE

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Background: *Mangifera indica* has been commonly used in medicine for its benefits as antidiabetic, antioxidant, anti-viral, cardiotonic, antihypertensive and anti-inflammatory. The *Mangifera indica* extract (MIE), rich in polyphenolic compounds, has been previously described as an antioxidant with anti-inflammatory and immunomodulatory activities in several experimental settings.
Objective: The aim of this study was to investigate whether water extract from the leaves of *Mangifera indica* can protect against dextran sulfate sodium (DSS)- induced acute colitis in a mouse model.
Methods: C57BL/6 mice were given fresh water with 3% DSS for 7 days to induce colitis. Mice were orally administered MIE (50; 100; 200 mg/kg) when treatment of DSS started. Body weight, colon length, and histological score were assessed to determine the effects on colitis. Myeloperoxidase (MPO) activity, TNF-α and other proinflammatory cytokines were also examined in the colon tissues. In addition, colonic expression of other proteins such as cyclooxygenase2 (COX-2) was determined by Western blot.
Results: MIE significantly attenuated the clinical signs of colitis such as loss of body weight and colon length and histopathologic (neutrophilic infiltration, necrosis) signs of damage. Moreover, MIE suppressed MPO activity, TNF-α production and COX-2 expression in colitic mice. Interestingly, protective activity of MIE in colitis was observed at doses of 50 and 100 mg/kg, but at the highest dose this effect was significantly reduced.
Conclusions: These results suggest the potential of *Mangifera indica* as a new anti-inflammatory compound used for inflammatory bowel disease treatment at controlled doses.
Grant from: Junta de Andalucía, Proyecto Excelencia POLFANAT.

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PARADOXICAL FACILITATION OF CATECHOLAMINE SECRETION, INDUCED BY THE COMBINATION OF ATP AND METHIONINE - ENKEPHALIN IN CHROMAFFIN CELLS STIMULATED WITH ACETYLCHOLINE AT 37°C

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The predominant secretion of noradrenaline (NA) or adrenaline (AD) in the medulla of adrenal gland varies with the type of clinical emergency (cardiogenic, anaphylactic, hemorrhagic or traumatic shock or hypoglycemic coma). This selective secretion is neurologically regulated by the cerebral cortex and hypothalamus but also has a peripheral component related to the increased expression of voltage-dependent Ca^{2+} channels (VDCC) of the L subtype, predominant in noradrenergic cells and PQ subtype predominant in adrenergic cells (RB Lomax, Am J Physiol 1997; 272: C476-C484). Since these VDCC are regulated by ATP via purinergic receptors (Gandia et al, J. Physiol 1993, 470: 55–72) and methionine-enkephalin (ME) (Albillos et al J Physiol 1996, 494: 687–95), we propose here the question whether the regulation of Ca^{2+} entry also affect the differential secretion of NA and AD-induced by the physiological neurotransmitter acetylcholine (ACh) in bovine chromaffin cells perfused with Krebs-Hepes at 37°C. Notably, ATP (1 μM) inhibits the secretion of NA and AD; ME (3 μM) also reduce the secretion by only 25%. Paradoxically, the combination ATP/ME increased 2.5 times the secretion of NA and 2 times that of AD. It's interesting to know whether this increase is antagonized by the combination of suramin (a nonspecific blocker of purinergic receptors) and naloxone (opioid receptor blocker). Together, these data suggest that the simultaneous stimulation of purinergic and opioid receptors on the plasma membrane of the chromaffin cells might activate complementary pathways that regulate the release of NA or differential AD. A calcium-cAMP interruption would be at the center of the regulatory process itself, so critical for survival in the above clinical situations that threaten the patient's life.
Acknowledgments: Fundación Teófilo Hernando, UAM/Servicio de Farmacología Clínica, H.U. Princesa FPU12/02220 to AW and FIS No. CA12/E to ARN.

NEUROPSYCHOPHARMACOLOGY

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TARGETED NANOPARTICLES ACROSS THE BLOOD BRAIN BARRIER

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Introduction: A major limitation of nanosystems is that the body recognizes them as foreign particles, so they are rapidly opsonized and removed from the bloodstream before fulfilling their function. Chemical modification of the surface of nanoparticles (NPs) can improve cellular uptake, prevent opsonization and increase plasma half-life. Functionalization of NPs surface with different ligands can also reduce phagocytosis by increasing the number of nanosystems that are able to get through the blood brain barrier (BBB). For this, we have studied the NPs passage across the BBB of different nanoparticles.
Methods: Three different formulations of NPs were prepared by nanoprecipitation using an acetone-water system and copolymer PLGA RG®502. NPs were then administered to rats: F1 (rhodamine-loaded NPs suspended in saline; F2 (rhodamine-NPs suspended in 1% polysorbate 80; and F3 (rhodamine-loaded NPs-polysorbate 80 in saline).

One hour after administration animals were sacrificed and cerebral cortex microphographs were observed.
Results and Discussion: F2 showed a slight increase in fluorescence whereas the best results were obtained with formulation F3 in which NPs were prepared with polysorbate 80. Due to the small size of the NPs it cannot be assure that the fluorescence observed is directly related to the presence of NPs however, it can be stated that in cerebral cortex a marked fluorescence increase was observed after administration of formulation F3, probably due to their passage through the BBB.

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BEHAVIOURAL CHARACTERIZATION OF 5-HT$_4$ RECEPTOR KNOCKOUT MICE: IMPLICATIONS FOR ANXIETY AND DEPRESSION

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New pharmacological targets for treating depression include 5-HT$_4$ receptors. Indeed, preclinical studies have reported that partial 5-HT$_4$ receptor agonists exhibit a faster onset of antidepressant actions versus conventional antidepressants.
This study was aimed to analyze the behavioural outcomes of 5-HT$_4$ receptors knockout (KO) mice in predictive animal models of depression/anxiety. Regarding anxiety-like responses, we did not detect statistical differences between wild-type (WT) and KO mice in central activity parameters on the open field test (% central time= 15.3 ± 1.4 in WT *vs* 15.9 ± 1.2 in KO mice; % central distance = 20.2 ± 1.3 in WT *vs* 21.8 ± 1.6 in KO mice). In the same way, the number of entries to the bright zone in the light dark box was also similar (entries= 11.9 ± 1.0 and 11.9 ± 0.6 for WT and KO mice, respectively). On the other hand, KO mice did not exhibit changes in different paradigms predictive of depression assessing behavioural despair (immobility time = 130.0 ± 6.7 in WT *vs* 140.0 ± 4.4 seconds in KO on the forced swimming test), anhedonic-like traits (% sucrose intake/total = 91.4 ± 0.9 in WT *vs* 91.0 ± 1.6 in KO on the sucrose preference test) and anxiety-like responses associated to depressive states (latency to eat = 201.9 ± 17.5 in WT *vs* 183.7 ± 12.9 in KO seconds on the novelty suppressed feeding test).
In conclusion, the lack of 5-HT$_4$ receptor does not appear to induce significant changes in the behavioural basal performance of rodents subjected to predictive paradigms of anxiety/depression manifestations. Further studies are needed to reconcile the above findings with the reported anxiolytic/antidepressant effects of 5-HT$_4$ receptors.
Supported by Ministerio de Ciencia (SAF07-61862), Ministerio de Economía y Competitividad (SAF2011-25020), ANR-SERFEED grant.

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THE CONTRIBUTION OF 5-HT$_4$ RECEPTORS TO THE ANXIETY/DEPRESSION MANIFESTATIONS IN TWO ANIMAL MODELS OF DEPRESSION

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New pharmacological targets for treating depression include 5-HT$_4$ receptors. Indeed, 5-HT$_4$ receptor agonists are reported as antidepressant drugs with a rapid onset of action. Depression is a multifactorial disease in which genetic-environment interactions are important. In this study, we have evaluated the behavioural consequences of the lack of expression of 5-HT$_4$ receptors in two models of depression/anxiety: olfactory bulbectomy (OBX) and chronic administration of corticosterone (CORT). 5-HT$_4$ receptor knock-out (KO) and WT mice (20 ± 2 g, 3 months old) were bulbectomized or treated with corticosterone (45 mg/ml in drinking water for 4 weeks).
One month after olfactory bulbectomy we did not observe any significant difference between WT and KO mice on the typical OBX-induced hyperactivity (peripheral distance = 21.6 ± 2.1 in WT *vs* 24.1 ± 2.1 m in KO) assessed in an aversive (400 lux) open-field arena. In the corticosterone model no significant differences were observed between KO and WT mice in anxiety-related parameters (central time = 28.1 ± 4.4 in WT *vs* 25.2 ± 3.3 s in KO; % central distance = 17.3 ± 1.8 in WT *vs* 15.1 ± 1.6 in KO). However, in the novelty suppressed feeding the latency to feed in KO was significantly higher than in WT mice (latency = 476.8 ± 31.0 *vs* 557.2 ± 17.9 s for WT and KO corticosterone-treated mice, respectively; $P < 0.05$, two-way ANOVA followed by Newman-Keuls post hoc test).
In conclusion, 5-HT$_4$ receptors appear to modulate depression/anxiety-related responses in some behavioural predictive paradigms (NSF) but not in others. Further studies are needed to evaluate the pharmacological effects of 5-HT$_4$ receptor agonists in these animal models of depression/anxiety.
Supported by Ministerio de Ciencia (SAF07-61862), Ministerio de Economía y Competitividad (SAF2011-25020), ANR-SERFEED grant.

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NEUROPROTECTIVE PROFILE OF THE NEW GLYCOLIPID COMPOUND IG20 IN RAT HIPPOCAMPAL SLICES

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Introduction and Objectives: IG20 is a sulfated glycolipid that promotes neurite outgrowth and myelination and inhibits glial proliferation (patent no: WO2014/006250 A1). Some preliminary data show that IG20 could interact with RhoGDIα pointing out a possible mechanism of action in the RhoGTPases family which takes part in axonal regeneration in the injured CNS (Vyas *et al*., 2005). With this preliminary data, the hypothesis was raised that, IG20 could exhibit neuroprotective properties; we have tested such hypothesis.
Methods: We have measured cell viability and ROS (reactive oxygen species) production in hippocampal slices stressed with different toxic stimuli that cause cell death through different pathways. Using MTT reduction as a probe, we studied the effect of IG20 over voltage dependent calcium channels and calcium signaling.
Results and Conclusions: We have found that IG20 exerts neuroprotection in hippocampal slices stressed with glutamate, veratridine or glucose and oxygen deprivation (OGD) followed by reoxygenation. From 0.3 to 10 μM, IG20 preincubation prevented up to 50% of veratridine-induced damage. Furthermore, when added after glutamate exposure, IG20 was able to prevent MTT reduction at the same concentration range. Regarding OGD, IG20 pretreatment (1–10 μM) protected 90%, compare to 70% of untreated slices. At 3 μM, IG20 treatment decreased ROS production in glutamate-treated slices. We characterized the ROS scavenging profile of IG20 and found it was not able to directly buffer ROS in the absence of tissue. We could not demonstrate that IG20 exerted anti glutamatergic effect, since it did not block cytosolic calcium transient evoked by glutamate. So far, we

are not sure about which is the mechanism involved in the neuroprotective action of IG20; conversely we raised many questions, yet to be answered, before a clear scenario on its mechanism of action can be proposed.

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EFFECTS OF D_1 RECEPTOR BLOCKADE ON THE BRAIN METABOLISM AND NEURODEGENERATION INDUCED BY THE LITHIUM-PILOCARPINE MODEL OF EPILEPSY

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Introduction: It is widely accepted that an imbalance between glutamatergic and GABAergic systems causes epileptic hyperexcitability. Furthermore, a reduction in cerebral metabolic activity is often observed during the interictal stages of epilepsy. On the other hand, the involvement of dopaminergic central system in the epileptogenesis is not fully understood. It has been suggested that dopamine receptor subtypes exert opposite functions on the regulation of convulsive activity. Whereas the D_1 blockade is described to have a protective role, the administration of haloperidol, a "selective" D_2 blocker can potentiate the occurrence of seizures.
Objectives: The aim of this study was to evaluate the effects of the acute administration of SCH23390, a selective D_1 antagonist, on the metabolic and neurochemical changes induced by the lithium-pilocarpine experimental model of temporal lobe epilepsy.
Materials and Methods: The lithium-pilocarpine model of temporal lobe epilepsy was used in adult male SD rats. Thirty minutes before the injection of pilocarpine, SCH23390 (0.2 mg/kg ip) was administered. Three days after the epileptogenesis induction, positron emission tomography (18F-FDG PET) was performed to evaluate the brain metabolic activity. Twenty four hours after PET, animals were sacrificed and different markers (GFAP immunohistochemistry and Fluoro Jade C staining) were analyzed to evaluate the eventual brain damage.
Results and Conclusions: Three days after the insult, a significant reduction in metabolic activity of brain areas involved in epileptogenesis was observed (16% and 14% in hippocampus and cortex respectively). In our model, the hypometabolism was not modified by previous administration of SCH23390.
On the other hand and regarding the neuronal integrity, SCH23390 was able to prevent the reactive gliosis (GFAP) and neurodegeneration (FJC) in the aforementioned areas.
According to these results, although D_1 blockade did not improve the metabolic impairment, SCH23390 prevented some signs of brain damage induced by lithium-pilocarpine model.
Study supported by SAF 2009-09020.

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STIMULATION OF IGF-II RECEPTOR INDUCES NEUROPROTECTION AGAINST OXIDATIVE DAMAGE ON ADULT CORTICAL NEURONAL CULTURES

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Reactive oxigen species (ROS) contribute to the oxidative damage found in neuropathological conditions related to enhanced glucocorticoid expression. Recent studies strongly suggest putative roles of Insulin-like Growth Factor II (IGF-II) in the development of and recovery from glucocorticoid and stress-related disorders. Our laboratory has demonstrated the involvement of IGF-II receptor (IGF-IIR) in redox homeostasis after glucocorticoid-induced toxicity. Thus, the IGF-IIR stimulation promoted antioxidant effects against corticoid induced damage, improving mitochondrial function.
As increased ROS production and mitochondrial dysfunction play crucial roles in major neurodegenerative disorders, we have examined the neuropotective properties of low IGF-II concentrations on adult rat cortical neuronal cultures under high corticosterone (CORT) toxicity, using the stain Fluoro-Jade B™ (FJ). A large and significant increase in FJ fluorescence intensity of CORT-treated cells was detected, whereas the addition of IGF-II completely prevented this increase ($P < 0.05$). The alternative and selective inhibiton of IGF-IR or IGF-IIR did not exert any change in the effect of IGF-II, whereas the inibition of both receptors together returned the fluorescence level to that found in CORT-treated cells. Patterns of IGF-II effects on both FJ staining and ROS production were very similar.
Recently, it has been demonstrated that some CORT effects on neurons are mediated by PKC phosphorylation, and the specific signalling pathway associated with IGF-IIR activation remains unclear, suggesting that both IGF-II and CORT, may share a common intracellular pathway involving PKC. In our study CORT activated PKC, 42% higher than control ($P < 0.05$), and IGF-II treatment restored the levels to those found in control cells. As conclusion, low IGF-II concentrations may contribute to the mechanisms of neuroprotection promoting antioxidant effects, reducing the neurodegeneration induced by oxidative insults.

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NEW DUAL NEUROPROTECTANTS BASED ON 3-SUBSTITUTED INDOLE DERIVATIVES TARGETING BOTH CALCIUM CHANNELS AND PHOSPHOPROTEIN PHOSPHATASE 2A

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Neurodegenerative diseases have been proposed to possess a multifactorial nature. Hence, there is a rising interest in the development of new neuroprotective compounds interacting with two or more biological targets implicated in the neurodegenerative progression. Ca^{2+} plays a pivotal role in a plethora of cellular events, so its cytosolic concentrations are finely controlled by intracellular organelles. When this accurate control is impaired, a cascade of pro-apoptotic events can occur. Thus, compounds tuning the cell Ca^{2+} signal have been classically studied for the treatment of neurodegenerative diseases. On the other hand, scientific community has recently paid attention to phosphoprotein phosphatase 2A (PP2A) due to its role in the dephosphorylating process of tau protein, taking into account that one of the hallmarks of Alzheimer's disease are the neurofibrillary tangles, mainly formed by aggregates of hyperphosphorylated tau protein.
With this background, we focused on an indole alkaloid, gramine, as a potential hit compound for the synthesis of new neuroprotectants, due to our observation that it blocked Ca^{2+} entry through voltage-activated Ca^{2+} channels (VACC) and protected neuronal cultures against toxic stimuli related to neurodegeneration, e.g. high glutamate exposure, rotenone/oligomycin A-induced oxidative stress, or okadaic acid-elicited tau hyperphosphorylation. Thus, we have synthesized twenty-five 3-substitued indole derivatives related to gramine that have shown, in some cases, improved neuroprotective profile in several *in vitro* models of neurotoxicity. In general, these compounds have reduced Ca^{2+} entry through VACCs, as observed in patch-clamp experiments; some of them also prevented the okadaic acid-induced PP2A inhibition, as shown in immunoprecipitation experiments. To get insights on chemical and pharmacokinetic characteristics of gramine derivatives and in

the interaction with PP2A, we also applied molecular modelling methodologies and *in silico* ADME-Tox tools. We hypothesise that the dual action on VACCs and PP2A can lead to a new promising approach for the treatment of neurodegenerative diseases.

This work was supported by grant P113/00789 from ISCIII (Instituto de Salud Carlos III, Spain) and grant SAF 2100-21795 from MINECO (Ministerio de Economía y Competitividad, Spain). The continued support of FTH (Fundacion Teófilo Hernando, Spain) is also acknowledged.

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EFFECT OF BUSPIRONE ON THE SUBTHALAMIC NUCLEUS ON AN ANIMAL MODEL OF PARKINSON′S DISEASE: AN ELECTROPHYSIOLOGICAL STUDY

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Introduction: The most effective treatment for Parkinson's disease, L-DOPA, induces dyskinesia (LID) after prolonged use. We have previously shown that in 6-hydroxydopamine lesioned rats rendered dyskinetic by prolonged L-DOPA administration, lesion of the subthalamic nucleus (STN) reduces LID and buspirone antidyskinetic effect. The aim of this study was to evaluate the effect of buspirone on STN neuron activity.

Methods: Single-unit extracellular recordings were performed *in vivo* on STN neurons from four different groups, i.e., control, chronically treated with L-DOPA, lesioned and lesioned chronically treated with L-DOPA (dyskinetic) rats and *in vitro* cell-attached recordings.

Results: In control rats buspirone administration decreased the firing rate in a dose-dependent manner (4 mg/kg i.p. 35%; and 8 mg/kg i.p. 67%). This effect was absent in 6-OHDA lesioned rats and was not modified by acute or prolonged L-DOPA administration. In addition, in control rats the $5HT_{1A}$ antagonist WAY-100635 and the D_3 antagonist PD128907 prevented the effect of buspirone. Conversely, in parasagittal slices containing the STN, buspirone induced excitatory, inhibitory and also biphasic responses being only the inhibitory effect prevented by WAY-100635.

Conclusion: Buspirone *in vivo* reduces the firing rate of the STN neurons through $5HT_{1A}$ and D_3 receptors whereas *in vitro* buspirone seams to show a more variable effect. Moreover, effect of buspirone was abolished in 6-OHDA lesioned rats, suggesting that the STN may not be directly involved in its antidyskinetic effect.

Support by UFI 11/32 and FIS PI12/00613 A.S. has a fellowship from the UPV/EHU.

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PHARMACOLOGICAL CHARACTERIZATION OF 5-HT7 SELECTIVE ARYLPIPERAZINE DERIVATIVES IN RAT PRIMARY ASTROCYTES

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Serotonin 5-HT7 receptors constitute a target of interest for the treatment of depression, schizophrenia, sleep disorders and migraine. This receptor is coupled to Gs, activating the adenylyl cyclase/cAMP signaling pathway, and G12 proteins.Progress in 5-HT7 research has been hampered along several years by the absence of selective compounds. In a previous study, we reported that the arylpiperazine derivative LP-211 exerts a long- lasting inhibition of [3H]-SB-269970 radioligand binding at human 5-HT7 receptors and an insurmountable antagonism of 5-carboxamidotryptamine (5-CT)-stimulated cAMP signaling in HEK293 cells stably expressing the receptors (Atanes et al., 2013). These results were unexpected as LP-211 has been extensively used as a selective 5-HT7 pharmacological tool, to which 5-HT7 agonist activity has been attributed in *ex vivo* and *in vivo* studies. The present work aims to characterize the pharmacological profile of this compound on a different native cellular background in order to clarify its mechanism of action. With this aim, we have established rat astrocytes primary cultures, where we have characterized endogenous 5-HT7 receptors by [3H]-SB-269970 radioligand binding (Kd = 13.5 ± 2.9 nM; Bmax = 15.8 ± 1.5 fmol/100,000 cells) and 5-CT-mediated cAMP signaling (Emax (% of basal) = 646.9 ± 26.9). Moreover, we have tested the effect of compound LP-211 and its analog MEL-9 in these primary cultures. The development of selective chemical probes and their pharmacological characterization in different experimental settings will foster our understand ing of the physiological functions of this receptor and its possible therapeutic potential in the central nervous system.

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EFFECT OF INTENSIVE AND REPEATED USE OF ETHANOL ON THE INTEGRITY OF BLOOD- BRAIN BARRIER IN MOUSE BRAIN. ROLE OF TOLL-LIKE RECEPTOR 4 (TLR4)

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Ethanol is the oldest and one of the most commonly consumed drugs of abuse. Recently, the age of first consumption of alcohol has fallen and during adolescence consumption is usually intense and mostly concentrated in a few days; a pattern termed *binge drinking*. Ethanol has been shown to produce brain damage and, although the mechanisms involved are not completely clear, TLR4 have been shown to play an important role. The Drinking in the Dark (DID) procedure is accepted as a model of *binge drinking* where singly-housed mice are maintained in reversed cycle lighting and drinking water is replaced by ethanol on each of 4 consecutive days; the first three days the exposure time is 2 h and on the 4th day it is extended to 4 h.

C57BL/6 WT and $TLR4^{-/-}$ KO mice (20–25 g) were subjected to 4 DID cycles and killed 24 h after the last ethanol exposure. By means of immunohistochemistry, the effect of ethanol on the blood-brain barrier (BBB) integrity and on neuronal death in the hippocampus was determined.

Ethanol increased IgG extravasation and reduced laminin and collagen-IV expression, reflecting an increase in BBB permeability. In addition, there was an increase in the staining with the fluorochrome Fluoro-Jade, indicating greater neuronal death. Similar studies in TLR4 *knock-out* mice showed no reduction in laminin or in collagen-IV and no increase in IgG extravasation. Moreover, ethanol did not induce neuronal death.

The BBB alterations and neuronal death produced by ethanol involve TLR4 signalling since these changes are not observed in the absence of this receptor.

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DISRUPTION OF BLOOD-BRAIN BARRIER INTEGRITY IN POSTMORTEM HUMAN ALCOHOLIC BRAIN

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Alcoholic human brain shows neuroimmune activation reflected by an increase in the expression of proinflammatory cytokines and chemokines, microglial markers and inflammasome proteins. Blood-brain barrier (BBB) integrity is altered by several factors including increased levels of inflammatory cytokines and free radicals which in turn, are involved in the phosphorylation of MAPKs and induction of metalloproteinases (MMPs). MMP-9 can digest several matrix proteins in the cerebrovascular basal lamina and promote the loss of BBB function by cleaving tight junction structural proteins.

We have now examined the BBB integrity in the dorsolateral prefrontal cortex (PFC) from postmortem human controls and alcoholics obtained from the New South Wales Tissue Resource Centre at the University of Sydney. Alcoholic patients with a history of chronic (34 ± 7 years) alcohol dependence uncomplicated by liver cirrhosis and/or nutritional deficiencies were included. Frozen samples or paraffin sections of Brodmann area were used. MMP-9/2 activity was measured by zymography and expression of MMPs and phosphorylated MAPKs by western blot. Laminin and collagen-IV expression and leukocyte recruitment was evaluated by immunohistochemistry.

Human alcoholic prefrontal cortex shows an increase in MMP-9 protein expression and gelatinolytic activity. No change was observed in MMP-2 activity. There was a reduction in the expression of collagen-IV and claudin-5 and a pronounced leukocyte infiltration of cortical parenchyma compared with controls. An increased phosphorylation of the MAPK proteins JNK1/2, ERK1/2 and p-38 was also observed.

Together, data indicate that prefrontal cortex of alcoholics shows alterations in BBB morphology that may have implications for disease pathogenesis and progression.

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EFFECT OF MDMA ON AQP4 EXPRESSION IN ASTROCYTIC ENDFEET AND TPA ACTIVITY IN RAT HIPPOCAMPUS

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3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') increases blood-brain barrier (BBB) permeability in hippocampus through an early, $P2X_7R$-mediated event which enhances MMP-9 and MMP-3 activities and degrades extracellular matrix. Astrocytic endfeet are in contact with basal lamina and together with the tight junctions form the BBB. Aquaporin-4 (AQP4) forms water channels localized predominantly in astrocytic endfeet and maintains water and ion homeostasis associated with neuronal activity. The tissue-type plasminogen activator (tPA) increases vascular permeability by interacting with low-density lipoprotein receptor-related protein (LRP) in perivascular astrocytes. We have examined the time-course of MDMA-induced changes in AQP4 expression and proteolytic activity and expression of tPA in hippocampus. Expression of the high-mobility group protein-1 (HMGB-1), as an enhancer of tPA activity, was also evaluated. Male Dark Agouti rats were sacrificed 1 h, 3 h, 6 h and 24 h after MDMA (12.5 mg/kg, i.p.). AQP4 expression was determined by immunohistochemistry in dentate gyrus (DG), CA3 and CA1. Hippocampal expression of tPA, plasminogen and HMGB-1 was determined by western blot and tPA activity by gel zymography. AQP4 expression in the astrocytic endfeet increased in CA3 and DG 6 h and 24 h, respectively, after MDMA injection. No change was observed in CA1. Hippocampal expression and activity of tPA increased 3 h after MDMA. At this time point there was a reduction in plasminogen expression. HMGB-1 expression also increased 3 h after MDMA.

These results suggest that MDMA induces changes in AQP4 functions such as regulation of water movement in hippocampus and these effects may be a consequence of increased tPA activity and HMGB1 expression.

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DIFFERENTIAL REGULATION OF SYNAPTIC PLASTICITY MARKERS AND BEHAVIOUR BY HISTONE DEACETYLASE INHIBITORS

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Introduction: Recent discoveries have awakened the interest of histone deacetylase (HDAC) inhibitors for their effects on neurodegenerative and psychiatric diseases. Vorinostat (suberoylanilide hydroxamic acid/ SAHA) and givinostat are non-selective class I and II HDAC inhibitors that have shown neuroprotective action in animal models. Similarly, sirtinol is a class III (sirtuins) HDAC inhibitor showing beneficial effects in neurodegenerative models. Other more selective HDAC inhibitors such as the class II HDAC inhibitor MC1568 and the Sirt2 inhibitor 33i have not yet been studied in the brain. Here we have studied comparatively the effect of these five compounds in histone acetylation, synaptic plasticity markers and behaviour.

Methods: Male mice (C57BL/6J 8–10 weeks of age) were treated i.p. with vorinostat (10 mg/kg), givinostat (10 mg/kg), sirtinol (1 mg/kg), MC1568 (25 mg/kg) and 33i (1 mg/kg) for 10 days (8 mice/group). Mice were tested for anxiety and memory test and brain protein expression including acetylation levels of histone 3 (AcH3) and 4 (AcH4), brain derived neurotrophic factor BDNF, c-AMP response element binding protein CREB, VGLUT1 and synaptophysin.

Resuts: Both non-selective HDAC inhibitors givinostat and vorinostat induced an increase in the acetylation state of histones 3 and 4 as well as the expression levels of the synaptic plasticity markers in both areas. Sirtinol, 33i and MC1568 affected differently to histone acetylation and synaptic plasticity markers. Behavioural studies revealed that while givinostat and vorinostat improved recognition memory in the novel object recognition test, sirtinol showed anxiolytic action in the elevated plus maze.

Conclusion: This study supports the potential interest of class I and III HDACs as targets for neurological and psychiatric disorders respectively. Yet, further studies using different doses of all these drugs should be carried out in order to fully describe their pharmacological profile and their potential interest for CNS disorders.

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SMALL SYNTHETIC HYALURONAN DISACCHARIDES AFFORD NEUROPROTECTION IN BRAIN ISCHEMIA RELATED MODELS

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High molecular weight glycosaminoglycanes of the extracellular matrix have been implicated in tissue repair. The aim of this study was to evaluate if small synthetic hyaluronan disaccharides with different degrees of sulfation (di0S, di4S and di4,6S) could improve cell survival in in vitro and in vivo brain ischemia related models. Rat hippocampal slices subjected to oxygen and glucose deprivation and a photothrombotic stroke model in mice were used. The three hyaluran disaccharides, incubated during the oxygen and glucose deprivation (15 min) and re-oxygenation periods (120 min), reduced cell death of hippocampal slices measured as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction, being the most potent di4,6S; in contrast, high molecular hyaluronan was ineffective.
The protective actions of di4,6S against oxygen and glucose deprivation were related to activation of the PI3K/Akt survival pathway, reduction of p65 translocation to the nucleus, inhibition of inducible nitric oxide oxidase induction and reactive oxygen species production, and to an increase in glutathione levels. Administered 1 h post-stroke, di4,6S reduced cerebral infarct size and improved motor activity in the beam walk test. In conclusion, di4,6S affords neuroprotection in in vitro and in vivo models of ischemic neuronal damage. Our results suggest that its neuroprotective effect could be exerted through its capability to reduce oxidative stress during ischemia. Its small molecular size makes it a more potential drugable drug to target the brain as compared with its high molecular weight parent compound hyaluronan.

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IMPLICATION OF AMYLOID –β PEPTIDE-INDUCED DECREASES OF VGLUT1 EXPRESSION IN THE COGNITIVE DEFICITS OF ALZHEIMER′S DISEASE

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Introduction: It is proposed here a novel mechanism by which amyloid pathology might alter glutamatergic transmission which, in turn, could be responsible for cognitive deficits in Alzheimer′s disase (AD).
Methods: A transgenic mice model of AD (Tg2576) has been used. Cognitive status was checked by the Morris water maze. Postmortem cortical brain tissues (BA 10) from a cohort of neuropathologically confirmed AD patients and age-matched controls were also used. Protein expression was measured by immunoblotting, and glutamate levels were measured by HPLC.
Results: Reductions in VGLUT1 expression accompanied by a decrease release of glutamate were found in primary cell culture from Tg2576 mice. In vivo, changes in VGLUT1 expression seem to parallel amyloid load in the hippocampus and precede cognitive deficits: VLUT 1 expression was significantly decreased, amyloid load was significantly increased and no cognitive deficits in the Morris water maze were observed in 8–9 months old Tg2576 mice, while older mice (16 months old) showed decreases in VGLUT1 expression, increases in amyloid load and cognitive deficits. In AD samples, VGLUT1 expression was decreased. A significant negative correlation was found between amyloid pathology (amyloid levels or senile plaque score) and decreased VGlut1 expression.
Conclusions: Amyloid pathology seems to contribute to decrease VGLUT1 expression and therefore to a decreased release of glutamate and hence, to the cognitive deficits in AD. Modulation of VGLUT1 activity may be considered as a possible therapeutic target for the treatment of AD.
This work has been supported by a grant from FIS (13/00858).

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INCREASED ACTIVATION OF JNK3 IN ALZHEIMER'S DISEASE

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Introduction: The pathogenesis of Alzheimer′s disease (AD) is still poorly understood. C-Jun N-terminal kinases (JNKs) have multiple functions including regulation of gene expression, inflammation, cell proliferation and apoptosis. JNK3 is a brain-specific JNK isoform that might play important roles under neurodegenerative conditions, such as AD. However, JNK3 specific localization and functions in the brain still remains unclear.
Methods: A transgenic mice model of AD (Tg2576) has been used to study the localization of pJNK3 in astroglia (GFAP), neurons (NeuN), microglia (CD11b), senile plaques (Aβ) or pTau, using immunohistochemistry double staining. Moreover, the expression of JNK3 in AD postmortem cortical brain tissues has also been checked by immunoblotting in a cohort of AD patients and age-matched controls.
Results: It has been found that immunostaining for pJNK3 was associated with the periphery of amyloid plaques. pJNK was also associated with hyperphosphorylated tau in these plaques. In contrast, there was no co-localization with GFAP, NeuN or CD11b. In human samples, pJNK3 levels were significantly increased in AD brains and its expression correlated with Aβ42 levels and cognitive status (as assessed by MMSE score).
Conclusions: The existence of high immunoreactivity of pJNK3 surrounding amyloid plaques as well as the increased levels of this protein in human AD brains and its correlation with cognitive status strongly suggests that JNK3 may play a role in Alzheimer-related neurodegeneration. However, the critical question is still the cell type responsible for the release of this inflammatory kinase.
This work has been supported by a grant from FIS (13/00858).

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ALCOHOL POTENTIATES THE REINFORCING EFFECTS OF MEPHEDRONE IN ADOLESCENT MICE. ROLE OF D_3 RECEPTORS

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Introduction: Mephedrone (Meph) is a synthetic β-keto-amphetamine whose recreational consumption as a psychostimulant has risen during the last years. As many other illicit drugs, this consumption occurs mainly during leisure days using a binge pattern and often associated to other drugs, especially alcohol (EtOH). This kind of drug consumption in the adolescence is a matter of concern because it is a crucial period in brain development and an increased vulnerability to drugs exists. The aim of this study was to assess whether moderate doses of EtOH could enhance the reinforcing effects of mephedrone, as well as the most relevant associated transcriptional changes.
Materials/Methods: Adolescent (4–5 weeks-old) male CD-1 mice were used. Meph and EtOH were dissolved in saline and administered s.c., either alone or mixed. Reinforcing effects were measured using the conditioned place preference paradigm (CPP), which measured the time spent in each of the two available compartments. Briefly, the mice were subjected to 1 day of pre-conditioning (free access to both compartments), and 8 days of conditioning (receiving saline or drug in alternate days and confined-compartments). On the 10^{th} and last day mice were given free access to roam around both compartments and the time spent in each one was recorded. 24 h after the test, the mice were sacrificed and the anterior striata were removed. Total RNA was extracted and retro-transcribed. cDNA was hybridized to GeneChip Mouse Gene 1.0 ST Affymetrix microarrays, which were appropriately analyzed to identify the genes whose expression was significantly

modified. Differentially expressed genes were further validated by real-time PCR.
Results: Meph (25 mg/kg) induced significant ($P < 0.01$) positive conditioning (preference score: 152.30 ± 50.85 *vs.* -24.01 ± 26.69 from saline), which increased a 70% when associated with 0.75 mg/kg EtOH (269.89 ± 41.76, $P < 0.001$). This dose of EtOH alone did not induce any positive conditioning. Microarray analysis reported significant modifications in expression of the D_3 dopamine receptor gene (Drd3) and several genes related with neurotransmission and synaptic plasticity. Real-time PCR validation of Drd3 expression gave a similar increase (around 45%) for the three drug-treated groups. The D_3 antagonist SB-277011A was tested on CPP and Drd3 expression induced by the doses of Meph, EtOH mentioned above and their association. The treatment completely prevented all drug-induced conditioning, as well as the increases of Drd3 expression.
Conclusions: Alcohol, even at non-reinforcing doses, potentiates the reinforcing effects of mephedrone, implying that an increased risk of developing addiction could be expected, if translated to humans. Dopamine D_3 receptors play a key role in the establishment of these effects.
Work supported by Plan N[nal] sobre Drogas (2012I102), Generalitat de Catalunya (2009SGR977; 2009SGR118) and Plan N[nal] de Investigación Científica (SAF2011-23582).

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IG20, A NEW GLYCOLIPID, INCREASES THE MEMBRANE EXCITABILITY AND EXOCYTOSIS IN CHROMAFFIN CELLS

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Introduction: We have synthesized a new glycolipid, IG20, which increases oligodendroglial myelination to promote growth. In this context, we planned the present study to see if IG20 also affected the release of neurotransmitters by exocytosis.
Material/Methods: In primary cultures of bovine chromaffin cells we monitored the release of catecholamine with amperometry, the recording of cell excitability and ion channel currents with patch-clamp techniques, and the changes of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) with fluo-4- based microfluorimetry.
Results: IG20 had the following effects: 1) Increased secretion of catecholamine induced by K^+; 2) dramatic increase in basal secretion, which was blocked in the absence of extracellular Ca^{2+}; 3) increase in cytosolic Ca^{2+} concentration; 4) blockade of secretion by nifedipine; 5) Blockade of Ca^{2+} - dependent K^+ current (BK channels); 6) gradual depolarization of the plasma membrane and action potential firing.
Conclusions: This pharmacological profile suggests that the IG20 is inserted into the plasma membrane thereby causing the inhibition of BK channels and cell depolarization. In so doing, L- type (α1D, Cav1.3) voltage- activated Ca^{2+} channels open to let Ca^{2+} entry and the triggering of the exocytotic release of catecholamine. This suggests that the glycolipid IG20 may facilitate neurotransmission, plasticity and neuronal repair processes in brain injury and spinal cord.

NATURAL PRODUCTS

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BIOACTIVE PROPERTIES OF STEVIA ETHANOLIC EXTRACT: ANTIPROLIFERATIVE EFFECTS ON HELA CELLS AND ANTIOXIDANT ACTIVITY

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Introduction: *Stevia rebaudiana* (Asteraceae), commonly known as stevia, is a plant with an increasing use due to its sweetener capacity and healthy benefits in diabetes.
Objective: To evaluate the antiproliferative activity on cervical cancer cells (HeLa) as well as the antioxidant capacity and toxicity of stevia ethanolic extract.
Methods: The antiproliferative effect was measured through the MTT method on HeLa cells. The antioxidant capacity of the extract was evaluated through the DPPH and xanthine oxidase procedures comparing the activity of the extract with C vitamin. The toxicity was studied in the invertebrate *Artemia salina* (brine shrimp lethality test). All experiments were conducted in triplicates.
Results: Stevia showed antiproliferative activity in a dose dependent mode on HeLa cells in the range of 7.81–250 μg/ml after 72 h of exposure. Cell survival was significantly reduced up to 10% at the highest dose. We could also confirm that stevia ethanolic extract had antioxidant capacity in terms of scavenging DPPH and superoxide radicals, although activity was not higher than C vitamin. On the invertebrate model of toxicity, we observed that the plant had no significant effects since the viability of brine shrimp was 90% at the highest studied dose (200 μg/ml), which may confirm the safety of this plant.
Conclusions: We can conclude that stevia posseses antioxidant properties and the extract is capable of preventing the proliferation of human tumour cells showing the potential and benefits of this plant in health promotion.

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PHARMACOLOGICAL STUDY OF *JASONIA GLUTINOSA* IN SMOOTH MUSCLE OF RAT DUODENUM

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Introduction: *Jasonia glutinosa (L.)* DC. ("té de roca", rock tea) is a traditional medicinal plant, endemic from the Iberian Peninsula (Spain) and southern France. It is known for its traditional use as a digestive plant. However, the mechanism has not been elucidated yet.

Objective: The aim of this study is to examine the effects of *Jasonia glutinosa* extract on rat duodenum smooth muscle to evaluate their popular use as spasmolytic and their possible mechanism of action.
Material and Methods: The preparation of the extract was carried out with a Soxhlet apparatus using ethanol as solvent. Pieces of male rat duodenum were cut into segments and suspended in an organ bath. Each segment was connected to an isometric transducer and mechanical activities were analyzed. The extract was tested at concentrations of 0.5–5 mg/ml.
Results: Rock Tea extract relaxed the duodenum spontaneous contractions and contractions by high [K^+] with an EC_{50} of ≈2.3 mg/ml and 0.9 mg/ml, respectively. In segments incubated in a Ca^{2+}-free high-K^+ medium, the presence of the extract at 5 mg/ ml reduced the maximum response to reintroduction of Ca^{2+} by approximately 62%. Pre-incubation of Rock Tea extract inhibited the contractions induced by BayK-8644 (10^{-5} M), a L-type calcium activator. Verapamil (10^{-6} M), a Ca^{2+}-channel blocker, had similar effects in all experiments
Conclusion: Relaxant effect of *Jasonia glutinosa* in duodenum may be produced by inhibition of L-type Ca2 + -channels and the blocking of the increase of intracellular Ca2 + . These actions suggest that this plant may prevent a wide range of digestive disorders.

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ANTIHYPERTENSIVE AND DIURETIC EFFECTS OF A *FRAXINUS EXCELSIOR L.* SEED EXTRACT ON SPONTANEOUSLY HYPERTENSIVE RATS

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Introduction: We investigate the effect of a *Fraxinus excelsior L.* seed extract (FESE) on spontaneously hypertensive rats (SHR), and we try to state the diuretic effect of this extract.
Methods: Male 17–20 week-old SHR were used. Water (negative control), 50 mg/kg Captopril (an antihypertensive drug), 10 mg/kg Furosemide (a classic loop diuretic), 1 mg/kg Torasemide (a potent loop diuretic) or 20 mg/kg FESE were intragastrically administered. Arterial blood pressure was measured by the tail cuff method before and 4 hours after the different administrations. The same products were additionally administered to 18 hour fasted SHR, and urine was collected for 4 hours after the different administrations to establish the urine volumetric excretion (UVE). Moreover, plasma samples were used to establish the corresponding fractional excretion (FE) of different ions (Na^+, Cl^-, K^+, Ca^{2+} and PO_4^{3-}) and metabolites (creatinine, urea and uric acid).
Results: FESE shows antihypertensive effect in the SHR. The decrease in arterial blood pressure caused by this extract was slightly lower than that of Captopril, but more accentuated than that of Torasemide. No differences were observed in the renal function of the different groups of rats. FRP and Torasemide significantly increased UVE. Torasemide significantly increased FE(Na^+) and FE(Cl^-), and showed a clear uricosuric effect. FESE showed slight natriuretic effects and significantly decreased FE(K^+).
Conclusions: We have proven the antihypertensive effect of FESE in SHR and we have demonstrated that this extract behaves as a potassium-sparing diuretic in these animals.
Supported by a CENIT Project (CEN-20091006).

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INHIBITORY EFFECT OF NARINGENIN AND NARINGIN ON IL-6 AND IL-8 RELEASE

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Introduction: Flavonoids are used as phlebotonics products that improve vascular permeability and venous deficiency but their mechanism of action are still unknown. We had previously demonstrated that Naringenin and Naringin exerted an inhibition of the IL-1β production with slights differences between them. The aim of this study was to evaluate the possible involvement of these flavonoids on the other pro-inflammatory cytokines release like TNF-α, IL-6, and IL-8.
Material/Methods: Whole blood aliquots were incubated and then, lipopolysaccharide (LPS) was added to all of them. Samples were centrifuged and each supernatant was collected to be tested. TNF-α, IL-6, and IL-8 released from monocytes were measured with specific immunoassay techniques.
Results: LPS (10 mg/ml) induced a significant increase on the TNF-α, IL-6 and IL-8 release compared to control values. This production was inhibited by Naringenin and Naringin at 0.5 mM, 1, and 2 mM concentrations. TNF-α inhibition average with Naringenin was 14.9%±2.53, 27.17 ± 1.89, 48.63 ± 3.18, and with Naringin was 28.71 ± 2.05, 50.57 ± 3.81, 66.74 ± 3.99 respectively. IL-6 inhibition averaged with Naringenin was 32.47 ± 1.96, 47.49 ± 2.87, 64.76 ± 3.34, and with Naringin was 30.03 ± 2.52, 42.95 ± 1.85, 51.05 ± 3.25 respectively. IL-8 inhibition averaged with Naringenin was 29.79 ± 2.96, 39.69 ± 3.87, 54.82 ± 4.34, and with Naringin was 32.11 ± 3.62, 40.33 ± 4.03, 57.04 ± 3.69, respectively.
Conclusions: Both flavonoids were able to inhibit the pro-inflammatory cytokines assayed in a dose-dependent way, suggesting that this mechanism could be correlated with the therapeutic use of these drugs.

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ANTI-PROLIFERATIVE AND CYTOTOXIC EFFECTS OF GRAPE POMACE AND GRAPE SEED EXTRACTS ON COLORECTAL CANCER CELL LINES

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Grape pomace is the main by-product from wineries and a promising source of bioactive compounds including anthocyanins, flavonols, flavan-3-ols and hydroxycinnamic acids. Some of these compounds exhibit antiproliferative actions on cell cultures (Amico et al. Nat. Prod. Commun. 4, 27–34, 2009). Based on these antecedents, we have investigated the antitumoral effects of grape pomace and grape seed extracts on colon cancer cells (Caco2, HT29) and fibroblasts (CRL2072), which were used to check specificity. Crude (c) extracts were prepared from white pomace (cW), red pomace (cR) and grape seeds (cG), their anti-proliferative and cytotoxic actions being studied with a battery of tests (Neutral Red, MTT, Alamar Blue and Lactate Dehydrogenase, LDH) and by direct morphological evaluation.
The three extracts reduced the viability and proliferation of Caco2 cells, cGs being the most effective and specific. HT29 cells were resistant to these actions. Purified extracts (p) were then prepared from the same sources (pW, pR, pG) and compared with the LDH test; again, all three extracts were active and pG was the most potent and specific on Caco2 cells. HT29 were more sensitive to these purified extracts. The biological activity of pR resided almost exclusively on compounds

present in the phenolic subfraction, rather than the anthocyanin subfraction.
Preliminary results on the mechanisms involved in these effects revealed downregulation of Myc gene expression in Caco2 and HT29 cells. These results suggest a possible antitumoral effect of the extracts and recommend further fractioning to discover the active molecules. Supported by Junta de Comunidades de Castilla-La Mancha (POII10-0061-4432).

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T-RESVERATROL PROTECTS ENDOTHELIUM-DEPENDENT RELAXATION IN ISOLATED RAT AORTA ACUTELY EXPOSED TO HIGH GLUCOSE

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Introduction: *Trans*-resveratrol (*t*-RV) is a polyphenol found in plants that show beneficial effects on cardiovascular diseases and diabetes. The goal of this study was to assess whether *t*-RV protects endothelium-dependent relaxation impaired by high glucose (HG) (D-Glucose 25 mM) in isolated rat aorta.
Methods: Aortic rings obtained from male Sprague-Dawley rats were mounted in an organ bath and pretreated for 3 h with D-Glucose 5 mM, HG and HG plus *t*-RV (1, 10 and 100 μM). Afterward, rings were pre-contracted with phenylephrine (PE) (0.1 μM) and endothelium-dependent relaxation to cumulative addition of acetylcholine (ACh) was evaluated.
Results: Pre-incubation of rings with HG decreased dramatically ACh maximal response (Emax) from 87.69 ± 2.59% ($n = 6$) to 40.54 ± 1.78% ($n = 6$). The inhibitory effect of HG was significantly reverted, in a concentration-dependent fashion when rings were preincubated with *t*-RV, as shown Emax values to *t*-RV 1 μM (53.71 ± 1.82%, $n = 6$), 10 μM (67.06 ± 1.79%, $n = 6$) and 100 μM (72.95 ± 1.85%, $n = 6$), respectively. No statistical differences in ACh pD_2 values were observed.
Conclusion: Our results show strong evidences that *t*-RV protect ACh-induced endothelium-dependent relaxation in isolated rat aorta affected by acute exposure to HG.

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ESTIMATION OF TOTAL FLAVONOID CONTENT IN *SIDERITIS HYSSOPIFOLIA*

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Introduction: The therapeutic of effects flavonoids can be largely attributed to their antioxidant properties. The aim of this study was to determine the total flavonoid content of the ether, butanolic, methanol, water-butanol, chloroform and final aqueous extracts obtained from the aerial parts of *Sideritis hyssopifolia,* using aluminum chloride colorimetric method.
Material and Methods: An aliquot (500 μL) of extracts, dissolved in 80% ethanol, were mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm against a blank with a spectrophotometer. A calibration curve was prepared with quercetin (1–10 μg/mL).
Results: Total flavonoid content of extracts was expressed as milligram of quercetin equivalents per gram of extracts (mg QE /g extract). The values of total flavonoid content obtained for the different extracts were: ether extract.- 59.744 mg QE/g extract; butanolic extract.- 79.712 mg QE/g extract; methanol extract.- 39.776 mg QE/g extract; aqueous butanolic extract.- 12.780 mg QE/g extract; chloroform extract.- 58.466 mg QE/g extract and final aqueous extract.- 8.722 mg QE/g extract.
Conclusions: The results from this study indicate that the butanolic extract showed the highest total flavonoid content, while the lowest was found in final aqueous extract.

[Correction added on 12 September 2014: *IBIOMED* has been added to the authors' affiliation details.]

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EFFECTS OF AN EGG WHITE HYDROLYSATE IN DIFFERENT ANTHROPOMETRIC AND CARDIOVASCULAR ALTERATIONS IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME INDUCED BY DIET

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Introduction: A dietary intervention, particularly with bioactive peptides, maybe an interesting option for the treatment of alterations associated to the metabolic syndrome (MS). The aim of this study was evaluate the beneficial effects of an egg white protein hydrolysate (EWH) in different anthropometric and cardiovascular alterations in an experimental model of MS induced by diet.
Material/Methods: Male Wistar rats were randomized in different experimental groups: Control group (standard diet + drinking water), MS group (high-carbohydrate, high-fat diet + 25% dextrose in drinking water), MS + EWH (high-carbohydrate, high-fat diet + 25% dextrose in drinking water with EWH) and control + EWH group (standard diet + drinking water with EWH). Experimental groups lasted 20 weeks and the EWH treatment (1 g/kg/day) was included during the last 12 weeks of the experimental period. Body weight, anthropometric parameters and solid and liquid intakes were weekly measured. At the end of the experimental protocol different cardiovascular parameters were evaluated: direct blood pressure and heart rate, cardiac function and aorta and mesenteric functionality.
Results: MS group had higher body weights and anthropometric parameters (that correspond to overweight) compared with those obtained in the control group. In relation of the cardiovascular parameters evaluated, only an increase in aorta and mesenteric bed contractile functions were founded in MS group. EWH diminished the anthropometric parameters in animals with MS, but it did not affect the observed alterations in vascular reactivity.
Conclusions: EWH could correct anthropometric parameters but not the vascular alterations associated to MS.

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OXIDATIVE STRESS AND INFLAMMATORY MARKET AFTER ORAL TREATMENT OF DIFFERENT DOSE OF HYDROXYTYROSOL

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Aim: To evaluate the effect of the oral administration of HT to two-month evolution diabetic rats on some biomarkers of vascular inflammation.

Material and Methods: The study was carried out on two-month evolution diabetic rats induced with streptozotocine. Seven groups of rats ($N = 12$ rats per group) were constituted: non diabetic rats (NDR), diabetic rats without treatment with HT (DR) and diabetic rats treated from the first day of diabetes with HT (0.5, 1, 2.5, 5 and 10 mg/kg/day p.o). At the end of the experimental follow up, some variables were determined: plasma concentration of oxidized LDL (oxLDL), malondialdehyde (MDA), reduced and oxidized glutathione (GSH and GSSG), VCAM-1 and myeloperoxidase (MPO).
Results: Plasma MDA was higher in DR than in NDR (0.27 ± 0.03 *vs* 0.6 ± 0.05 nmol/mg prot, $P < 0.0001$); HT reduced MDA concentration with all the doses ($P < 0.0001$). Both GSH and GSSG were not modified with HT administration ($P < 0.1$). oxLDL concentration was higher in DR than in NDR (13.1 ± 1.3 *vs* 21.8 ± 2.1 ng/mL, $P < 0.0001$); HT reduced oxLDL concentration with all the doses ($P < 0.0001$). VCAM-1 concentration was higher in DR than in NDR (0.3 ± 0.03 *vs* 1.1 ± 0.09 ng/mL, $P < 0.0001$); HT reduced VCAM-1 concentration with all the doses (45-55% inhibition $P < 0.0001$). MPO concentration was higher in DR than in NDR (4.1 ± 0.2 vs 12.3 ± 1.2 ng/mL, $P < 0.0001$); HT reduced MPO concentration with all the doses (43–74% inhibition $P < 0.0001$).
Conclusions: hydroxytyrosol reduced oxidative stress and immflamatory biomarkers in this experimental model.

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EFFECT OF NITRODERIVATIVES OF HYDROXYTYROSOL ON OXIDATIVE STATUS AND CATECHOL-*O*-METHYL TRANSFERASE ACTIVITY IN RAT′S TISSUE

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Objective: To evaluate the antioxidant activity of nitrohydroxytyrosol (NO-HTy), nitrohydroxytyrosyl-acetate (NO-HTy-A) and ethyl nitrohydroxytyrosyl-ether (NO-HTy-E) after chemical damage in rat brain slices as well as the inhibition of catechol-*O*-methyl transferase (COMT) activity with a putative effect against Parkinson disease.
Materials and Methods: The protective effects of nitroderivatives against oxidative stress were studied *in vitro* in hippocampus rat brain slices by induction of *tert*-butylhydroperoxide (t-BOOH) and ferrous salts. Moreover, the inhibition of COMT activity by HTy-nitroderivatives, free hydroxytyrosol and the commercial inhibitor RO-410690 was assessed through *ex vivo* study by intraperitoneal administration in rats with a single dose of 20 mg/Kg for five days and later measurement of intracellular dopamine and its metabolite levels in the corpus striatum by HPLC with electrochemical detection.
Results: The results indicated that oxidative variables were significantly changed in comparison to control samples after chemical damage. Treatment with the nitroderivatives and their precursors produced a decrease of ROS generation (with a maximum inhibition of 66% respect to induced control samples for NO-HTy-A) and malondialdehyde (MDA) (with a maximum inhibition of 73% respect to induced control samples for NO-HTy-E). Data also showed that intraperitoneal systemic administration produced a clear and statistically significant increase in the intracellular levels of dopamine and its metabolite, 3,4-dihydroxyphenylacetic acid (increase up to 50% respect to control samples).
Conclusions: The new nitroderivative compounds, synthesized from natural olive oil phenol, hydroxytyrosol, show protective activity against oxidative stress as well as remarkable activity in the DOPAC and DA metabolism suggesting a putative effect against Parkinson disease.

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EFFECTS OF ANTHOCYANIDINS ON ASTROCYTES OVER OXIDATION MARKERS AND CELL INJURY INDUCED BY MPTP NEUROTOXIN

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Searching for natural antioxidant products, anthocyanidins are poliphenolic compounds included in flavonoid group that previously showed antioxidant and neuroprotective effects in *in vitro* and *in vivo* models. These compounds can be found in several foods and medicinal plants as active substances.
The objective of this work is to evaluate the antioxidant effect of pelargonidin, malvidin and cyanidin at different concentrations (5, 25 y 100 μM) over some oxidation′s markers. The human astrocyte glioblastoma, cellular line U373, was used for a cellular injure model using Fenton′s reagent ($FeSO_4$ 0,5 mM/ H_2O_2 1 mM)) to measure glutation levels (GSH), and to evaluate nitrite release induced by interferón-γ (IFN- γ). Moreover we have evaluated the possible protective effect of these compounds over cell viability (MTT) on 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) injure. MPTP is a neurotoxin precursor to MPP+, which causes permanent symptoms of Parkinson′s disease.
Anthocyanidins could reduce significantly GSH depletion induced by Fenton′s reagent at different concentrations (C5, M5, M25, P5 and P100). Therefore, malvidin and pelargonidin seemed to have best results than cyanidin. In the presence of IFN-γ all anthocyanidins reduced significantly nitrite levels, noting that every compound at studied concentrations did not show significantly differences with untreated cells.
Moreover, anthocyanidins seemed to reduce partially the Parkinson′s like effect induced by MPTP. All compounds, exhibited significantly higher cell viability than cells treated with MPTP alone.
These results suggest that anthocyanidins could contribute to antioxidant and neuroprotective effects of natural drugs and foods in which they are present.

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ANTI-INFLAMMATORY EFFECTS OF MITRAPHYLLINE ON MACROPHAGE ACTIVATION: SKEW TOWARD M2 PHENOTYPE

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Introduction: Mitraphylline is the major pentacyclic oxindolic alkaloid presented in Cat′s Claw, widely used in the Peruvian Amazon to alleviate inflammation. Nowadays, there is no scientific data that prove this anti-inflammatory effect. One possible action is attributed to Mitraphylline by the inhibition of NF-κB pathway, which modulates several pro-inflammatory cytokines, such as TNF-α. TNF-α is strongly involved on macrophages activation in inflammatory processes. M1 or classically activated macrophages have anti-microbial and cytotoxic properties, therefore M2 or alternative activated are anti-inflammatory and reparative macrophages. Macrophage polarization plasticity has important therapeutic implications.
Material/Methods: The purpose of this study is to characterize the anti-inflammatory properties and the inmunomodulatory effect of Mitraphylline, on M1/M2 polarization macrophage subsets. In vitro studies with primary human monocyte-derived macrophages were performed at different doses of Mitraphylline and at different time points. We evaluated the RNAm expression of different pro-inflammatory cytokines, protein production and secretion of several markers involved on M1/M2 macrophage polarization.

Results: Mitraphylline shifted inflammatory M1 to anti-inflammatory M2 macrophages, being shown by inhibiting NF-κB, iNOS, IL-6, TNF-α (−36%, −40%, −25%, −63%; $P < 0.001$) and increasing CD200r, Arg-1, IL-10 (+18%, +31%, +15%; $P < 0.05$). By using Western blot, iNOS and Arg-1, as two most specific markers for M1 and M2, was inhibited or induced in those cells. The polarization of M2 macrophages was associated with the decrease of inflammatory cytokine IL-1β, TNF-α and MCP-1 (−23%, −51%, −11%; $P < 0.05$).
Conclusion: Our results demonstrate that Mitraphylline has a therapeutic potential, possibly by inducing the polarization toward M2 macrophages and inhibiting inflammatory responses.

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PHOTOPROTECTIVE EFFECT OF GLYCOSYLGLYCEROLS AND GLYCOLIPIDS FROM MICROALGAE IN HACAT KERATINOCYTES

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Background: Chronic exposure of the skin to ultraviolet B (UVB) radiation induces reactive oxygen species (ROS) production, which play a crucial role in the induction of skin cancer. Therefore, strategies for skin protection comprise the use of natural products to counteract oxidative stress. In this line, some compounds isolated from marine microalgae have shown to have antioxidant, anti-inflammatory, and anti-tumor properties in different experimental models.
Objective: This study was designed to assess the photoprotective effects of glycosylglycerols and glycolipids from Cyanophycea and Micractinium sp. on UVB-mediated responses in human immortalized HaCaT keratinocytes.
Methods: HaCaT cells were treated with different compounds such as glucosylglycerol (GG) and glucosylglycerol acetylated (ITC23.5), and the glycolipids (GL) sulfoquinovosyldiacylglycerol (ITC23.12), and digalactosyldiacylglycerol (IPT10A2) derivatives, at 30 or 50 μg/ml for 1 hour prior to UVB irradiation (50 mJ/cm^2). Cell viability was measured by LDH assay. We also assessed the intracelular ROS production by measuring the intensity of the oxidation-sensitive fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA).
Results: LDH assay indicated that UVB irradiation strongly increased cell death and LDH levels. Pretreatment of HaCaT cells with GG and GL, especially at the dose of 30 μg/ml, markedly reduced LDH enzyme activity, compared with only UVB-exposed cells (GG: 44%, ITC23.5: 36%, ITC23.12: 28% and IPT10A2: 40%). In addition, UVB-induced ROS intracellular levels were also reduced after pretreatment with these compounds, at all the doses assayed.
Conclusions: The results suggest that the microalgae extract have a protective effect on the UVB-injured keratinocytes by increasing cell viability, and by inhibiting intracelular ROS accumulation.
This work was supported by ALGALIMENTO Project, IPT-2011-1370-060000, "Development of a production chain and hypersaline marine microalgae and derived products market oriented food", Ministry of Economy and Competitiveness. Project INNPACTO 2011.

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DETERMINATION OF POLYPHENOL CONTENTS IN TEA AND HERBAL INFUSIONS

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Introduction: Tea and herbal infusions are some of the most commonly consumed beverages all over the world, and one of the major source of phenolic compounds in our diet. The aim of the present study was to determine and compare the content of total polyphenols in several herbal and tea infusions available in retail trade.
Material and methods: Samples of six different herbal and tea infusion brands were analyzed by using a Folin-Ciocalteu colorimetric assay. Briefly, an infusion (two samples each) with 200 mL boiling water was prepared (remaining 10 min at 90°C). Absorbance was determined at 765 nm. Total polyphenolic content was expressed as GAE (Gallic Acid Equivalent)/g dry sample.
Results: Among the infusions examined, green tea exhibited the highest polyphenol content (146.80 mg GAE/g), whereas infusions from linden/orange tree leaves contained the lowest concentrations (30.36 mg GAE/g). For other infusions (pu-erh tea, tea with cinnamon, thyme or sage herbal infusions), values were comparatively lower, and varied from 56.60 to 74.37 mg GAE/g.
Conclusions: Infusions studied are a valuable source of phenolic compounds and have a high potential antioxidant activity, especially green tea.

[Correction added on 12 September 2014: This abstract was moved from Page 57 to Page 48. *IBIOMED* has been added to the authors' affiliation details.]

MOLECULAR PHARMACOLOGY

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INVESTIGATION OF THE DIMERIZATION INTERFACE OF THE SEROTONIN 5-HT$_{2A}$ RECEPTOR BY COMPUTATIONAL AND EXPERIMENTAL APPROACHES

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Dimerization of G protein-coupled receptors (GPCRs) regulates many aspects of GPCR function. Several studies point to different domains taking part in the dimer interface of class A GPCRs, including transmembrane domains (TM) 4/5, TM5/TM6, and TM1/TM2/helix 8. We have previously reported homodimerization of 5-HT$_{2A}$ receptors (Brea et al., 2009), a GPCR targeted by antipsychotics and other drugs. Here we investigate the 5-HT$_{2A}$ receptor dimerization interface by a combination of computational and experimental approaches.

Our computational modeling study identified a number of amino acids in TM regions of the human 5-HT$_{2A}$ receptor possibly involved in dimerization contacts. Eight selected amino acids were mutated and different mutant receptor constructs were generated fused to bioluminescence or fluorescence resonance energy transfer (BRET, FRET) donors (*Renilla luciferase* (Rluc), cerulean) or acceptors (YFP, citrine). The expression of the different receptor mutants was quantified by luminescence measurements, and their ability to bind selective ligands was assessed by radioligand binding of [^{3}H]-ketanserin. BRET assays were established for the assessment of dimerization.

The expression levels of the receptor mutants ranged from 58% to 196% of wild type, adequate for their use in RET assays. BRET experiments revealed citrine as a suitable acceptor partner for Rluc/coelenterazine *h* luminescence as previously shown for YFP (BRET$_{max}$ = 817.7 and 760.4 mili netBRET units, BRET$_{50}$ = 1.14 and 0.71, for Rluc/YFP and Rluc/citrine wild type receptor pairs, respectively). To date, our results indicate that the mutant receptors and constructs generated will be useful tools for the elucidation of 5-HT$_{2A}$ receptor dimer interfaces.

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AGE-RELATED CHANGES IN THE INHIBITION OF SUPEROXIDE ANION LEVELS FROM PERITONEAL LEUKOCYTES BY ALLOPURINOL AND APOCYNIN

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Reactive oxygen species (ROS), especially those produced by phagocytic cells, play an important role in the oxidative stress associated with aging. Despite of allopurinol (xanthine-oxidase (XO) inhibitor) and apocynin (NADPH-oxidase inhibitor) are mainly used in vitro to block ROS generation, its efficiency has been scarcely studied in aging. The aim of this study was to quantify the inhibitory effects of these compounds on the generation of superoxide anion ($O_2^{\cdot-}$) in peritoneal leukocytes from different age groups of mice. Adult, mature, old and long-lived (6, 13, 18 and 30 months-old, respectively), ICR-CD1 female mice were used.

Peritoneal leukocytes suspensions were extracted and the levels of superoxide anion ($O_2^{\cdot-}$) in basal conditions and in the presence of allopurinol (100 μM) or apocynin (100 μM) were assessed in both total peritoneal leukocytes and isolated macrophages. In basal conditions mature and old mice showed a significant increase of the $O_2^{\cdot-}$ levels in macrophages and total peritoneal leukocytes with respect to adult mice, whereas long-lived mice showed similar levels to those in adults. Allopurinol decreased significantly the $O_2^{\cdot-}$ levels in all groups, showing similar inhibition percentage in macrophages and total population of leukocytes.

However, apocynin inhibition was higher in macrophages than in total peritoneal leukocytes in adult, mature and long-lived mice. In conclusion, these different effects of allopurinol and apocynin must be taken into account in the ROS inhibition studies, since there are cell type and age-related differences in the involvement of XO and NADPH-oxidase in the generation of $O_2^{\cdot-}$.

Support: MINECO-(BFU2011-30336), RETICEF-(RD12/0043/0018)-ISCIII-FEDER(UE), UCM-Research-Group-(910379ENEROINN).

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T_{EM} LYMPHOCYTES ARE THE SPECIFIC TARGET OF THE SCORPION TOXIN VM24

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Introduction: In autoimmune diseases such as multiple sclerosis, autoreactive T cells exhibit a T_{EM} (effector memory) phenotype. For T_{EM} lymphocytes activation, proliferation, migration and cytokine secretion calcium signaling is essential, a process regulated through Kv1.3 potassium channels. As the peptidic toxin Vm24 isolated from the Mexican scorpion *Vaejovis mexicanus smithi* is a potent and selective blocker of Kv1.3 channels, we propose that it is a good candidate for the treatment of autoimmune diseases.

Objective: Evaluate the immunomodulatory potential of the Vm24 toxin on human purified CD4$^+$ T_{EM} lymphocytes.

Material/Methods: CD4$^+$ T_{EM} lymphocytes (CD4$^+$, CD45RO$^+$, CCR7$^-$) were purified (>95% purity) from peripheral venous blood of healthy volunteers by negative magnetic cell sorting. TCR-specific cell stimulation was performed with plate-bound OKT3 monoclonal antibody (2 μg/cm^2) in the presence or absence of Vm24 toxin (1 nM). Activation (CD25 expression) of the cells was assessed at 24 hours and proliferation (CFSE dilution) after 96 hours of stimulation by flow cytometry.

Results: Data obtained indicated that incubating the cells in the presence of Vm24 toxin (1 nM) resulted in a 40% inhibition in CD25 expression, as well as a 30% inhibition in proliferation, and that this effect was dose-response dependent.

Conclusions: Kv1.3 channel blockade by Vm24 toxin in purified CD4$^+$ T_{EM} cells decreases activation and proliferation, highlighting a potential use for this peptide as a selective immunomodulator for these lymphoid cells.

Acknowledgements: Partially financed by grant SEP-CONACyT 153496. JIVB is a recipient of a CONACyT schorlarship CVU 515949.

HORMONAL PHARMACOLOGY AND METABOLISM

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PPARβ/δ REGULATES FGF21 IN HEPATOCYTES

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Introduction: Fibroblast growth factor (FGF) 21 is a novel hormone that has profound effects on metabolic parameters such as glucose and lipid homeostasis and represents a promising potential therapy for the treatment of type 2 diabetes. FGF21 is primarily produced by the liver, where its expression is partially under the control of PPARα and its endogenous ligands such as fatty acids. However, little is known about the role of PPARβ/δ on FGF21 regulation.
Material/Methods: Studies were conducted in liver from wild-type and PPARβ/δ-deficient mice and in primary culture of hepatocytes.
Results: PPARβ/δ-deficient mice showed 3-fold increase ($P < 0.001$) in hepatic FGF21 mRNA levels and in plasma FGF21 levels ($P < 0.01$) compared to wild type mice. In agreement with this, GLUT1 expression was significantly increased ($P < 0.05$) in adipose tissue. No changes were observed neither in plasma free fatty acids, nor in hepatic expression of PPARα-target genes (acyl-CoA oxidase or medium chain acyl-CoA dehydrogenase) nor in ATF-4 expression, suggesting that they were not involved in the increase in FGF21. Interestingly, PPARβ/δ-deficient mice exhibited reduced hepatic protein levels of PGC-1α and Rev-Erbα, a condition previously associated with increased FGF21 levels, suggesting that this mechanism could be responsible for the increased levels of this hormone. Treatment of a mouse primary culture of hepatocytes with the PPARβ/δ agonist GW501516 also increased FGF21 expression, which was associated with increased expression of hydroxymethylglutaryl-CoA synthase 2, reported to upregulate the expression of this gene.
Conclusions: Overall, our findings suggest that modulation of PPARβ/δ activity in hepatocytes regulates FGF21 expression through different mechanisms.
Acknowledgements: This work was supported by funds from Spain′s Ministerio de Economía y Competitividad (SAF2012-30708).

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DELETION OF THE INSULIN RECEPTOR SUBSTRATE 2 (IRS2) MODIFIES THE ACTIONS OF DOPAMINE IN MOUSE ADIPOCYTES

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Introduction and Objectives: Disruption of Irs2 in mice promotes the progression to type 2 diabetes mellitus, because of insulin resistance in peripheral tissues and significant reduction in pancreatic ß-cell mass. This diabetic model displays gender dimorphism, related to life expectancy and metabolic profile. Moreover, dopamine has pleiotropic actions as neurotransmitter or hormone. Insofar that the biogenic amine regulates endocrine functions such as pancreatic insulin release and some hormonal actions on adipocytes, also plays a role in the regulation of glucose homeostasis.
Our aim was to compare the effects of dopamine on lipolysis of white adipose tissue (WAT) in wild type and Irs2 knockout (KO) mice in order to elucidate the pathophysiology of metabolic syndrome and related disorders.
Material/Methods: WT and $Irs2^{-/-}$ mice of both sexes were used to evaluate the effects of dopamine (10^{-9}–10^{-5} M) on lipolytic activity, measured by the glycerol release (basal and stimulated) from visceral adipocytes. Results are expressed as mean±s.e.m. and were considered to be statistically significant if $P < 0.05$ after analyzing with the GraphPad software.
Results: As previously described, there were significant differences between WT and $Irs2^{-/-}$ animals in basal lipolytic activity ($P < 0.01$ for male and $P < 0.05$ in female mice). In both sexes, on comparing glycerol release of WT mice with their corresponding KO groups the latter exhibited a significant decrease in the area under the concentration-response curve (expressed in arbitrary units) for dopamine.
Conclusions: Corroborating preceding observations, the reduced response to dopamine in the $Irs2^{-/-}$ mice suggests a dysfunctional adrenergic signaling in this diabetic model.

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STUDY OF POTENTIAL BIOMARKERS AND TARGETS IN HUMAN MORBID OBESITY: PLEIOTROPHIN AND MIDKINE

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Pleiotrophin (PTN) and midkine (MK) are two closely related cytokines that have been proposed to be involved in different human pathologies, including obesity and related comorbidities. PTN is one out of 11 proteins overexpressed in the human omental adipose tissue relative to subcutaneous adipose tissue (Hoggard et al., Obesity 6: 1158-1167, 2012), while serum MK levels have been found elevated in overweight/obese subjects and correlated with body mass index (Fan et al., PLoS One 9: e88299, 2014).On the basis of these findings we have followed the evolution of serum levels of both cytokines in a group of morbid obese patients undergoing bariatric surgery ($n = 23$) and their matched normoweight controls by using ELISA kits. Concerning PTN, the data obtained with two kits of different vendors (Cusabio, MyBioSource) were highly variable and inconsistent, which prevented the obtention of reliable results. MK levels (determined with a validated kit provided by Cellmid, Australia) were similar in controls and patients before surgery, but were significantly increased (56%) one year after bariatric surgery, when patients exhibited a great improvement of many clinical, metabolic and hormonal parameters. Stratification of the patients on the basis of pre-existent food craving did not reveal any difference of MK levels between subtypes of obesity. The significance of the association between MK elevations and surgery remains to be established, however this result strongly suggests that MK could be envisaged as a biomarker of treatment outcome and/or a potential target in morbid obesity.
Supported by Instituto de Salud Carlos III (PI10/00440).

GASTROINTESTINAL/RESPIRATORY PHARMACOLOGY

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RADIOGRAPHICAL ANALYSIS OF MORPHINE EFFECT ON GASTROINTESTINAL MOTOR FUNCTION IN CONSCIOUS RATS

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Introduction: Opioids are commonly used for the treatment of moderate to severe pain. However, several side effects may occur upon opioid administration, including nausea, emesis and constipation. Morphine-induced nausea and vomiting is associated with gastric dysmotility. In rodents, which do not vomit, X-rays allow the non-invasive evaluation of drug-induced alterations in gastrointestinal motility, but the effects of opioids, including morphine, have not been characterized so far.
Aim: To determine, using radiographical methods, the alterations in gastrointestinal motility induced by acute morphine in conscious rats.
Methods: Male Wistar rats received morphine (1.25, 5 or 10 mg/kg, ip) or saline. Thirty min later, barium sulfate (Barigraf®, 2.5 mL, 2 g/mL in water) was administered per os. X-rays were obtained (60 kV, 7 mA; exposure time: 0.06 s) at different time-points after contrast and were semi-quantitatively and morphometrically analyzed as previously described (Abalo et al., 2013).
Results: Morphine dose-dependently delayed motor function in all gastrointestinal regions. This effect was more pronounced in the large intestine, whereas in the stomach, only the highest dose tested (10 mg/kg) consistently delayed motor function.
Conclusions: Morphine depresses gastrointestinal motor function in conscious rats. Gastric dysmotility associated to more intensive treatment may contribute to the emetic effects of morphine in humans. X-rays are useful to evaluate the effect of drugs on gastrointestinal motor function.
Acknowledgements: Supported by: SAF2012-40075-C02-01. Pharmacology and Nutrition is Unidad Asociada I+D+I, at Consejo Superior de Investigaciones Científicas (CSIC).

ONCOLOGIC

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CELL KINETIC IN PROXIMAL COLON: DOSE-RESPONSE EFFECTS OF DIETS SUPPLEMENTED WITH 0.7% OR 7% *PLANTAGO OVATA* HUSK IN HEALTHY MICE AND IN THE EARLY STAGES OF COLORECTAL CARCINOGENESIS

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Introduction: *Plantago ovata* husk is a soluble fermentable fiber whose intake is frequent in humans as it is used in the prevention and the treatment of digestive diseases. However, the role of the fiber in diet is still being investigated with regard to its dosage. Our aim is to assess the dose-response effects of diets supplemented with 0.7% (human equivalent dose) or 7% of *Plantago ovata* on cell proliferation in proximal colon (PC) in healthy mice and in early stages of colorectal carcinogenesis.
Material/Methods: ICR:CD1 female mice which consumed a standard control diet or diets supplemented with 0.7% or 7% *Plantago ovata* husk for 9 weeks were exposed to protocol of carcinogenesis with azoxymethane (AOM) (10 mg/Kg body weight for 6 weeks). AOM non-treated mice were considered to be healthy. In week 2 after the last carcinogen injection, AOM exposed and non-exposed mice were euthanized. Tissue sections of PC were immunostained with antibody anti-Ki67 (proliferation marker). Intestinal crypt length as well as epithelial cells and Ki67-positive nuclei number per crypt were recorded using an image analysis system. Statistical study was carried out using SPSS 19.0®.
Results: In healthy mice, both doses of fiber showed a significant stimulatory effect on cell proliferation but only in caudal region of PC when compared to animals fed with standard diet. However, the human equivalent dose only induced a significant increase of the mean number of Ki67-positive cells in the basal region (proliferation compartment) of intestinal crypts whereas the highest dose also significantly increased the proliferating cells in the upper region (mainly maturation compartment) of crypts. In the early stages of colorectal carcinogenesis, 7% of fiber showed a significant inhibitory effect on cell proliferation in cranial region of PC when compared to AOM action. This fiber high dose also decreased the mean number of Ki67-positive cells in the caudal region of PC, but significant differences were not demonstrated. The supplement with 0.7% of fiber only significantly increased the mean number of epithelial cells but only in the normal proliferation compartment of crypts in caudal region of PC.
Conclusions: These results suggest that the effects on the cell kinetic of a diet supplemented with *Plantago ovata* depend on the dose and the location in proximal colon. This is an interesting finding when animal models are used to investigate the effects of a dietary fiber on digestive diseases.

[Correction added on 12 September 2014: *IBIOMED* has been added to the authors' affiliation details.]

PHARMACOGENETICS AND PHARMACOGENOMICS

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EFFECT OF POLYMORPHISMS ON THE PHARMACOKINETICS, PHARMACODYNAMICS, AND SAFETY OF RISPERIDONE IN HEALTHY VOLUNTEERS

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Introduction: Risperidone is an atypical antipsychotic drug that is indicated for the acute and maintenance treatment of schizophrenia. We aimed to identify polymorphisms in *CYP2D6* capable of predicting the pharmacokinetics, pharmacodynamics, and adverse effects of risperidone.
Methods: Genotyping was performed in 70 healthy volunteers receiving a single 1-mg oral dose of risperidone. Risperidone and hydroxy-risperidone plasma levels were measured using high-performance liquid chromatography combined with tandem mass spectrometry. Prolactin concentration was quantified by direct chemiluminescence.
Results: Poor CYP2D6 metabolizers showed higher risperidone peak plasma concentration –C_{max}–, area under the curve –AUC–, and half life –$t_{1/2}$–, as well as lower clearance. They also showed lower C_{max} and AUC and higher $t_{1/2}$ for hydroxy-risperidone. Furthermore, individuals with a mutant *VKORC1* genotype had a lower risperidone AUC and $t_{1/2}$ and higher clearance. The hydroxyl-risperidone AUC was lower in individuals with the *COMT* mutant genotype. Risperidone increased prolactin levels (iAUC and iC_{max}), which were higher in women than in men. The most frequent reactions were somnolence (47.1%), headache (21.4%), and dizziness (17.1%). Women had neurological effects and headache more frequently than men. The incidence of headache was associated with polymorphisms in the *AGTR1* and *NAT2*; neurological effects were associated with *CYP2C19*. Both prolactin levels and adverse effects were not associated with *CYP2D6* polymorphisms.
Conclusions: Differences in the pharmacokinetics of risperidone are due to polymorphisms in *CYP2D6*, *COMT*, and *VKORC1*. Differences in adverse reactions can be explained by gender and polymorphisms in *CYP2C19*, *AGTR1*, and *NAT2*.

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RELATIONSHIP BETWEEN CYP2D6 GENOTYPE AND HALOPERIDOL PHARMACOKINETICS AND EXTRAPYRAMIDAL SYMPTOMS IN HEALTHY VOLUNTEERS

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Introduction: This study aimed to elucidate the relationship between *CYP2D6* genotype and haloperidol pharmacokinetics and induced extrapyramidal symptoms (EPSs).
Materials and methods: Twenty five healthy subjects were included in this randomized, placebo-controlled, single-dose (5 mg) crossover and doubleblind clinical trial, selected according to their *CYP2D6* genotype and classified as poor metabolizers ($n = 8$), extensive metabolizers ($n = 10$) and ultrarapid metabolizers ($n = 7$).
Results: We confirm that *CYP2D6* genotype partially determines haloperidol metabolism and the rate of EPSs measured as wakefulness activity by actigraphy. The best predictor of wakefulness activity was the model including haloperidol area under the plasma concentration–time curve, sex and tranquilization, which explained 48.3% of the total variance.
Conclusion: Other markers need to be identified in order to explain the observed variability of haloperidol response and to develop pharmacogenetic predictors of haloperidol-induced EPSs.
Pharmacogenomics (2013) 14(13), 1551–1563; amalialafuente@ub.edu

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PHARMACEUTICAL CARE AND PHARMACOGENETIC APPROACH FOR A BETTER UNDERSTANDING OF TACROLIMUS METABOLISM

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Pharmacogenetics and pharmaceutical care are raising fields that are becoming essential in the pharmaceutical area. We present the cases of two kidney-transplanted males treated with Tacrolimus as immunosuppressant therapy and with Omeprazole as gastrointestinal prevention. Both of them received the service 'medication review with follow up' which comprises the study of their pharmacotherapeutic needs as well as the development of a health care plan and monitoring tailored to the patient.
Patient 1 presented fluctuating Tacrolimus levels, gastrointestinal complications and muscular pain. Patient 2 had no problems related to the immunosuppressant therapy. They used other drugs such as Mycophenolic Acid, Prednisone and Acetaminophen. The main metabolic way of Tacrolimus is CYP3A5, and for Omeprazole is CYP2C19 (also metabolized by CYP3A5). Tacrolimus is transported by a glycoprotein P transporter encoded by ABCB1 gene. Differences in Tacrolimus plasmatic levels fluctuation could be based on differences in the genotype of those CYP.
Patient 1 carries a single nucleotide polymorphism (SNP) in CYP2C19 gene that has been associated with an increase in Omeprazole plasmatic levels and inhibition of glycoprotein P transporter. A competitive inhibition between both drugs for CYP3A5 could result in prominent changes in Tacrolimus plasmatic level with minimal changes in doses drug.
A change in antiacid therapy was proposed (substitution of Omeprazole for Rabeprazole), for which there is evidence of lower incidence in its metabolism profile. Tacrolimus plasmatic concentrations were immediately stabilized.
To determine the existence of a genetic change in those CYP; CYP3A5, CYP2C19 and ABCB1, samples were genotyped using a PCR-RFLP.

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GENETIC POLYMORPHISMS CAN PREDICT KNEE OSTEOARTHRITIS PROGRESSION. RESULTS FROM THE ARTHROTEST MULTICENTER ASSOCIATION STUDY

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Objective: To develop a genetic prognostic tool based on Single Nucleotide Polymorphisms (SNPs) for radiologic progression prediction in primary knee OA (KOA) patients.
Material/Methods: Cross-sectional, retrospective, multicentric(31 sites), association study in 595 Spanish KOA patients.Inclusion criteria: Caucasian patients aged ≥40 years, with two anteroposterior X-rays available and initial Kellgren-Lawrence grade 2 or 3.Patients who progressed to KL4 or were referred for total knee replacement in ≤8 years since the diagnosis were classified as progressors to severe disease.A unique expert viewer measured radiologic progression from all X-rays.774 SNPs were analyzed.SNP genotyping was performed with Illumina Golden gate or KASPar chemistry.Clinical variables of the initial disease stages (gender,BMI,age at diagnosis,OA in the contralateral knee and OA in other joints) were registered as potential predictors.Variables with an association of $P < 0.05$ were included on the multivariate analysis using forward logistic regression.
Results: Two hundred and eighty-two patients fulfilled DNA and X-ray quality control criteria. 23 SNPs and age at diagnosis were significantly associated to KOA severe progression in the exploratory cohort ($N = 220$; $P < 0.05$).The predictive accuracy of the clinical variable was limited (AUC=0.66).Combining only genetic variables a good accuracy was also obtained (AUC = 0.78).When genetic variables were added to the clinical model (full model-Arthrotest®) the predictive ability was significantly improved (AUC = 0.82) and confirmed on the replication cohort ($N = 62$; two-sample Z-test; $P = 0.190$).
Conclusions: An accurate prognostic tool to predict primary KOA progression has been developed based on genetic and clinical information.SNPs predict radiologic progression more accurately than clinical variables.This model could help clinicians optimize patients' therapeutic care and personalize disease management.

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ROLE OF *TAP1* AND *TAP2* POLYMORPHISMS IN AMOXICILLIN-CLAVULANATE HEPATOTOXICITY (AC DILI)

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Introduction: The transporter associated with antigen processing (TAP) is an important component of the immune system, translocating cytosolic peptides into the endoplasmic reticulum prior to the assembly of HLA-peptide complexes destined for the cell surface. The TAP transporter is a heterodimer consisting of TAP1 and TAP2. *TAP1* and *TAP2* polymorphisms have been associated with autoimmune diseases. Due to the demonstrated role of the immune system in AC DILI we aimed to analyse the role of selected *TAP* polymorphisms in Spanish AC DILI patients.
Material/Methods: Five *TAP1* (rs1057141, rs2127679, rs41550019, rs41561219, rs1135216) and 3 *TAP2* (rs1800454, rs241448, rs4148876) polymorphisms were genotyped in 104 Spanish AC DILI patients and 142 controls.
Results: No significant differences in genotype distribution or allele frequency between DILI patients and controls were found for any of the polymorphisms. Homozygotes for the major allele were most frequent in both groups: *TAP1* Ile393Val: AA (69% patients, 71% controls), Ala430Val: CC (94%, 94%), Val518Leu: CC (89%, 88%), Val578Ile: CC (92%, 93%), Asp697Gly: AA (70%, 73%) and *TAP2* Val379Ile: GG (77%, 76%), Gln687Stop: AA (56%, 55%), Arg651Cys: CC (92%, 90%). Due to low minor allele frequencies in the analysed polymorphisms only *TAP2* rs241448 reached sufficient statistical power (80%) to detect allelic differences with an odds ratio of 2.
Conclusions: Our findings do not support any associations between the selected *TAP1* and *TAP2* polymorphisms and enhanced risk of AC DILI development. Confirmation in a larger cohort is necessary due to low frequency of some of the alleles.
Financing: FIS PI12-00378, P10-CTS-6470, CIBERehd by ISCIII.

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TYK2: A POSSIBLE TARGET TO TREAT MODERATE-TO-SEVERE PSORIASIS

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Introduction: *TYK2* gene encodes a member of the Janus Kinase (JAK) family that promotes IL17 transcription and plays a main role in immune responses. Recently, a JAK inhibitor (tofacitinib) and two anti-IL17 agents (ixekizumab and secukinumab) have been developed as new alternatives to treat moderate-to-severe psoriasis resistant to ustekinumab and anti-TNF drugs. A single nucleotide polymorphism (SNP) in *TYK2* gene (rs12720356*A) has recently been associated with psoriasis, but impact of mutation is insufficiently evaluated.
Aims: To examine the association of rs12720356 (Ile684Ser) in *TYK2* in Caucasian patients with moderate-to-severe psoriasis compared to healthy controls.
Material/methods: We analyzed rs12720356 in 191 moderate-to-severe plaque psoriasis patients and 197 healthy controls. The technology employed was Ilumina Veracode genotyping platform. We tested Hardy-Weinberg equilibrium with SNPStat program. Univariate analysis was performed with R program 3.0.2. (SNPassoc package). The univariate results were adjusted by rs12191877 (SNP strongly associated with psoriasis, HLA-C*0602 allele, in previous studies). The optimal model was selected by the Akaike Information Criterion (AIC).
Results: The minor allele frequencies of rs12720356 (G allele) were 0.09 and 0.04 in controls and cases, respectively. The optimal model was additive (OR = 0.42; 95% CI = 0.23–0.78; $P = 0.010$). Results of adjusted by *HLA-C* univariate analysis show a relation between G allele carriers in rs12720356 in *TYK2* and protection to psoriasis (OR = 0.26; 95% CI = 0.13–0.54; $P = 0.00017$).
Conclusion: Our results show that *TYK2* is related to psoriasis. So, TYK2 could be a potential target to develop new drugs to treat moderate-to-severe psoriasis.

CLINICAL PHARMACOLOGY, SIDE EFFECTS AND TOXICOLOGY

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CT-1 PREVENTS GENTAMICIN-INDUCED ACUTE KIDNEY INJURY

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Introduction: Gentamicin (G) is a widely used aminoglycoside antibiotic whose most important side effect is its nephrotoxicity. Cardiotrophin-1 (CT-1) is a member of the IL-6 family of cytokines that seems to protect several organs such as the liver against toxic or ischemic damage. The purpose of this study was to assess the effect of recombinant human cardiotrophin-1 in the development of acute kidney injury (AKI) caused by G.
Material/Methods: The study was performed in male Wistar rats, divided in 4 experimental groups (6 animals/group): C) rats receiving saline solution for 6 days. G): rats receiving G (150 mg/kg/day, i.p.) for 6 days. CT-1): rats receiving CT-1 (100 μg/Kg/day i.v.) for 7 days. G +CT-1): rats receiving G and CT-1 for 6 days. In C and CT- 1 groups, plasma creatinine concentration was not modified.
Results: In G group, there was an increase in plasma creatinine and urea, a decrease in creatinine clearance, and a significant increase in urinary excretion of AKI markers such as N-acetyl -glucosaminidase (NAG), Kidney Injury Molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL). G+CT-1 group showed significantly lower plasma urea and creatinine levels, higher creatinine clearance and lower excretion of NAG, NGAL, and KIM-1 than G group. Histological renal damage was less severe in G+CT-1 group that in G group.
Conclusions: In conclusion, administration of CT-1 together with G prevents most of the AKI symptoms induced by G. The results of this study have potential clinical application as CT-1 is near of being used as a drug.

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URYNARY REGIIIβ: A DIFFERENTIALLY MARKER OF ACUTE KIDNEY INJURY INDUCED BY GENTAMICIN

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Introduction: Nephrotoxicity poses a considerable health and economic problem worldwide. Overall, it is estimated that nephrotoxicity is responsible for 10-20% of the acute renal failure cases. A critical aspect for the optimal clinical handling of AKI is an early diagnosis. Important progress has been made in the last decade towards an increasingly earlier detection based on novel and more sensitive urinary markers. However, AKI diagnosis may still be improved in an individual-drug basis, for enhanced theranostics and a more individualized medicine.
Material/Methods: We decided to study the regenerating islet-derived protein III beta (REGIIIβ) as potentially differential urinary markers of gentamicin nephrotoxicity in an animal model of AKI induced by gentamicin, cisplatin and saline as control. Renal function was monitored by serum creatinine, BUN, creatinine clearance, proteinuria. Renal morphology and tissue integrity were assessed by histological studies and urine renal markers.
Results: REGIIIβ appears in the urine of rats with overt renal injury induced by gentamicin, but it is not present in the urine of rats with a similar degree of renal damage inflicted by cisplatin. The treatment with gentamicin doses slightly increased to gene expression as early as on day 3 and urinary excretion on day 4, when no detectable kidney injury has occurred yet. REGIIIβ urinary levels differentiate the nephrotoxicity caused by gentamicin from that caused by cisplatin.
Conclusions: The REGIIIβ is an potentially differential or etiological urinary markers of gentamicin′s nephrotoxicity. They will be help to better delineate the pharmacological profile of gentamicin and, turn, to improve its clinical utility.

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HERBALS AND DIETARY SUPPLEMENT (HDS) PRODUCTS: A GROWING CAUSE OF HEPATOTOXICITY IN THE SPANISH DILI REGISTRY

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Introduction: HDS products including androgenic anabolic steroids (AAS) is a growing cause of drug-induced liver injury (DILI). We aimed to determine HDS-DILI incidence and the phenotype and outcome of AAS hepatotoxicity.
Material/Methods: AAS-induced DILI cases included in the Spanish-Latin-American DILI Registry were analyzed for demographic, clinical-biochemical parameters and outcome.
Results: Sixty-four out of 1023 DILI cases included in the registry over 20 years were attributed to HDS, 25 (39%) by AAS and 39 (61%) by other HDS. Eighty percent (20/25) of the AAS and 41% (16/39) of the other HDS cases were identified in the last 4 years. All AAS cases were male with a mean age of 32 years (range: 20-49). Classified into type of liver injury (60% hepatocellular and 40% cholestatic), the main causative agents were stanozolol in both groups followed by methylepitiostanol. The mean peak total bilirubin (TBL)

value in the hepatocellular group was 21xULN (range: 2-37) and 32xULN (range: 6-54) in the cholestatic group (p = 0.029). Six patients with cholestatic injury and high TBL values (22-54xULN) developed acute renal impairment (AKI) with serum creatinine 1.6-8.50 mg/dL. A TBL value >21.5xULN was found to be associated with AKI (serum creatinine ≥1.5 mg/dL) (AUROC: 0.87, sensitivity: 100% and specificity: 63%) in cases with cholestatic damage.
Conclusions: HDS use is a rapidly growing cause of DILI and represents a major health concern. AAS-induced liver injury presents a characteristic phenotype in which hepatocellular damage with high TBL values predominates. Cholestatic damage and high TBL values increase the risk of renal impairment during AAS hepatotoxicity.
Funding: AEMPS.FIS PI12-00620.CIBERehd-ISCIII.

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DRUGS IN SPECIAL SITUATIONS. EVIDENCE LEVELS TO GUIDE PEDIATRIC PRESCRIPTIONS

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Introduction: A treatment option for patients suffering from a disease for which no satisfactory authorised alternative therapy exists, may be the Compassionate Use Programme. On the other hand, although off-label prescribing is common, it is often done in the absence of adequate supporting data.
Objectives: 1) to describe the extent of the compassionate and off-label drugs use in hospitalized pediatric patients; 2) to evaluate the evidence level of the above prescriptions; 3) to determine the degree of the new drug authorizations and the variations on SmPC (Summary of Products Characteristics) after three years.
Methods: Cross-sectional study including all applications in 2011. Setting: Reina Sofía University Hospital. Analysis: Review of SmPC and evidence according to the NICE. Number of active clinical trials (source: ClinicalTrials.gov).
Results: A total of 190 applications and 82 different indications, mainly from Pediatrics (34%). Only 30% of petitions was based on somewhere randomized clinical trial meanwhile the rest had a low level of evidence. There were nine indications without references (everolimus-polycystic disease or nilotinib-leukemia). Currently, we haven't found active research for 40% of the indications. Only five drugs have achieved SmPC variations (methotrexate for juvenile idiophatic arthritis, sildenafil for pulmonary hypertension, deferasirox for hyperferritinemia, and raltegravir and tenofovir for HIV). As compassionate use were requested decitabine, figitumumab, parenteral vitamin A and cisapride, none of which is currently authorized.
Conclusions: Our findings identified a high volume of prescriptions in the absence of good evidence (suppositional or investigational, but not supported uses). Clinical research should focus on these drugs and diseases.

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TOXICOKINETIC-TOXICODYNAMIC RELATIONSHIP IN CASES OF PYRETHROID EXPOSURE

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In human medicine, the prevalence of pyrethroids in formulations for lice control has increased in the last decade. Pyrethroids act primarily on the nervous system. The commonly accepted mechanism of action is the prolongation of the open state of voltage-dependent sodium channels in nervous tissue. Pyrethroids are classified as Type I or Type II according to both chemical structure and biological effects of high-dose acute exposures. The objective of this work was to analyse TK-TD relationship, correlating brain pharmacokinetic parameters with neurochemical effects for the pyrethroids Type II λ-cyhalothrin and deltamethrin.
Two experiments were carried out: (1) male Wistar rats treated with λ-cyhalothrin or deltamethrin (20 and 26 mg/kg, per os) were killed at different time period after treatment and plasma samples and brain regions isolated, homogenized and extracted in acetonitrile to determine pyrethroid levels by HPLC-UV; (2) male Wistar rats treated with λ-cyhalothrin or deltamethrin (8 and 9 mg/kg, per os, 6 days) and with corn oil (control animals) were killed 24 h after dosing, brain regions isolated and contents of DA, 5-HT and metabolites quantified by HPLC-ED.
Both pyrethroids were extensively absorbed, distributed to SNC tissues and slowly eliminated. Significant differences in the kinetic parameters between nervous tissues and plasma were observed. Nervous tissue accumulation of both pyrethroids was reflected by the AUC tissue/AUC plasma ratios. Hypothalamus presented the higher T½β, Cmax, and AUC. Both pyrethroids caused a statistically significant decrease in the DA and 5-HT levels in the brain regions. The major depleting effect was observed in the hypothalamus.
Our results demonstrate that information regarding TK-TD relationship improves the scientific basis for risk decisions of pyrethroids.
This work was supported by Consolider-Ingenio 2010 Ref. CSD/2007/00063 (FUN-C-FOOD), and project Ref. UCM-BSCH/GR35/10-A.

RECEPTORS

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CYTOSOLIC AND ENDOPLASMIC RETICULUM CALCIUM SIGNALS EVOKED BY TWO TYPES OF CHONDROITIN SULFATES

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Introduction and objectives: Chondroitin sulfate (CS) is an ubiquitous glycosaminoglycan of the cellular membrane and extracelular matrix; particularly, nervous tissues (NT) is highly enriched with CS leading to the formation of perineural net (PNN), a lattice-like structure that enwraps soma and proximal dendrites of certain subpopulation of neurons. In NT, CS is synthesized by neurons and glia. The present work aims to elucidate the effect of CS over calcium signaling that underlie synaptic transmission.
Material and methods: We have measured membrane potential and ionic currents through patch-clamp techniques. Furthermore we performed cytosolic and endoplasmic reticulum (ER) calcium measurements by using Fluo 4AM and genetically ER-targeted chameleon. The cellular model used was the primary culture of hippocampal cells.
Results and Conclusions: We found that both CS from bovine traquea and CS from shark cartilague induce activation of AMPA/Kainate

receptors in primary hippocampal neurons. They are able, in addition, to promote a cytosolic calcium transient and calcium release from ER. The receptors involved in this calcium signaling were AMPA/Kainate receptors and group I metabotropic glutamate receptors. The involvement of metabotropic receptor was corroborated by transfecting hippocampal cells with a fluorescent protein directed to ER and monitoring ER calcium concentration. This discovery adds a new target to the list of proteins reported to interact with CS, and could explain the functions attributed to this glycosaminoglycan.

We thank the continued support of Fundación Teófilo Hernando and Bioibérica S.A.

TEACHING IN PHARMACOLOGY

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QUESTIONS ORDER MANIPULATION WITH RESPECT TO LEARNED-KNOWLEDGE ORDER MAY CONDITION THE SCORE IN MULTIPLE-CHOICE QUESTION ASSESSMENT

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Multiple-choice questions-test with one-true answer is widely use in pharmacology assessment to determine the student's depth of understanding on a topic because it is an easily graded method to evaluated large number of students simultaneously in a short time, and it provide an immediate feedback for the students. But, is it an effective and safe assessment?

Aim: To evaluate the questions order and question's answer order manipulations impact in the exam's score of analgesic and sedative drugs assessment.

Methods: Prospective controlled study was done in Anaesthesia (Medicine Degree, N = 398) and Pharmacology (Podiatry Degree, N = 145) students. A 45 multiple-choice questions/one true-answer test was done in each course (2011-13). The students were randomized in three groups: A= questions' and answers' orders were similar to which knowledge's were taught in class; B= questions order was different and answers′ order was similar to which knowledge's were taught in class; C= questions' and answers' order were different from that of which knowledge's were taught in class. The percentages of hits obtained and the time employed in completed the test by the students were evaluated.

Results: Group A completed the test with a slight high percentages of obtained hits and short time than group B and C: i) Hits (%): 88,9 ± 15% vs 81,6 ± 17% vs 78,6 ± 5,7%, respectively; ii) Time (min): 51,9 ± 8,6 min vs 62,6 ± 11,9 min vs 75,55 ± 13,4 min, respectively, ($p < 0.05$).

Conclusion: Test's questions order manipulation with respect to learned knowledge order may result in different scores not related with the real student's knowledge at the assessment.

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DEVELOPMENT OF A MINI-GRAPHIC ATLAS OF MECHANISMS OF ACTION OF DRUGS MADE BY STUDENTS

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Introduction: We proposed a practical activity addressed to students of Pharmacology and Pharmacotherapy in the 4th year of Pharmacy Degree with the main goal of favouring their learning. The knowledge of mechanisms of action of drugs is crucial in pharmacology leading to understand the indications, clinical and adverse effects of each of the major drug classes.

Material/Methods: The students organized in groups, made a graphical mechanisms of action of the major drugs classes specified by the teacher, who oversees this work through various tutorial sessions, conducted in the classroom and warned in advance. The level of student satisfaction was assessed through surveys.

Results: Finally, the activity was completed with the selection of the best graphical mechanisms evaluated by the teachers and the subsequent development of a "Mini-graphic atlas of mechanisms of action of drugs" designed by students. The students highly appreciated the increase of their knowledge after having developed this activity.

Conclusion: The results suggest that students achieve an ability to apply knowledge on pharmacology and pharmacotherapy in a creative way and help to reach learning aims.

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ACQUISITION OF SKILLS AND ABILITIES IN PHARMACOLOGY: PREFERENCES OF UNDERGRADUATE MEDICAL STUDENTS

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Introduction and Objectives: In pharmacology teaching at medical schools the problem-based learning (PBL) is a pedagogical technique that, by a student-centered approach, promotes the development of a number of important skills, abilities and competencies in order to prepare those future professionals for attending their patients. The aim of this study intends to investigate, through systematized surveys, the impact of PBL in pharmacological training of undergraduate medical students, by comparing this innovative educational methodology with other teaching techniques that we use in practical classes.

Material/Methods: The medical students participated voluntarily on an anonymous survey that encompassed ten questions about five of their corresponding practical classes in pharmacology matters. Those questions distinguished undoubtedly the lessons previously presented at the pharmacology lab, by computed-assisted instruction (CAI) or by PBL. For this last one, pharmacokinetic problems or pharmacological clinical cases were employed, encouraging the students to resolve the specific situation proposed.

Results: Of a total number of 350 medical students participating in the survey, 179 were studying Basic Pharmacology and 171 Clinical Pharmacology, respectively at the 2nd and 3rd course of their medical core curriculum. At 2nd course, a clear majority of students preferred the pharmacology lab practical classes (77.1%) over CAI or PBL. Moreover, at 3rd course students showed their welcome to repeat more PBL exercises (69.1%).

Conclusions: In undergraduate medical students, there is a tendency to prefer the PBL methodology when their knowledge in pharmacology are more advanced.

Supported by Proyecto de Innovación y Mejora Docente (ID2013/353), Universidad de Salamanca (convocatoria 2013-2014).

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UNDERSTANDING DRUG MECHANISMS OF ACTION: EFFECTIVENESS OF A LEARNING MULTIMEDIA TOOL

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Introduction: New technologies and internet offer attractive possibilities to improve and facilitate teaching/learning process. The objective of this study was to evaluate a learning innovation developed in the subject *Pharmacology* at the University of León (second course, degree of Physical Therapy), based on the use of multimedia animations. This interactive help tool was employed to explain drug mechanisms of action along the course (one semester).
Material and methods: Animations have been searched in internet, displayed at classroom, and their links provided to students. At the end of the innovative experience, a voluntary questionnaire was offered to undergraduates, scoring their opinion from 1 (bad) to 5 (excellent).
Results: A 93% return rate of students' questionnaire was achieved. Animations have been displayed by almost all the undergraduates, although 40% of students followed them without listening or reading English explanations attached. This educational tool have made mechanisms of action easier to understand for 86% of students. Moreover, a high (70%) or very high (23%) level of satisfaction has been perceived among them, increasing their interest for Pharmacology in about 88% of the students.
Conclusions: A good acceptance of the educational experience performed has been observed among students, and their use should be implemented to improve students' motivation and interest for this subject.

[Correction added on 12 September 2014: *IBIOMED* has been added to the authors' affiliation details.]

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TUTORING AS TOOL TO IMPROVE LEARNING OUTCOMES IN PHARMACOLOGY

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Introduction: Tutoring plays a vital role in enhancing students' academic and personal development. Nevertheless, this learning methodology currently remains underused and underestimated at Spanish University. Thus, the aim of this study was to find out new ways to manage tutoring in the subject *Veterinary Pharmacology* (second course of the Degree in Veterinary Medicine) and their influence on academic results of our students.
Material and methods: A pilot study (called TUBAVET) with two types of tutoring (three classroom-based sessions and three self-evaluation on line questionnaires) have been carried out in this subject over the semester. To evaluate the effectiveness of tutoring, students' scores and their level of satisfaction were statistically studied.
Results: Thirty-three students have taken part in this pilot experience. Average scores obtained in self-evaluation on line questionnaires on Pharmacology ranged from to 7.4 to 9.4. Moreover, 90.1% of tutored students passed the course in comparison to 81.5% in tutorless group (Chi-square test, $P > 0.05$). Average final examination scores tended to be higher in the tutored group (7.4 ± 1.1) than in the tutorless one (6.6 ± 1.1). "Good" or "excellent" qualifications were also higher (48.5 and 12.1%, respectively) among the students who took part in this study compared to those tutorless (22.2 and 4.6%, respectively) (Chi-square test, $P < 0.05$). Level of satisfaction degree was also high in tutored group.
Conclusions: Enhancement of tutoring improves the academic performance of students, as well as their motivation and interest for this subject.

[Correction added on 12 September 2014: *IBIOMED* has been added to the authors' affiliation details.]

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ASSESSING STUDENTS' OPINION AND SATISFACTION ON PHARMACOLOGY IN NURSING DEGREE

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Introduction: The European Higher Education Area has brought a big change in Nursing education framework. The survey was conducted to gather information on the development of the learning process and to assess attitudes and opinions of the students attending the subject Pharmacology in Nursing Degree (Campus of Ponferrada, University of León) as a way to assess their academic improvement and success.
Material and methods: Information was collected by using a voluntary five-point Likert scale structured questionnaire, which was administered to all students enrolled in the subject.
Results: 42 students (87% female and 13% male) have collaborated in this study. Most of them had no (53%) or little (36%) previous knowledge on Pharmacology. They think that this subject is very useful in Nursing curricula (86%), giving it a considerable (62%) or great (29%) importance in the second course of the degree, although they would increase neither theory nor practical classes in it. On the other hand, in their opinion, the subject has got a high (71%) or very high (12%) level of difficulty. They spent studying Pharmacology between 3-5 h (24%) and 6-8 h (38%) and, in general, they were satisfied (57%) or very satisfied (26%) with the subject. No significant differences were found between genders.
Conclusions: Findings indicate that most students have a favorable opinion of Pharmacology, and are satisfied with this subject, although the level of difficulty appreciated by them remains as a challenge to overcome.

[Correction added on 12 September 2014: *IBIOMED* has been added to the authors' affiliation details.]

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RESOLUTION OF CLINICAL CASE STUDIES IN THE DEGREE OF PHARMACY BY USING "AULA VIRTUAL" OF UNIVERSITY OF MURCIA

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Information and Communication Technologies (ICTs) have become one of the main pillars for professors who teach at the University. These tools facilitate the contact between teacher and students, making it quicker and easier, avoid the needing of a meeting. So what this virtual mood of solving questions is much more comfortable. In the University of Murcia this tool is a version of Sakai named Aula Virtual. On the other hand, one of the biggest lacks nowadays in the University is to make teaching more practical. One opportunity of making it real is by solving clinical case studies. For these reasons, the aim of this study was to make that the students will find easier the theoretical part through the resolution of clinical case studies.
"Aula Virtual" of University of Murcia contains several forums where the students can change opinions and to interact. We set five clinical case studies, one for each month with two extra clues. This work

counted as a percentage in their marks depending on the time spent on solving it properly (0.2 first week, 0.15 second week, 0.12 third week) Normally, in practical classes the students do not involve properly. By this system we have obtained a 96% of participation. The average score was 0.18. Most of the students have improved their final marks compared to the years before.
We highly recommend to include new ways of teaching taking advantage of these new tools. It makes that the students appraise the subject and learn more.

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LEARNING TO RESEARCH IN PHARMACOLOGY USING WEB 2.0 TECHNOLOGIES

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Introduction: Web 2.0 is today essential as an information channel for the communication. Its main tools are: blogs, twitter, podcasting, wikis, etc. These tools and communication technologies are used by students, teachers and patients in multiple ways to learn and to communicate. Therefore, these tools also need to be implemented in higher education.
Aims: Introduce Web 2.0 communication tools in the teaching of pharmacology as a way to motivate and to teach students for the realization and publication of papers.
Methods: In Pharmacology and Clinical Pharmacy subjects of 4th course of the degree in Pharmacy are scheduled workshops and seminars to acquire teaching skills of scientific communication. Students acquire these skills through the preparation of a literature review article later published in an online journal for pharmacy students. Published papers are discussed in the university blog and shared on Facebook and twitter.
Results: Forty-nine papers made by our pharmacy students have been published in the journal on line. The papers have had an average of 2705 visits (minimum 141 - maximum: 5212). Articles has been published in the pharmacy blog (http://blog.uchceu.es/farmacia/), with an average of 1078 visits (minimum 330, maximum: 3335) and on our Facebook page (https://www.facebook.com/farmaciaUCHCEU)
Conclusion: In general it can be concluded that the integration of social networks of Web 2.0 represents a current form of self-learning, which encourages reflection and requires students to be active in the construction of knowledge.
The introduction of this technology in education presents a new challenge for teaching at the University.

OTHERS | OTROS

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ASSESSMENT AND COMPARISON OF THE ANTIOXIDANT ACTIVITIES OF COMMERCIAL DIETARY SUPPLEMENTS USED TO IMPROVE MALE INFERTILITY

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Introduction and objectives: Couple infertility commonly results from the synergistic negative influence of several factors. External factors, related to life-style and environmental exposure as well as oxidative stress play an important role in the pathophysiology of male infertility. High levels of semen Reactive Oxygen Species (ROS) can cause sperm dysfunction, sperm DNA damage and reduced male reproductive potential. This observation has led clinicians to treat infertile men with antioxidant supplements. To date, most clinical studies generally demonstrate that dietary antioxidant supplements are beneficial in terms of improving sperm function and DNA integrity. The aim of this study was to investigate the antioxidant properties of five commercially available dietary supplements used in Spain to improve male infertility.
Materials and methods: Antioxidant activities of the dietary supplements (AndroMÁS, Androferti, Seidiferty, Aquilea Fértil and Gesta Dha) were determined using two analytical methods: oxygen radical absorbance capacity (ORAC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). Two batches of each supplement were analyzed.
Results: AndroMÁS and Seidiferty showed significant antioxidant properties as measured by the ORAC assay with ORAC values of 0.25 and 0.24 μmol Trolox/mg supplement, within the range reported in the literature for dietary antioxidant supplements (0.018- 3.18 μmol of Trolox equivalent/mg of supplement). The DPPH radical scavenging activity was highest in AndroMÁS, Androferti and Seidiferty (97.2%, 93,0% and 60.5%, respectively).Based on the results of various antioxidant activity methods, the greatest antioxidant capacity of the tested dietary supplements was found in AndroMÁS.
Conclusions: This study suggests that the antioxidant properties showed by AndroMÁS and Seidiferty could contribute to the therapeutic efficacy on male infertility demonstrated in male patients who received these supplements.

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PITX2 DECREASES L-TYPE CA^{2+} CURRENT AND INCREASES THE SLOW DELAYED RECTIFIER K^+ CURRENT IN CARDIAC CELLS

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Introduction: An increase in Pitx2 expression has been associated with an increased risk of atrial fibrillation (AF). On the other hand, AF produces profound changes in the expression of voltage-gated Ca^{2+} and K^+ channels. Here we analyzed the effects of Pitx2 on L-type Ca^{2+} and K^+ channels.
Methods: Currents were recorded in mouse atrial cultured HL-1 cells transfected or not with the cardiac Pitx2 isoform (Pitx2c) by using whole-cell patch-clamp. L-type Ca^{2+} current ($I_{Ca,L}$) was recorded by using Ba^{2+} as charge carrier (I_{Ba}).
Results: Pitx2c significantly reduced peak I_{Ba} density by 40.2% without modifying time- and voltage-dependent properties of the current. Regarding voltage-gated K^+ channels, under control conditions 2 groups of cells were identified based on the predominant voltage-gated K^+ current exhibited. In most of the cells (≈80%), a rapid delayed rectifier current (I_{Kr}) sensitive to dofetilide could be recorded. In the rest of the cells (≈20%), I_{Kr} was absent and the predominant current was an outward current sensitive to 4-aminopyridine (2 mM), similar to the ultrarapid delayed rectifier K^+ current (I_{Kur}) recorded in human atrial

myocytes. In the presence of Pitx2c, only 10% of the cells exhibited I_{Kur} and the rest exhibited a voltage-gated, dofetilide-resistant, K^+ current with very slow activation kinetics that was completely abolished by the selective I_{Ks} blocker HMR-1556 (1 μM).
Conclusions: Pitx2c decreased $I_{Ca,L}$ and increased I_{Ks} suggesting that this transcription factor could contribute to the reduction of $I_{Ca,L}$ and the increase of I_{Ks} that characterize the AF-induced electrical remodeling.

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NAV1.5 N-TERMINUS EXHIBITS A PDZ BINDING DOMAIN AND INCREASES THE KIR2.1 CHANNEL DENSITY

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Introduction: The N-terminal domain of Nav1.5 channels (Nter), a 132 aminoacids peptide increases the Nav1.5 current ($I_{Nav1.5}$) density. Additionally, there is a reciprocal modulation of the expression of Nav1.5 and Kir2.1 and Kir2.2 channels. Thus, we tested whether Nter is able to increase the expression of Kir2.x channels.
Material and Methods: Currents were recorded using the patch-clamp technique in Chinese hamster ovary cells transiently transfected with wild type or site directed mutated Nter, Kir2.1, Kir2.2, and Nav1.5 channels.
Results: Cotransfection of Nter with Kir2.1 and Kir2.2 channels significantly increased Kir2.1 current ($I_{Kir2.1}$) and $I_{Kir2.2}$. Conversely, co-transfection of Nter with Kir2.3 channels, did not significantly modify $I_{Kir2.3}$. Nter did not increase the current density generated by Nav1.5 channels lacking their C-terminal PDZ domain (Nav1.5ΔPDZ). Noteworthy, Nav1.5ΔPDZ channels still co-immunoprecipitated with syntrophin. We identified in the Nter peptide a sequence which could act as a "PDZ-like" binding domain (18-RESLA). Site directed mutagenesis demonstrated that mutants of all residues, except p.S20A Nter, increased $I_{Kir2.x}$. Finally, adult rat ventricular myocytes were enzimatically dissociated, cultured, and infected with either a control adenoviral construction (Ad-GFP) or an Nter codifying adenoviral construction (Ad-Nter). Results demonstrated that myocyte infection with Ad-Nter significantly increased both the inward sodium (I_{Na}) and inward rectifier (I_{K1}) currents.
Conclusions: The N-terminal domain of Nav1.5 channels exerts "chaperon-like" effect increasing $I_{Nav1.5}$ and $I_{Kir2.x}$ densities, this effect depends on the residue Ser20 which probably determines the binding to syntrophin via an "internal" PDZ binding domain.

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GRAPHENE DERIVATIVES AS SCAFFOLD FOR EX VIVO SURVIVAL AND MATURATION OF DOPAMINERGIC SN4741 CELLS

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Carbon nanomaterial Graphene (G) can form a three-dimensional porous structure with efficient bioconjugation and cell differentiation properties, providing a promising scaffold for neural regeneration.
Aims: To study this putative new application of G, we cultured a clonal substantia nigra dopaminergic neuronal progenitor cell line (SN4741) in presence of G as scaffold.
Methods: Cells were cultured in DMEM/10% FCS to about 80% confluence and incubated with different concentrations (0.001–1 mg/ml) of three chemically different G derivatives (G oxide (GO); partially reduced GO (PRGO) and fully reduced GO (FRGO)) and two different presentation matrixes as powder and films. Cell viability was measured by the MTT assay. To study cellular characterization, morphology and assessment of cell engraftment into G films, we analyzed the immunostaining of the neuronal marker NeuN, the anti-rat Beta-3-tubulin antibody, and the anti-rabbit DCX as immature neuronal marker. Reactive oxidative species (ROS) and the mitochondrial membrane potential after JC-1 incubation were measured by flow cytometry. Lactate dehydrogenase was measured in the culture supernatant.
Results: We found similar increase of survival and metabolism (30-40%) at low concentrations of PRGO and FRGO (0.05-0.01 mg/ml) compared with the higher concentration (1 mg/ml), no changes were seen in the GO group. PRGO or FRGO films showed an increased in the effective anchorage capacity to nest into the G matrix and in the maturation of the dopaminergic SN 4741 cells.
Conclusions: G scaffolds could offer a powerful platform for neural stem cells, direct cell conversion techniques and neural tissue engineering.

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THE EFFECTS OF TREATMENT ON DISEASE SYMPTOMS AND PROGRESSION OF STRUCTURAL CHANGES IN KNEE OSTEOARTHRITIS PARTICIPANTS FROM THE OSTEOARTHRITIS INITIATIVE PROGRESSION COHORT

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Introduction: To explore the effects of commonly used medications for treatment of knee osteoarthritis (OA) on structural progression.
Material/Methods: Participants (n = 600) were selected from the Osteoarthritis Initiative (OAI) progression cohort (http://www.oai.ucsf.edu/) (n = 1390) who met the following criteria: 24 consecutive months of follow-up with clinical and imaging data including radiographs and magnetic resonance imaging (MRI) of the target (highest WOMAC pain) knee. Data for joint space width (JSW) were obtained from the OAI database and cartilage volume was measured using a fully-automated MRI.
Results: Participants reported taking (+) (n = 300) or not taking (−) (n = 300) OA treatment (analgesic/NSAID, etc.) over 24 months, with or without glucosamine and chondroitin sulfate (Glu/CS). The +analgesic/NSAID subjects had higher WOMAC scores ($P < 0.0001$) and smaller JSW ($P = 0.013$) reflecting more severe disease at the onset of the study (T0). In the analgesic/NSAID group, subjects taking Glu/CS had a smaller loss of JSW at 12 months ($P = 0.057$) and cartilage volume at 24 months in the medial central tibial plateau ($P = 0.022$ univariate and $P = 0.025$ multivariate analysis). In the +analgesic/NSAID group, those taking Glu/CS had significantly lower WOMAC scores (pain, $P < 0.0001$; stiffness, $P = 0.037$; disability, $P = 0.0004$) and higher KOOS scores at T0 as well as a smaller cartilage volume loss in the tibial plateau at both 12 ($P = 0.029$) and 24 months ($P = 0.033$).
Conclusions: In both the + and analgesic/NSAID groups, participants who took Glu/CS had reduced loss of JSW and cartilage volume over 24 months. These effects of Glu/CS on structural changes support results from previous studies.

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ANTIFUNGAL ACTIVITY IN VITRO OF STATINS: A COMPARATIVE STUDY OF SIMVASTATIN, FLUVASTATIN, LOVASTATIN, PRAVASTATIN AND ATORVASTATIN AGAINST *CANDIDA*

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Introduction: Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which catalyzes the biosynthesis of sterols. This inhibition may affect fungal growth and multiplication reducing ergosterol biosynthesis. The objective of this study was to evaluate the antifungal activity of statins against strains and isolates from 3 species of *Candida* causing invasive fungal infections.
Material and methods: Five statins, atorvastatin, fluvastatin, pravastatin, lovastatin and simvastatin, were studied against 3 strains of *Candida glabrata,* 4 of *Candida krusei* and 3 of *Candida lusitaniae,* by the CLSI M27-A3 broth microdilution method, with fluconazole as positive control.
Results: Simvastatin was the most active statin against *Candida glabrata*, *Candida krusei* and *Candida lusitaniae* (MICs: 6.35, 42.22 and 1.71 μg/ml, respectively) ($P < 0.001$), followed by lovastain, fluvastatin, atorvastatin and pravastatin which showed species-dependent antifungal activities. The lowest MICs of statins, except for pravastatin, were observed against *Candida lusitaniae* (range, 1.71-9.33 μg/ml) ($P < 0.001$).
Conclusions: Simvastatin, lovastatin and fluvastatin exhibit higher species-dependent antifungal activities than atorvastatin and pravasatin. *Candida lusitaniae* was the most susceptible species. Even the antifungal activity of statins was observed at higher concentrations than clinically achievable, the results reported here may represent a first step when considering the use of these drugs as antifungal agents, probably in combination with other drugs. In vivo studies will be required to confirm clinical relevance.

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ALCOHOL AND TOBACCO CONSUME DURING PREGNANCY AFTER NATURAL AND IN VITRO FERTILIZATION CONCEPTION

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Women's use of alcohol and tobacco in pregnancy is associated with an increased risk of foetal loss, and birth defects and low weight. It has also been reported that alcohol use in women decreases the success of infertility treatment, such as in vitro fertilization (IVF). Our aim was to determine the life-style behaviour of women during natural and in vitro fertilization pregnancy.
Methods: Prospective surveys on pregnant women have been done. Women in different trimester of their pregnancy were encouraged to complete a self-administered survey, in which participants reported epidemiological data, obstetric data, tobacco and alcohol (type, amount, and frequency consumed) and other possible toxic compounds intake/consume during their pregnancy.
Results: Four hundred and forty-six women, aged 31.5 ± 0.2 years old, 48.2% with previous children and 0.48 ± 0.04 with previous abortion, have been included. The 89.9% were Spanish, 56.5% were outside-home workers, and 34.5%/56.9% had university/high school studies. Women were in their first (FT) (41%), second (ST)(26.7%), third trimester (TT)(22.2%) of pregnancy and puerperium (P)(10.1%); and after natural 94.6% and IVF 5.4% conceptions. Their labour deliveries were 48.9%eutocic, 17.8% instrumental vaginal and 33.3% caesarean. Toxic consume-intake ranking order was (Natural/IVF conception): i) Tobacco FT 48.9%/42.9% > ST 43.4%/23.1% > TT 39.8%/4.20%* > *P* 33.3%/0.0%; ii) Alcohol FT 41.8%/16.7%* > ST 28.7%/8.3%* > TT 21.3%/0.0% > *P* 11.9%/0.0% iii) Marijuana FT 3.6%/0.0% > ST 2.2%/0.0% > TT 1.2%/0.0% > *P* 0.0%/0.0% ($P < 0.05$). No other toxic consume were detected.
Conclusion: The reductions of alcohol and tobacco consumption during pregnancy were boosted after assisted reproduction techniques.

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