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PLENARY SPEAKERS

PL001

HOW DO WE MORE RAPIDLY TRANSLATE NEW GENETIC INSIGHTS, INTO NOVEL THERAPIES FOR PATIENTS?

Bountra C.

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PL002

CIRCADIAN RHYTHM AND MOLECULAR CLOCKS IN PHARMACOLOGY

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Circadian rhythms play a fundamental role in mammalian physiology. Circadian rhythms are synchronized through communication with the master clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN synchronizes peripheral clocks in various organs. Nevertheless, isolated peripheral tissues and even cultured cells maintain circadian rhythmicity in the absence of input from the SCN. The molecular clock components (Bmal1, CLOCK, Cry and Period genes) are responsible for this cell autonomous rhythmicity. This core clock machinery is found in most tissues and has been estimated to mediate the circadian transcription of 20% of active genes. Circadian dysregulation has been implicated in aging, and in several disorders, such as metabolic diseases, mood disorders, and neurodegenerative diseases. Thus, the circadian clock itself may become a therapeutic target for age-related diseases. Indeed, some behavioral and pharmacological strategies ("clock drugs") change clock gene expression and rhythms and may restore circadian rhythms in patients, hence mitigate disease symptoms and age-related decline. Furthermore, circadian systems have been shown to influence pharmacokinetics and pharmacodynamics. And interestingly, some randomized clinical trials showed that circadian-based treatments (chronotherapy) had relevant patient outcomes. Integration of chronotherapeutics to clinical trial design may improve the success of drug candidates.

PL003

ACTIVITIES OF EPHAR, EACPT AND UEMS TO SUPPORT TRAINING OF BASIC AND CLINICAL PHARMACOLOGISTS IN EUROPE

Griesbacher T.

Otto Loewi Research Centre, Medical University of Graz, Graz, Austria

The European Certified Pharmacologists (EuCP) Programme was launched in July 2014 by the Federation of European Pharmacological Societies (EPHAR) with the intention to identify experts in the field of pharmacology (both basic and clinical) whose competency profile, in addition to their personal specialised scientific expertise, covers expert knowledge in all major fields of the discipline. EACPT, the European Association of Clinical Pharmacology and Therapeutics, declared its participation in the EuCP Programme in July 2015. National certification schemes of two EPHAR member societies (Italy and Austria) have already been approved by the EuCP Programme: the programmes differ in structure and reflect the flexibility of the EuCP Programme with respect to the respective national conditions. Thus, these first submitted national programmes can also serve as 'case studies' for other participating member societies to develop their national EuCP rules. Information on the EuCP Programme and all approved national EuCP programs are published on the EuCP website (eucp-certification.org). Currently, several further national societies of Pharmacology (Croatia, Finland, Germany, Netherlands, Norway, Spain, Sweden, Switzerland) are already working on national EuCP programmes or have started to explore possibilities to do so in their organisations. UEMS, the European Union of Medical Specialists has founded a new section of Pharmacology in 2016, which will represent all medical specialists in this discipline. The UEMS Section of Pharmacology is currently information about training regulations in those countries were (Clinical) Pharmacology is recognised as a regulated medical specialty, either as stand-alone medical specialty or as additive or sub-specialisation to other specialties such as Internal Medicine. Once the survey is completed, the results shall be used to compile harmonised UEMS European Training Requirements for this medical discipline.

PL004

NEUTROPHIL BREACHING OF VENULAR WALLS *IN VIVO*: NOVEL INSIGHTS, CONCEPTS & MECHANISMS

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Migration of leukocytes to sites of injury or inflammation is a crucial component of both innate and adaptive immunity but can also contribute to the pathogenesis of inflammatory disorders. However despite much research there has been a disappointingly slow progress in fruitful targeting of leukocyte trafficking for development of anti-inflammatory drugs, indicating a need for better understanding of the intricacies of targeted pathways. Within our group we aim to investigate the mechanisms of leukocyte transmigration through imaging of inflamed tissues by multiple methods, including the application of high resolution confocal intravital microscopy. With this approach we have characterised the profile and dynamics of leukocyte transmigration through venular walls and as a result have noted previously unreported physiological responses (eg sub-endothelial cell crawling[1]) and have identified potential pathological events such as neutrophil reverse transendothelial cell migration, a response that we have associated with dissemination of systemic inflammation.[2,3] Such studies have demonstrated that detailed analysis of leukocyte-vessel wall dynamics are likely to identify novel and disease-specific phenomena that could promote a change in thinking towards development of new therapeutic strategies.[4]

References:

1. Proebstl, et al., *J Exp Med* 209: 1219–1234 (2012).
2. Woodfin, A et al., *Nat Immunol* 12:761–769 (2011).
3. Colom et al., *Immunity*, 42:1075–1086 (2015).
4. Nourshargh & Alon, *Immunity*, 41: 694–707 (2014).

PL005

PHARMACOGENOMICS AND PERSONALISED MEDICINE FOR DRUGS OF THE CARDIOVASCULAR SYSTEM

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Drugs used to treat cardiovascular conditions are among the most widely prescribed worldwide. Current state of the art in the pharmacogenomics for cardiovascular drugs shows a prominent role for genetic variation for a number of commonly used cardiovascular drugs, including anticoagulants, clopidogrel, beta blockers, CETP inhibitors, and statins. The pharmacogenomic hallmarks for these drugs and drug classes will be presented and the evolution of the field in the last 2–3 decades will be assessed. The prospect of incorporating this information into routine clinical care is being constantly raised, however, it still mostly remains an open question. So, has the accumulated knowledge been translated to solid clinical applications yet? Is it really used in clinical practice? Which drugs, in which patients? If not, what are the missing links and the barriers that should be overcome to reach wide use of cardiovascular pharmacogenomics? Will some answers come from pharmacoepigenomics and other omics? The real question to be addressed: is cardiovascular drug pharmacogenomics and therapy personalization a reality, a hype, or an uphill long-distance track we must strive to continue successfully for the benefit of our patients and society?

PL006

THE NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) CONUNDRUM: HOW TO DEFINE IT?

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There is unanimous agreement that increased levels of obesity are driving an epidemic of NAFLD already affecting 25–40% of the adult population worldwide. And yet ask a basic scientist or medical researcher why only a small percent of NAFLD individuals develop steatohepatitis or NASH -that is, the variant of the disease that associates with increased mortality- and chances are, you will get as many different answers as the number of people you ask. I would argue, that the ectopic accumulation of fat in the liver is a physiological adaptation, an evolutionary advantage to anticipate periods of prolonged food shortage rather than a condition increasing the risk of developing liver injury. Accordingly, the liver has a limited capacity to export triglycerides (TG) which plateaus when intrahepatic TG content exceeds 6%. This causes the accumulation of TG when there is an increase of systemic fatty acids transported to the liver. VLDL-TG export is coupled to one carbon metabolism (1CM), a main metabolic integrator of the liver's nutritional status. Inputs in the form of amino acids, nutrients and glucose are processed through the 1CM pathway to generate outputs for a variety of biological processes such as VLDL-TG formation, the maintenance of redox homeostasis, epigenetics, and cell growth. Disruption of 1CM in mice by deletion of MAT1A -the main liver enzyme involved in S-adenosylmethionine (SAMe) synthesis- induces NASH, which is reversed by SAMe treatment. A metabolomics study of a large cohort of biopsied NAFLD patients revealed that the serum lipidomics signature in around half of the cases was remarkably similar to that obtained in SAMe deficient mice. Moreover, NASH patients frequently show reduced MAT1A mRNA levels. These findings provide functional evidence that alterations in 1CM is a driver of steatohepatitis affecting about half of the NAFLD patients, as well as the proof of concept for the assessment of the therapeutic activity of SAMe in human NASH.

SYMPOSIA
PLENARY ROUNDTABLE: OPEN INNOVATION IN DRUG DISCOVERY

PLRTS001

ASTRAZENECA'S OPEN INNOVATION PROJECT – LESSONS FROM THE FIRST SEVEN YEARS

Smith D.

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There is increasing realisation within big pharma that the best biology is happening externally and that if this is to be accessed as drug discovery collaborations then we must be more open with our data and share our compounds. AstraZeneca's (AZ) experience in this field started in 2011 with a collaboration with the UK MRC to reposition AZ compounds which had failed in their original indication in phase II or III. Such compounds were shown to hit their targets and to have low adverse effects. We have followed this up by expanding the program into new modules: "preclinical toolbox" (access to AZ tool compounds for mechanistic studies), "target innovation" (finding new chemical start points by accessing AZ screening compound collections), "new molecule profiling" (partnering molecules from academic chemists), "R&D challenges" (calls for solutions to AZ challenges with prizes) and "data library" (access to unpublished preclinical data sets). In this talk I will discuss how the programme has gone so far and what we have learnt from our experience in Open Innovation.

PLRTS002

ALMIRALLSHARE, A CROWDSOURCING PLATFORM TO SHARE SCIENCE AND INNOVATION

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Innovation is the main driver for growth of the pharmaceutical industry. However, the research and development of innovative medicines has become a difficult and extremely expensive business. To overcome this challenge, Pharma companies are embracing open innovation models to establish a reliable framework where knowledge and breakthrough ideas are converted into valuable solutions for patients. In this sense, at Almirall, we have been gradually implementing different open innovation initiatives, from joint research public-private projects to the launch of a crowdsourcing web platform, AlmirallShare, designed to share key dermatological challenges, such as translational preclinical models, with the scientific community, and find innovative solutions in skin health. This presentation will summarize some of our open innovation initiatives, as well as AlmirallShare first results.

PLRTS003

OPEN INNOVATION. THE J&J APPROACH

Gómez A.

Johnson & Johnson Innovation

Open Innovation has become the new paradigm in drug discovery and, with the creation of Johnson & Johnson Innovation (JJI) in 2012, is one of the main pillars of J&J´s strategy. JJI seeks to positively impact human health through innovation, creating a global network that generates transformational healthcare solutions through value-creating partnerships. JJI looks for disruptive innovation throughout the world through its family of complementary teams which include JLabs, the Innovation Centers, JJDC and Janssen Business Development and across the three sectors of J&J: pharma, consumer and medical devices & diagnostics.

PLRTS004

OIDD AS A NEW COLLABORATIVE MODEL IN DRUG DISCOVERY

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The drug discovery process is continuously evolving and pharmaceutical companies have designed different strategies for effective collaborations in research. With the globalization, the XX century was full of acquisitions and strategic alliances. In the XXI century, the advance of the personalized medicine is eradicating the traditional business model built on blockbusters. The unmet medical needs and the pressure to increase productivity while cutting costs have led to novel partnership models.[1] Current trends focus on external innovation, with an increased interaction between academic and private sectors. Many companies have implemented Open Innovation platforms to attract external talent, share knowledge and leverage synergies that create value.[2] In this ecosystem, the Lilly OIDD (Open Innovation Drug Discovery) program offers a variety of engagement modes to the affiliated investigators in both chemistry and biology, and has had an excellent acceptance globally.[3] In chemistry, 935 research groups are submitting compounds for testing, while in biology, 35 scientists are utilizing Lilly´s BIC cassette for validating novel biological targets. Although scientists in academia and industry have different goals, drivers and expectations, all recognize the benefits of working together. Industry needs to gain additional expertise to foster innovative research and translate these ideas into new treatments. On the other hand, scientists at public institutions are interested in testing and validating their hypothesis using company specific technologies, and obtaining additional funding. While operational differences between both partners exist, complementary expertise and the establishment of a trusting relationship are some of the key factors involving successful collaborations.

Keywords: open innovation, collaboration, drug discovery, pharmaceutical industry

References:

[1] S. M. Paul, D. S. Mytelka, C. T. Dunwiddie, C. C. Persinger, B. H. Munos, S. R. Lindborg, A. L. Schacht, *Nat. Rev. Drug Discov.* **2010**, *9*, 203–214.

[2] K. Schwartz, B. Huff, *Visions* **2010**, 19–22.

[3] a) M. Alvim-Gaston, T. Grese, A. Mahoui, A. D. Palkowitz, M Pineiro-Nunez, I. Watson, *Curr. Top. Med. Chem.* **2014**, *14*, 294–303; b) G. P. Carroll, S. Srivastava, A. S. Volini, M. M. Piñeiro-Núñez, T. Vetman, *Drug Discov. Today* **2017**, *22*, 776–785.

PLRTS005

DISCOVERY PARTNERSHIPS WITH ACADEMIA; COMBINING THE BIOLOGICAL EXPERTISE OF ACADEMIA WITH THE DRUG DISCOVERY EXPERTISE OF GSK TO DISCOVER NEW MEDICINES

Mossakowska D.

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PLRTS006

THE INNOVATION ECOSYSTEM OF ESTEVE

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Open Innovation is about expanding the innovation potential of organizations by opening them up to new ways of working with external partners. Although the routes to developing open innovation are not identical across companies, the journey involves several similar steps, starting by the cultural change – often from a generalized skepticism to ideas 'not invented here' – and followed by identification of internal and external resources to leverage open innovation, development of relationships and new ways of working, and finally building and integration with the ecosystem. Finding the optimum 'balance' of open Innovation – 'balance' of open vs. closed innovation – is always a challenge because increasing too much the number of external partnerships could actually become counter-productive. A project-by-project approach might reach the best configuration or balance.

ROUNDTABLE: PHARMACOLOGY OF CARDIO-METABOLIC DISEASES

RT001

NEW THERAPEUTIC APPROACHES IN THE TREATMENT OF TYPE 2 DIABETES MELLITUS

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Cardiovascular (CV) diseases are the leading cause of death in patients with type 2 diabetes mellitus (T2DM) and T2DM is a known risk factor for CV diseases, increasing the risk of developing/worsening macrovascular complications (coronary heart disease, stroke, heart failure). For decades T2DM treatment focused on improving glycemic control. However, even when aggressive HbA1c lowering is effective for reducing the risk of microvascular complications, it produces modest benefit for CV diseases. Furthermore, several studies have suggested that some antihyperglycemic agents increase CV risk, despite being effective at lowering blood glucose in type 2 diabetes. For this reason, since 2008, all new antihyperglycemic agents should demonstrate that their use will not result in an unacceptable increase in CV risk. Recent data from postmarketing Cardiovascular Outcome Trials, have questioned the cardiovascular safety of some dipeptidyl peptidase-4 inhibitors and sulfonylureas and insulin, while pioglitazone, glucagon-like peptide-1 receptor agonists (liraglutide, sematilide) and sodium-glucose cotransporter-2 inhibitors (canagliflozin, empagliflozin) have demonstrated beneficial effects on macrovascular complications. The mechanisms underlying these beneficial effects are discussed, even each drug shows a specific profile of cardiovascular benefit that is unrelated to the reduction in HbA1c and is not a class effect. Thus, when choosing the treatment strategy in patients with T2DM at high CV risk, not only the glucose-lowering effects, but also the overall benefits and risks of CV disease should be taken into consideration.

RT002

TARGETING MITOCHONDRIA IN HEART FAILURE

Enríquez J.A.

CNIC

RT003

WHAT IS NEW ON CARDIOVASCULAR PREVENTION 2018?

Juanatey J.R.G.

Galician Health Service

ROUNDTABLE: BACK TO THE FUTURE: 50 YEARS OF SPANISH PHARMACOLOGY

RT004

1968–2018. RISE AND FALL OF PHARMACOLOGY?

Palacios J.M.

Fundación Kaertor

In the last 50 years the world changed dramatically. And so did Pharmacology. This is related not only to scientific changes in the discipline itself, but also in basic sciences such as Biochemistry and Molecular Biology, in Medicine, and to the new tools generated during the period. Changes in government funding and regulations and in the Pharmaceutical Industry were crucial. The classical physiological methodologies were progressively substituted by biochemical methods. At the same time the introduction of an important number of molecules in the treatment of mental disorders, and the development of propranolol, based in receptor subtypes, were determinant. A golden era in Pharmacology was ending. The "Physiological to Biochemical era" was substituted by the Molecular one, when simple radioligand binding techniques led to the characterization of many new subtypes of receptors, their purification and mechanisms of signal transmission. This had an important impact on the industrial model of drug R&D. During the 80s the introduction of the DNA recombinant techniques led to the isolation of the genes for receptor proteins, understanding of their molecular structure, and of the many new, unsuspected receptor families. The Molecular Genetics was followed by the Genomics era, with the completion of the Human Genome Project in 2003. Changes in Industry were also radical. In one side the Biotech phenomenon. In the other, the consolidation of Industry, with a wave of Mergers and Acquisitions and (paradoxically?) a crisis of productivity. A questioning of the reductionist approaches, the need of a Systems-Integrative Pharmacology, and a change of the R&D model towards a new "Open innovation", defines the last recent years.

RT005

FIVE DECADES OF EVOLUTION IN VASCULAR PHARMACOLOGY RESEARCH

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During the past five decades, landmark discoveries have evolved our understanding in vascular pathophysiology. Questions in the 1970s were focused on the ability of the arteries to contract or relax in response to certain stimuli, their dependence on calcium, the receptors involved and the neurotransmitters that were released from the perivascular nerve endings. In fact, change in the vessels` diameter was thought to be mostly regulated by the autonomous nerve system and hormones. In 1980, Furchgott and Zawadzki changed our focus in blood vessel research with the discovery of endothelium-derived relaxing factor (EDRF) and the posterior acceptance that EDRF was nitric oxide. That was the beginning of a new era of research that exceeded the vascular field. The new position acquired by endothelial cells in the control of vascular function led to study the paracrine regulatory role of other vascular cells, including perivascular adipose cells. In the following decades, research in vascular biology targeted 3 facets, vasomotor tone, inflammation, and the balance between thrombosis and thrombolysis. Different studies indicated that cardiovascular risk factors were related with reduced nitric oxide bioavailability and impaired endothelial function. Consequently, clinical studies began to validate the importance of preclinical vascular biology research in the treatment of cardiovascular diseases. More recently, it has become evident that the immune system and the microbiome play a critical role in the development of cardiovascular diseases. In addition, evidence have revealed complex interactions between vascular cells and circulating factors including stem, progenitor, and differentiated cells, microRNAs, long noncoding RNAs, DNA, hormones, proteins, and lipids, that underlie vascular dysfunction, structural remodeling, and, ultimately, adverse clinical events. The experimental approaches used in this field have changed accordingly. We shifted from using classic pharmacological techniques in isolated organs to using cell cultures, molecular biology and omics technologies, which has also made the research much more expensive. Therefore, researchers devote a huge part of their time seeking for public and private resources to fund the human and material resources that research in vascular pharmacology requires today. This growing complexity has also promoted a changing paradigm evolving from working alone or in very small groups to work collaboratively and in different structures, such as CIBERCV, where basic and clinical groups collaborate to solve clinical problems. Then, what directions will vascular biology research take over the upcoming years?, and how will we advance the field? It is expected that the young researchers of our country will open new avenues with the hope of finding new therapies for patients suffering from cardiovascular diseases.

RT006

THE PAST TO THE FRONT; AND THE FUTURE. . ., WHERE HAS IT GONE?

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Back to the past to envisage the future. This seems to be the aim of this round table. Concerns about current times usually lead humanity to turn its sight to the past looking for answers. The relatively slow pace of drug discovery in the last decades, the increasing costs of drug development, the demographic threats to the sustainability of health systems and the uncertainties generated by the present economical-political crisis have challenged most strategies underlying successful drug research, ultimately casting doubts about an effective progress in pharmacotherapeutics. As a consequence, terms like standardized vs personalized medicine, knowledge-oriented vs application-oriented research, start-up vs big pharma companies, open innovation vs in-home innovation, promoting health vs drug-assisted health improvement are frequently posed as unavoidable dilemmas. Possibly, the answers, my colleagues, are in the wind. If so, the task of the pharmacologist's community should then be to continuously track its changes in direction.

Keywords: drug discovery, open innovation, personalized medicine

References:

[1] N. Author1, N. Author2, N. Author3, *J. Our. Nal.*, **Year**, *Vol.*, page–page. (Helvetica 9 pt).

[2] N. Author1, N. Author2. Book title. ed. n°, Publisher, Publisher City. Year.

RT007

THE NEW DRUG REGULATORY AGENCIES AND THE STAKEHOLDERS ENTER THE SCENE. BEYOND SCIENCE OR REGULATORY SCIENCE?

de Andrés - Trelles F.

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RT008

COLABORACIÓN UNIVERSIDAD-EMPRESA EN I+D DEL MEDICAMENTO: EXPERIENCIA DEL INSTITUTO FUNDACIÓN TEÓFILO HERNANDO DE LA UAM

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Half a century is a good time span to make a reflection on the evolution of pharmacology in general and on my own experience in particular. Fifty years ago, I was a medical student at "Universidad Complutense de Madrid" and at present I am emeritus professor at "Universidad Autónoma de Madrid" (UAM). Which drugs I was taught in 1968 and which are teaching to our students in 2018? An overview of the Goodman and Gilman's "The Pharmacological Basis of Therapeutics" of the third edition of 1965 and the 12th edition of 2017 rapidly tell us the enormous revolution in drug therapy achieved in the last 50 years which obviously, has evolved in parallel with profound and extensive new knowledge on disease pathogenesis, diagnosis and therapy. Our Department of Pharmacology and Therapeutics at the Medical School of UAM emerged under the initiative of professor Pedro Sánchez García about 50 years ago. For years our main interest focused the mechanisms underlying drug actions and the use of drugs as tools to clarify physiological mechanisms. At the turn of the 1990s our first collaboration Academy-Pharma emerged; this was a project with Laboratorios Alter on new dihydropyridine derivatives for cardiovascular diseases. In the next years, collaborations with several other pharma companies were established i.e. Prodesfarma, Ferrer, Janssen, Lilly, Viñas, Bioibérica or Zambón. The "Fundación Universidad-Empresa" facilitated the management of founds. From this time onwards, convergent basic and applied pharmacology developed in parallel. At this time, a solid platform of pharmacologists was building up at the department to mention but a few, Jesús Marín, Mercedes Salaices, Carlos Félix Sánchez Ferrer, Luis Gandía, Concha Peiró, Carmen Montiel, Almudena Albillos, Jesús Frías, Antonio Rodríguez Artalejo, Francisco Abad o Manuela García López. To ease the management of research projects, research fellowships, and personal recruitment, the "Fundación Teófilo Hernando" was created in 1996. The Pharmacology Department was honoring the memory of don Teófilo ever since in 1981 his family donated a sculpture of professor Hernando that was located at the Department hall. Since then, the memory of Teófilo Hernando is honored with the Commemorative Lecture. About 10 years later, the "Instituto Teófilo Hernando de I+D del Medicamento" (ITH) was officially approved by UAM. In 2011, a UAM spin off to ease the translation of knowledge from ITH to society, was also created. With this triad of institutions, with a headquarters located at the Scientific Park of Madrid, Cantoblanco, UAM, we intend to potentiate the drug discovery process from a multidisciplinary point of view, on the grounds that basic and applied pharmacology are mutually enriching. In this frame, FTH displayed during the last 10 years a high activity in the design and coordination of all phases clinical trials. This serve to support the activities of basic and clinical pharmacology as well as the training of young experts in all phases of drug discovery and development. Two masters and several courses and "Cátedras de Patrocinio" strengthens the interaction among Academy and Pharma Industry.

ROUNDTABLE: NATURAL PRODUCTS PHARMACOLOGY

RT009

PHARMACOLOGICAL EVALUATION OF THE OLIVE CONSTITUENTS OLEUROPEIN AND HYDROXYTYROSOL IN CARDIOVASCULAR DISEASES

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Epidemiological data support a lower incidence of cardiovascular diseases in the Mediterranean area compared with the rest of Europe. These findings were attributed to the traditional Mediterranean diet, with its characteristically high consumption of olive oil and olives. The main constituent of the leaves and unprocessed olive drupes of Olea europaea is oleuropein, and the majority of the polyphenols found in olive oil or table olives are products of its hydrolysis (e.g. hydroxytyrosol). Studies support that oleuropein has been recognized as a powerful hypotensive, hypoglycemic and antioxidant agent. In light of the above considerations and of increasing interest in the Mediterranean diet, we evaluated the effect of hydroxytyrosol in an animal model of metabolic syndrome and we observed that hydroxytyrosol ameliorates metabolic, cardiovascular and liver changes in a rat model of diet-induced metabolic syndrome. Additionally, we investigated the effects of oleuropein in the cardiovascular system of animal models by exploring its effects after chronic administration of nutritional doses in normal and hypercholesterolemic rabbits on myocardial infarct size reduction, on oxidative stress, on cholesterol and triglyceride levels. Furthermore, we investigate its mechanism of action concerning the reduction of cholesterol and triglycerides and its role on the induction of pharmacological postconditioning along with the associated intracellular signaling pathway changes after acute administration of two pharmacological doses of oleuropein in an ischemia-reperfusion normal fed rabbit model. We also evaluated the potential cardioprotective activities of oleuropein in cardiotoxicity induced by acute and chronic doxorubicin treatment in rats. Results from our studies indicate that the natural constituent oleuropein in nutritional dose is a potent antioxidant that decreases total cholesterol and triglyceride levels (through activation of PPARα) and considerably reduces infarct size in vivo. Additionally, in pharmaceutical dose induces pharmacological postconditioning in vivo in a dose dependent effect through activation of molecular pathways involved in ischemic postconditioning. Finally, oleuropein successfully treats doxorubicin induced acute and chronic cardiomyopathy in a complex manner including energy metabolism impairment, NO homeostasis, oxidative stress reduction and interference with signaling molecules. Our in vivo data encourages the future use of oleuropein for the treatment of coronary heart disease and doxorubicin induced heart failure.

RT010

COMBINING NATURAL PRODUCTS AND PHENOTYPIC DISCOVERY PLATFORMS TO DELIVER UNIQUE NOVEL MOLECULES FOR PHARMACOLOGICAL DEVELOPMENT

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Microbial natural products (NPs) have been one of the most prolific sources of new leads for the discovery of novel drugs to respond to unmet needs in infectious diseases, cancer and immunological disorders, with a large number of molecules and analogs today in clinical practice. NPs present a unique chemical space and architectural complexity, and their potency and selectivity is the result of an extended evolutionary selection to create biologically active molecules with the required properties to interact and potentially inhibit bacterial targets. MEDINA is a research center focused on the discovery of novel bioactive NPs with one of the richest and most diverse NPs collections that are at the origin of our collaborative drug discovery programs. Our research in microbial natural products is focused on the identification of novel compounds with biological activity as potential new leads to respond to unmet needs in major therapeutic areas including infectious and neglected parasitic diseases, cancer and neurodegeneration. In the course of these integrated screening programs combining the use of phenotypic assay platforms, we have identified different novel families of molecules with interesting new chemistry and biological activities currently in development that will be discussed in the context of current discovery efforts.

RT011

MARINE TOXINS AS DRUG LEADS

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Marine toxins are a large group of compounds with very different privileged structures. Their mode of action cover many targets, but mechanistically they can be regarded as neurotoxin and non-neurotoxic. Those with neuronal targets belong to several groups, each with a defined reference compound, namely saxitoxin, tetrodotoxin, brevetoxin, spirolide, ciguatoxin, domoic acid. Non neurotoxic toxins are yessotoxin, azaspiracid, pectenotoxin, palytoxin, maitotoxin, each with partially understood mechanisms of action. This presentation will discuss about the target for each toxin group, and their potential application in the therapeutic field, specially cancer, Alzheimer and inflammation.

ROUNDTABLE: PHARMACOLOGY TEACHING

RT012

TEACHING OF UNDERGRADUATES IN PHARMACOLOGY IN SPAIN: WHERE ARE WE? WHERE ARE WE GOING?

Bellido I.

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Health science curricula have changed under European Higher Education Area (EHEA) recommendations, resulting in less education in the basic sciences, such as Pharmacology. Currently, in all Degrees in any discipline in Health Sciences in all Spanish Universities, and following the recommendations of teaching within the EHEA, and the Spanish Society of Pharmacology on courses about Pharmacology in the Health Sciences Degrees' core-curricula, there is at least one course on Pharmacology, usually with 6 ECTS. In the teachers' words, Pharmacology courses developing implies a considerably greater effort than that required by traditional teaching, because these Pharmacology courses requiring a personalized teaching in association to the use of new ICT in classrooms full of students, in absence, in the most of the cases, of adequate material, and economic and personal supports. In addition, once the effectiveness of these new EHEA-study plans in the learning of the students have been evaluated, and following the new advances discovered in all the disciplines, including Pharmacology, there are many voices asking for the revision of the new EHEA curricula, regarding the distribution of credits and the courses contents. In what refers to Pharmacology, just 6 ECTS seem to be insufficient in most degrees in Health Sciences. And there are topics that are not included in these new curricula in most of the grades and that should necessarily be included as: Prescribing abilities and Pharmacological advice, Translational pharmacology, Pharmacogenetic, Pharmacoepidemiology (and pharmacovigilance), Nanopharmacology, Pharmacobioinformatic, Environmental pharmacology, Paediatric and Geriatric and Rare Disease pharmacology, Pharmacoeconomy, between others.

Keywords: teaching, undergraduates, pharmacology

RT013

DIGITAL TECHNOLOGIES TO TEACH AND LEARN PHARMACOLOGY

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The digital technology is altering learning and teaching and can be used with different purposes. In this presentation I will present some of the digital technologies implemented in teaching pharmacology in the Faculty of Pharmacy of the University of Coimbra. The digital technologies were used in different contexts, such as inverted learning, *peer* teaching and assessment. Results of student's perception about the relevance of use of these methodologies to their learning and engagement to Pharmacology will be also presented and discussed.

RT014

TEACHING PHARMACOLOGY, CLINICAL PHARMACOLOGY, AND PHARMACOGENOMICS IN GREEK MEDICAL AND PHARMACY SCHOOLS

Manolopoulos V.G.

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The landscape regarding teaching pharmacology, clinical pharmacology and pharmacogenomics in Greece will be presented. In Greece there are Pharmacology departments in each of the seven medical schools, from which more than 1000 students graduate each year, with a total of approx. 40 Faculty members (about a third medically qualified). Four also include a Therapeutic Drug Monitoring Laboratory service. In addition there are pharmacology departments in the 3 Pharmacy schools, with 10 faculty members. Most teaching is done in traditional didactic style although some PBL has been incorporated in some curricula. Recently, in Thessaloniki, a software which teaches pharmacology with game-like features has been introduced and is currently being tested. In only one medical school (in Thessaloniki) there is a separate Clinical Pharmacology department. Teaching of basic and clinical pharmacology occurs in two semesters, usually in late second and third year. An elective course in Clinical Pharmacology and Therapeutics at the final or prefinal year exists in three schools, although in two it is run by pathologists. At the postgraduate level, tracks with emphasis on pharmacology exist in several Masters' programs. In addition, there are 2 dedicated Masters' programs in clinical pharmacology. One at University of Thessaloniki on "Clinical and Industrial Pharmacology", and the other in our department in Alexandroupolis titled "Clinical Pharmacology and Therapeutics". Finally, our department runs since 2003 the only undergraduate course dedicated to Pharmacogenetics and Personalized Pharmacotherapy.

RT015

BASIC AND CLINICAL PHARMACOLOGY IN ITALY: CURRENT CHALLENGES AND OPPORTUNITIES

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Pharmacological Sciences have grown rapidly and extensively during the last forty years, becoming one of the most advanced Academic Areas in Italy, considering the number of enrolled students and the quality of research and teaching. Italian and foreign students have the chance both to achieve intellectual development and to acquire several professional skills. Faculty research interests include bench-to-beside approach, basic pharmacological research and clinical pharmacology, contributing to forward preventive medicine, diagnostic and therapeutic applications to human diseases with a close contact between pre-clinical and clinical approach. Today, just like yesterday, the ultimate goal of pharmacology is to identify new therapeutic targets, to develop and to produce drugs ensuring the safety of patients and efficient usage. In Italy, Clinical Pharmacology is an academic specialty, which is obtained after a 5-year period of specialisation training after which the title of Specialist in Medical Pharmacology is awarded. This specialisation allows entry into the national health system workforce, integrated with, but separated from, that of other professionals who might be drug experts, such as hospital pharmacists. Five years ago, there was a reform within the Medical Pharmacology Schools. Pharmacovigilance, as well as pharmacoepidemiology, regulatory affairs and pharmacoeconomics, plays a relevant role and a significant interest in several national research programmes, in collaboration with the Italian Medicines Agency and the Regional Centres of Pharmacovigilance. Notwithstanding the relative scarcity of pharmaceutical industrial research by the pharmaceutical companies in Italy, several joint research activities with the industry are currently in progress.

ROUNDTABLE: IMMUNOINFLAMMATION

RT016

EMOTIONAL AND ENVIRONMENTAL REGULATION OF THE IMMUNE AND INFLAMMATORY RESPONSE

D`acquisto F.

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Affect and emotion are defined as "an essential part of the process of an organism's interaction with stimuli." Similar to affect, the immune response is the "tool" the body uses to interact with the external environment. Thanks to the emotional and immunological response, we learn to distinguish between what we like and what we do not like, to counteract a broad range of challenges, and to adjust to the environment we are living in. Recent compelling evidence has shown that the emotional and immunological systems share more than a similarity of functions. This talk will provide an overview of the crosstalk between these two systems and the need for a new scientific area of research I called affective immunology (www.affectiveimmunology.com). Research in this field will allow a better understanding and appreciation of the immunological basis of mental disorders and the emotional side of immune diseases.

RT017

IMMUNOMETABOLIC ALTERATIONS IN ARTHRITIS

Mobasheri A.[1,2,3,4,5]

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Metabolism plays a key role in the regulation of inflammatory processes. Under adverse micro-environmental conditions, mammalian cells undergo a switch in metabolism from a resting regulatory state to a highly metabolically and activated state in order to maintain energy homeostasis. This metabolic shift occurs when oxygen levels are low, limiting the metabolism of pyruvate by the tricarboxylic acid (TCA) cycle in mitochondria during oxidative phosphorylation. However, in some instances this occurs under aerobic conditions. We now know that this metabolic shift occurs in cancer and many degenerative and inflammatory conditions thus representing a potential threat to cell function and survival. This phenomenon also leads to an increase in metabolic intermediates for the biosynthesis of inflammatory and degradative proteins. These metabolic intermediates are pro-inflammatory and turn activate key transcription factors and inflammatory signalling pathways involved in catabolic processes and the persistent perpetuation of drivers of disease pathogenesis. Environmental cues such as the availability of nutrients (i.e. glucose) and oxygen are sensed by the mechanistic target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and hypoxia-inducible factor 1 α (HIF-1α) which together can determine cell activation and differentiation. Therefore, this metabolic switch supports inflammation and catabolic pathways. This presentation will focus on aberrant immunometabolism in osteoarthritis (OA) [1] and rheumatoid arthritis (RA) [2] with a particular focus on metabolic dysfunction in chondrocytes and synoviocytes. This presentation will also review the role of impaired metabolism in arthritis therapeutics, highlighting areas for future research, such as the potential to target metabolic pathways and mediators therapeutically.

Keywords: Immunometabolism, osteoarthritis, articular cartilage, synovium, chondrocyte, synoviocyte, homeostasis, ageing, obesity, diabetes, diabesity, adipokine, glycolysis, oxidative phosphorylation, cell signalling, nutrients, glucose, oxygen

References:

[1] Mobasheri A, Rayman MP, Gualillo O, Sellam J, van der Kraan P, Fearon U. The role of metabolism in the pathogenesis of osteoarthritis. Nat Rev Rheumatol. 2017 May;13(5):302–311. https://doi.org/10.1038/nrrheum.2017.50.

[2] Weyand CM, Goronzy JJ. Immunometabolism in early and late stages of rheumatoid arthritis. Nat Rev Rheumatol. 2017 May;13 (5):291–301. https://doi.org/10.1038/nrrheum.2017.49.

RT018

INNATE IMMUNE RESPONSES IN JOINT TISSUES AND THEIR PHARMACOLOGICAL INHIBITION

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The increasing incidence of musculoskeletal pathologies in developed countries has caused a dramatic impact on Europe health system. In fact, 20% of the European population receives chronic treatments to cope with these pathologies. It is widely accepted that the higher incidence of several of these pathologies, such as osteoarthritis (OA), rheumatoid arthritis (RA), and osteoporosis (OP), is related to aging, sedentary lifestyle, metabolic alterations, pollutants, and alterations in the level of sex hormones. However, inflammation has also been implicated in the onset and outcome of these pathologies. Innate immune responses from musculoskeletal tissues contribute to the development and maintenance of this inflammation. Accordingly, in the last years we have explored the pharmacological inhibition of the innate immune receptor, toll like receptor 4 (TLR4), as a therapeutic target to block the inflammatory and catabolic processes observed in the context of OA joint. Also, we have investigated the integration of these responses with tissue specific anabolic signals.

ROUNDTABLE: NOVEL MECHANISMS FOR TREATING CANCER

RT019

NANOMEDICINE: A NEW APPROACH TO GLIOBLASTOMA THERAPY?

Ceña V.[1,2]

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Glioblastomas (GBMs), are the most common type of primary brain tumor, causing about 4% of death cases associated to cancer.[1] Glioblastoma cells are genetically unstable leading to a highly infiltrative, angiogenic and resistant to chemotherapy neoplasm. This, along with the fact that chemotherapy is not effective in the long term, leads to a poor prognosis with a median survival of only about 14 months from diagnosis, and a 2-year-survival rate as low as 3–5%.[2] This poor prognosis even when GBM patients are treated according to standard care makes essential to search for novel therapeutic approaches. Interference RNA (RNAi) technology is a very effective gene silencing mechanism that is also a very promising therapeutic tool since it can knockdown proteins involved in the pathogenesis of different diseases by targeting their mRNA.[3] One example is cancer where RNAi-mediated knockdown of proteins involved in cancer cell survival has been proposed to potentiate antitumoral actions of drugs,[4] establishing another potential therapeutic approach for cancer treatment. Nanoparticles are generally used as delivery agents for drugs and/or siRNA.[5] Although NPs have the same limitations as other xenocompounds to cross the BBB, several strategies have been devised to improve BBB crossing to increase delivery to the central nervous system, so increasing the efficiency of different therapeutic compounds aimed to treat glioblastoma.

Keywords: cancer, glioblastoma, nanoparticles, siRNA, nanomedicine

RT020

PROTEIN-PROTEIN INTERACTIONS: EMERGING ONCOTARGETS IN THE RAS-ERK PATHWAY

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Given the implication of aberrant RAS-ERK signaling in the development of a large number of tumor types, this route is under intense scrutiny to identify new anti-cancer drugs. Most avenues in that direction have been primarily focused on the inhibition of the catalytic activity of the kinases that participate in this pathway. Although promising, most of these have had limited clinical repercussion due to undesired toxicity and/or drug resistance problems. As an alternative path, new efforts are now being devoted to the targeting of protein-protein interactions involved in the flow of RAS-ERK signals. Many of these, have shown promising results in preclinical models, including ERK dimerization that we have recently identified as a potential target for antineoplastic intervention. Following this rationale, we are now focusing on the interactions between ERK and scaffold proteins in order to unveil novel points of therapeutic interest.

RT021

NOVEL PLAYERS INVOLVED IN CHROMATIN FUNCTION AS TARGETS FOR ANTICANCER THERAPIES

Real F.X.[1,2]

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The TCGA and ICGC cancer genome projects, together with technological development in massive parallel sequencing have led to the discovery of novel categories of genes mutated in cancer that had not been previously identified. Many of them code for proteins involved in chromatin biology, including enzymes involved in the post-translational modification of histones, histone readers, chromatin remodelers and the cohesin complex. The mechanisms through which these alterations contribute to cancer is not well established in all cases but their relevance as therapeutic targets is acquiring increasing support. I will discuss recent work in the field, including that from our laboratory focusing on the NURF component BPTF and the STAG2 cohesin.

ROUNDTABLE: CNS PHARMACOLOGY

RT022

OPIOID-BASED STRATEGIES FOR THE TREATMENT OF CHRONIC PAIN

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Opioid agonists such as morphine are the gold standard for the treatment of severe pain. Therefore, their clinical effectiveness is undisputed in acute or cancer pain, however, their use is limited by adverse effects (sedation, apnoea, respiratory depression, addiction…) that mainly result from activation of opioid receptors located in the central nervous system. Also non-steroidal anti-inflammatory drugs are burdened by detrimental side effects such as gastrointestinal bleeding, ulcers, or cardiovascular complications. Thus, novel analgesics are urgently needed. Preclinical research studies have identified an array of new molecular targets that are involved in the establishment and maintenance of chronic pain and may represent interesting targets for pharmacological intervention. Among these targets the opioid system has always played a key role and either its endogenous ligands or its receptors have been widely studied. Opioid receptors are expressed by central and peripheral neurons and by neuroendocrine (pituitary, adrenal), immune, and ectodermal cells. Interactions between immune cell–derived opioid peptides and opioid receptors have been examined extensively, particularly with regard to the induction of analgesia. In addition, the inhibition of endogenous opioid peptides catabolism has been used a novel pharmacological approach. Preventing the extracellular degradation of endogenous opioid peptides by enkephalinase inhibitors, both in central and peripheral nervous systems, has been shown to elicit analgesia in many experimental models of pain and in some human trials. More recently new sciences have drawn the attention of pharmaceutical companies. Nanomedicine is a rapidly advancing field and nanoparticles containing opioid agonists to treat several types of pain have been evaluated. New opioid formulations (liposomes, nanocarriers…) are being developed. The success of some of these new compounds or formulations is based on pharmacokinetic improvements and on the lack of side effects. Unfortunately, even the most sophisticated galenic, pharmaceutic, and pharmacologic strategies have not succeeded in preventing abuse of opioids. Overall, more studies on opioid analgesia are required.

RT023

FROM BEDSIDE TO BENCH TO CLINIC TRIALS: IDENTIFYING NEW THERAPEUTIC TARGETS FOR SCHIZOPHRENIA TREATMENT

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Schizophrenia is a severe and chronic mental disorder. Since the unintended discovery of the antipsychotic activity of chlorpromazine (1950s), the keystone of schizophrenia treatment is based on dopaminergic receptor (D2) blockade action. Clinicians are fully aware that the "one-size-fits-all" strategy used today in the pharmacological treatment of schizophrenia is not adequate. There is a current deadlock in the field of psychopharmacology with little advantage in the latest years. A new paradigm has emerged based on the investigation of D2 postsynaptic downstream pathways and transcriptional patterns, raising the possibility that molecular effects play a critical role in clinical response. Comparison of gene expression profiles in drug-naïve accurately ill patients before and after antipsychotic treatment is an alternative approach. Our data have revealed that six genes (ADAMTS2, CD177, CNTNAP3, ENTPD2, RFX2, and UNC45B) were overexpressed in patients and reverted to control levels of expression after treatment. These results may represent unknown molecular mechanisms behind schizophrenia symptoms and the molecular mechanisms of antipsychotic drugs. Recent results from our group on animal and cellular models seem to provide support to these initials results in humans. Alterations in gene expression could provide relevant information of the molecular basis of the disease and the mechanisms of action of the antipsychotics. There is an urgent need to expand the horizon of pharmacological research by elucidating new mechanisms related to antipsychotic actions.

RT024

PHARMACOGENOMICS REGULATED BY EPIGENETICS

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Brain disorders are known to be associated with genetic changes, but there is more to it. Epigenetic modifications are gaining momentum in trying to explain disease etiology. We apply genome-wide transcriptomic profiling of lymphoblastoid cells lines (LCLs) from healthy individuals and compare them to patients for identifying genes that predict sensitivity to drug response. In one study, we looked at Alzheimer's disease (AD) where brain deposits of amyloid-β (Aβ) plaques and phosphorylated tau neurofibrillary tangles are observed. However, doubts about the central role of Aβ in AD pathology have been raised, as Aβ is a common component of extracellular brain deposits found, also by in vivo imaging, in non-demented aged individuals. Using Aβ sensitivity phenotyping followed by transcriptomic profiling we observed reduced expression levels of RGS2, a key regulator of G-protein-coupled receptor signaling and neuronal plasticity. Data mining confirmed reduced RGS2 expression in postmortem AD brain tissues and peripheral blood. RGS2 is suggested as a novel AD biomarker (alongside other genes) toward early AD detection and future disease modifying therapeutics.

ROUNDTABLE: CARDIOVASCULAR DISEASES

RT025

BASIC SCIENCE AND TRANSLATIONAL POTENTIAL OF HYDROGEN SULFIDE IN THE CARDIOVASCULAR SYSTEM

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Hydrogen sulfide (H_2S) has emerged as an important endogenous gasotransmitter, which regulates homeostasis and affects the function of most organs in the body. Endogenously produced hydrogen sulfide is generated through cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS), which use cysteine as a substrate, and 3-mercaptosulfurtransferase (3-MST), which utilizes 3-mercaptopyruvate as a substrate. CBS expression is higher in neuronal tissues, while CSE predominates in the heart and blood vessels. H_2S production and metabolism has been shown to be deregulated under pathophysiological conditions, contributing to the development and progression of disease. H_2S exhibits a variety of effects in the cardiovascular system that impact on endothelial, smooth muscle and cardiomyocyte function. H_2S promotes endothelial cell growth, migration and organization into capillary networks, enhancing angiogenesis. In addition, H_2S acts on smooth muscle cells to reduce vascular tone leading to hypotension. Both endogenously produced and exogenously administrated H_2S exhibit cardioprotective actions by exerting anti-apoptotic, anti-inflammatory and anti-oxidant effects. H_2S ameliorates the damage that occurs after ischemia-reperfusion in many organs, in addition to the heart. H_2S also protects against the development of heart failure and atherosclerosis. The actions of H_2S in cells and tissues are mediated by modulation of ion channel activity, inhibition of phosphodiesterases and activation of kinases that leads to altered transcription factor activity. Most importantly, H_2S has the ability to trigger posttranslational modifications of proteins by sulfhydrating cysteine residues to modify protein function. Better understanding of the regulation of H_2S production and signaling in physiological and pathophysiological conditions will help harness the therapeutic potential of this gasotransmitter.

RT026

ISCHEMIC CONDITIONING OF THE MYOCARDIUM: TARGETS, COMORBIDITIES AND TRANSLATIONAL STUDIES

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Cardiovascular disease (CVD) is the main cause of mortality and morbidity in the world, accounting for the death of 7.2 million men and women every year. While treatment of the patients that suffer from acute myocardial infarction (AMI) is directed toward opening of the occluded artery as quickly as possible, logistics usually prevent recanalization before a significant amount of cardiac muscle has died. However, the process of myocardial reperfusion can paradoxically induce myocardial injury and alleviate the benefits of the reperfusion. To solve this problem and to improve clinical outcome and prevention of the lethal reperfusion injury, an intervention is needed that would make the heart muscle resistant to necrosis. In the last two decades, a remarkable scientific effort has focused on the limitation of infarct size. Important input from experimental studies has led the way in this direction. Ischemic preconditioning (IPC), postconditioning (PostC) and remote conditioning are the most powerful endogenous mechanisms that limit myocardial infarct size. Despite intensive research, there are currently no effective cardioprotective therapies in clinical practice. The challenge has been to successfully translate novel cardioprotective therapies discovered in the laboratory setting into the clinical setting. Therefore, the recognition and a better understanding of the underlying signal transduction of ischemic conditioning may provide an important paradigm for cardioprotection and its translation to clinical use of pharmacological interventions. Additionally, the majority of experimental studies have used healthy juvenile animal MI models, which do not adequately reflect the clinical setting given that most CVD patients are middle-aged and have co-morbidities (such as diabetes, hyperlipidemia, hypertension). These factors have been shown to confound the efficacy of cardioprotective therapies and need to be taken into consideration when evaluating novel cardioprotective therapies in the experimental and clinical setting. Herein we summarize basic and translational studies for evaluating targets of ischemic conditioning mainly focused on the role of NO pathway in postconditioning, in comorbidities and in remote conditioning.

ROUNDTABLE: GPCR PHARMACOLOGY

RT027

A MOLECULAR PHARMACOLOGY INSIGHT OF 5-HT2A RECEPTOR PHARMACOGENOMICS BASED ON THE H452Y SINGLE NUCLEOTIDE POLYMORPHISM

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The serotonin 2A (5-HT_{2A}) receptor is a GPCR that presents a wide and heterogeneous distribution in human brain where it is implicated in numerous neurological functions such as perception, cognition, memory and mood among others. Within the CNS, 5-HT_{2A} receptors are the main target of a group of drugs known as atypical antipsychotics used for the treatment of schizophrenia. Clozapine has been historically the archetype for atypical antipsychotic drugs although similar compounds are currently used in the clinical practice. Therefore, blockade of 5-HT_{2A} receptors seems to be an important aspect for the treatment of schizophrenia. To date, there have been described seven SNP within the coding region of 5-HT_{2A} receptor human gene and of these five are non-synonymous. The most frequent 5-HT_{2A} receptor SNP, with an allele frequency of about 10% of the population, is the one encoding for the substitution of a histidine at position 452 by a tyrosine (H452Y).[1] Although previous human genetics studies concluded that there is not statistical association of the 452Tyr allele with schizophrenia, several investigations, including a comprehensive meta-analysis, demonstrated a significant association between patients carrying the 452Tyr allele and poor response to clozapine.[2] Taking into account this background, we have conducted molecular pharmacology and phosphoproteomics investigations in cells heterologously expressing both genetic versions of 5-HT_{2A} receptors, i.e., 452His and 452Tyr allele. Significant differences between both genotypes in terms of receptor functionality were found regarding the phosphoproteomic-kinase profile, more particularly in relation to p-ERK and p-Akt responses, that could explain the discrepancies observed previously in patients submitted to antipsychotic treatments.

Keywords: 5-HT_{2A} receptor, Single Nucleotide Polymorphism (SNP), antipsychotics, schizophrenia, phosphoproteomics

References:

[1] Sanders-Bush, E., Fentress, H. & Hazelwood, L. Serotonin 5-ht2 receptors: molecular and genomic diversity. Mol Interv 3, 319–330 (2003).

[2] Arranz, M.J., et al. Meta-analysis of studies on genetic variation in 5-HT2A receptors and clozapine response. Schizophr Res 32, 93–99 (1998).

RT028

ILLUMINATING THE ADENOSINE A_{2A}-DOPAMINE D_2 RECEPTOR OLIGOMER IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative condition in which basal ganglia functioning are altered. Thus, dopaminergic neurons projecting from the substantia nigra to the striatum are selectively lost and, within other consequences, motor control disturbances occur. The main treatment consists of dopaminergic agents (i.e. L-DOPA), which mainly target dopamine D2 receptors (D2R) expressed in the striatum. However, a number of adverse effects appear after prolonged treatments, thus there exist a need for finding out novel drugs solving this question. Interestingly, the existence of a direct receptor-receptor interaction (i.e. oligomerization) between D2Rs and adenosine A2A receptors (A2AR), in which reciprocal negative allosteric interactions occur, have led to investigating the later receptor as a novel target for PD treatment. Accordingly, A2AR antagonists would help to modulate the therapeutic effect of dopaminergic agents and also controlling the appearance of adverse effects. Indeed, inverse agonists (i.e. caffeine), limiting the constitutive activity of A2AR, would be good candidates, as we have found in a mice model of PD. To our knowledge, only one A2AR antagonist has been approved for human use and introduced into clinics, and apart from efficacy issues the reason would be the incidence of A2AR-mediated side effects. Photopharmacology is a novel approach that allows the spatiotemporal control of receptor function. Hence, we have developed a light-sensitive caged A2AR antagonist (MRS7145) to photocontrol the effects in brain (striatum) fiber-optic implanted mice. Interestingly, this kind of tool would also help to characterize the neurophysiology of the striatal circuitry, namely the interaction between dopamine and adenosine receptors. In this sense, it is also important to elucidate the stoichiometry of D2R-A2AR complexes in pathological conditions. Thus, we have provided evidences for the existence of native D2R/A2AR oligomers in rat striatum and human caudate-putamen. And, in addition, we have shown that a change on the proportion of oligomer formation is observed, both in parkinsonian rats and in PD human necropsies. Overall, the fine-tuning modulation, either by the use of light-dependent A2AR antagonists or by adjusting drug-concentrations based on receptors' expression, of D2R-A2AR complexes may evolve as a very promising target for PD management.

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RT029

QUANTIFYING BIASED AGONISM AT G PROTEIN-COUPLED RECEPTORS

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Compelling data in the literature from the recent years leave no doubt about the pluridimensional nature of G protein-coupled receptor (GPCR) function and the fact that some ligands can couple with different efficacies to the multiple pathways that a receptor can signal through, a phenomenon most commonly known as functional selectivity or biased agonism. Biased signaling can be used within a therapeutic framework and currently constitutes a central topic in drug discovery programs.[1] Obviously, an accurate evaluation of biased agonism is needed. Nowadays, transduction coefficients ($\log(\tau/K_A)$), based on the Black and Leff operational model of agonism,[2] are widely used to calculate biased agonism.[3] Notwithstanding its common use, this scale may present confounding results when partial agonists are present in the pharmacological assay. Moreover, because of the definition of the Black and Leff model, scales based on it do not account of receptors showing constitutive activity and, consequently, cannot be applied to inverse agonists. In this communication, we will show our recent efforts to develop complementary methods for the analysis of biased agonism that can be applied to partial[4] and inverse agonists.[5]

References:

[1] Smith, J. S., Lefkowitz, R. J. & Rajagopal, S. Biased signalling: from simple switches to allosteric microprocessors. *Nature reviews. Drug discovery* **17**, 243–260 (2018).

[2] Black, J. W. & Leff, P. Operational models of pharmacological agonism. *Proc. R. Soc. Lond. B. Biol. Sci* **220**, 141–162 (1983).

[3] Kenakin, T., Watson, C., Muniz-Medina, V., Christopoulos, A. & Novick, S. A simple method for quantifying functional selectivity and agonist bias. *ACS Chem. Neurosci* **3**, 193–203 (2012).

[4] Burgueño, J. *et al.* A Complementary Scale of Biased Agonism for Agonists with Differing Maximal Responses. *Sci Rep* **7**, 15389, https://doi.org/10.1038/s41598-017-15258-z (2017).

[5] Hall, D. A. & Giraldo, J. A method for the quantification of biased signalling at constitutively active receptors. British journal of pharmacology, https://doi.org/10.1111/bph.14190 (2018).

RT030

DISCOVERY AND PHARMACOLOGICAL CHARACTERIZATION OF AN A2A RECEPTOR ANTAGONIST FOR IMMUNE-ONCOLOGY DEVELOPMENT

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High levels of extracellular adenosine in the tumor microenvironment promote tumor immune evasion by suppressing Th1 cytokine production and cytolytic activity of T and NK cells. We defined the expression of the four adenosine receptors in primary human immune cells by nanostring. A_{2A} was the main adenosine receptor expressed by T lymphocytes and monocytes, and the only one in mature monocyte-derived dendritic cells and NK cells. We further demonstrated that selective A_{2A} agonists suppressed cytokine production by human activated T lymphocytes and monocytes, highlighting that A_{2A} is the main receptor mediating adenosine signaling in these cells. We demonstrated that A_{2A} antagonists initially designed for Parkinson's disease but repurposed for immuno-oncology dramatically lost potency in a high adenosine environment. We therefore developed EOS100850, a novel non-brain penetrant inhibitor of A_{2A} with sub-nanomolar Ki and selectivity versus A_1, A_{2B}, and A_3 receptors. EOS100850 potently inhibited A_{2A} signaling in human T lymphocytes independently of adenosine concentrations, and rescued cytokine production in the presence of high concentrations of A_{2A} agonists. iTeos A_{2A} antagonist EOS100850 potently rescued Th1 cytokine production in human whole blood treated by A_{2A} agonists, and increased $CD8^+$ T cell cytotoxicity in a co-culture assay of effector $CD8^+$ T cells and target cancer cells. ITeos EOS100850 insurmountable A_{2A} receptor antagonist, uniquely designed to address the challenge of counteracting elevated adenosine concentrations in tumors, was tested in mouse A20 lymphoma model. Combined with anti-PD-L1, iTeos A2a antagonist showed significant enhancement of antitumor activities compared with anti-PD-L1 alone. In addition, 5/10 complete tumor regressions and 3/10 tumor free survivals were observed in this combination group. ESO100850 represents a novel, potent, and best-in-class A_{2A} blocker that has been specifically optimized for immuno-oncology indications.

ROUNDTABLE: RARE DISEASES PHARMACOLOGY

RT031

THE EVOLVING ORPHAN DRUG DEVELOPMENT MODEL

Mingorance A.[1,2]

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Back in the 80s and 90s, few companies considered developing medicines to treat rare diseases, which are those affecting <5 in 10,000 people in Europe. Nowadays, the orphan drug market has become increasingly attractive, and it is hard to find a drug development company that is not considering one or more orphan indications. The reasons behind this market evolution are multiple. Oversaturation and pressure around pricing and reimbursement for the large indications has led many companies to set their eyes on the smaller, untapped, rare indications. Legal incentives to orphan drugs including commercial exclusivity after launch, and the possibility of financing orphan drug development through venture financing, have also contributed to creating a more attractive market in the orphan space. At the same time, the recent advances in genomics have helped us understand that most common diseases are in fact a collection of smaller (often rare) diseases with similar presentation, and the advances in other technologies such as gene editing also make it now possible to target those smaller, genetically-defined, patient populations. This presentation will review these forces and the multiple business models for pursuing orphan indications that they offer, and discuss some of the unique scientific and business aspects that make the orphan space unique, including the crucial central role of rare disease patient organizations.

RT032

DRUG REPURPOSING IN FANCONI ANEMIA THERAPEUTICS

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Fanconi anemia (FA) is a rare inherited disease characterized by bone marrow failure (BMF), malformations, predisposition to leukemia and head-and-neck squamous cell carcinoma (HNSCC) and chromosome fragility due to impaired DNA repair. The only cure of BMF is hematopoietic stem cell transplant. Gene therapy clinical trials are also ongoing [1]. Up to now, no effective drugs has been established to prevent or delay the age-of-onset of BMF or solid tumors beyond tumor resection, because of the extreme toxicity of conventional chemotherapy that suffer FA patients. We adapted and scaled down to 96-well plates the flow cytometric micronucleus assay [2] to identify already approved drugs preventing chromosome fragility in FA cells. In addition we performed a high content screening of all EMA- and FDA approved anticancer drugs to find non-genotoxic chemotherapies to be repurposed to treat FA-HNSCC. The selected hits were evaluated for safety and efficacy in vivo in FA mice and in FA-HNSCC tumor xenografts in immunodeficient mice. Importantly, we managed to find a drug that significantly extended the lifespan and prevented premature ageing of FA mice. In addition, we identified drugs currently used in cancer therapy that induce cancer specific lethality in FA-HNSCC with strong efficacy in vivo in tumor xenograft. The corresponding applications for orphan drug designation have been submitted to EMEA in May 2018.

Keywords: Fanconi anemia, repurposing, drug screening, head and neck squamous cell carcinoma, therapy

References:

[1] M. Bogliolo and J Surralles, *Curr Opinion Genet & Dev*, **2015**, 33, 32–40.

[2] G. Hernández, Ramírez MJ, Minguillón J, Quiles P, Ruiz de Garibay G, Aza-Carmona M, Bogliolo M, Pujol R, Prados-Carvajal R, Fernández J, García N, López A, Gutiérrez-Enríquez S, Diez O, Benítez J, Salinas M, Teulé A, Brunet J, Radice P, Peterlongo P, Schindler D, Huertas P, Puente XS, Lázaro C, Pujana MÀ, Surrallés J. *Nature Commun*. **2018** 9:967.

RT033

AXOPATHY AND MITOCHONDRIA IN RARE GENETIC NEUROPATHIES AND COMMON NEURODEGENERATIVE DISEASES

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Charcot-Marie-Tooth disease (CMT) is composed of rare genetic peripheral neuropathies that involve both myelin and primary axonal defects. There are more than 80 associated genes in CMT and related neuropathies, and the pathogenic mechanisms involve a wide variety of cellular pathways such as gene transcription, membrane metabolism and transport, vesicle transport, the endoplasmic reticulum or mitochondria. The availability of drugs and genetic / cellular therapies is still very scarce. Axonal damage is present in CMT but also in the pathophysiology of most neurodegenerative diseases, so that research in CMT is not only of interest for peripheral neuropathy therapy but also defines molecular and cellular pathways that could be relevant in the therapy of common diseases associated with neurodegeneration such as Parkinson's disease or Alzheimer's disease, and other rare and devastating disorders such as Huntington's disease or amyotrophic lateral sclerosis. In this paper we address mitochondria and their relationship with other cellular structures, such as the endoplasmic reticulum, as a major pharmacological target within the axon and neurons to address the therapeutics of CMT disease, and also as a model to investigate axonopathy as a major druggable lesion in neurodegenerative diseases.

SYMPOSIUM I: PHARMACOGENETIC IMPLEMENTATION IN THE SPANISH HEALTH SYSTEM PART I: PSYCHIATRIC DISEASES

S1-001

TO EVALUATE THE EFFECTIVENESS OF PHARMACOGENETIC (PGX) TESTING IN THE SELECTION OF DRUG THERAPIES IN PATIENTS WITH MAJOR DEPRESSIVE DISORDER (MDD)

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A 12-week double-blind randomized clinical trial in 316 adult patients with MDD (DSM-IV-TR) was conducted. Patients were genotyped with the Neuropharmagen test and randomized to PGx-guided treatment or treatment as usual (control group). The primary endpoint was the proportion of patients achieving a sustained response (Patient Global Impression of Improvement, PGI-I ≤ 2) within the 12-week follow-up period. Additional assessments included the 17-item Hamilton Depression Rating Scale (HDRS-17), Frequency, Intensity, and Burden of Side Effects Ratings (FIBSER), Clinical Global Impression-Severity (CGI-S) scale, Sheehan Disability Inventory (SDI) and Treatment Satisfaction with Medicines Questionnaire (SATMED-Q). Between-group differences were evaluated using $\chi 2$-test or t-test as per data type. At 12-weeks, the PGx-guided treatment group had a higher proportion of responders compared to TAU (47.8% vs 36.1%, $P = 0.0476$, OR = 1.62 [95%CI 1.00–2.61]), although a sustained response was still not observed beginning at 4 or 8 weeks. Subjects in the PGx-guided group showed a higher reduction in HDRS-17 at 6 weeks ($P = 0.0364$) and a trend at 12 weeks ($P = 0.0771$). Effects were larger in patients with a baseline HDRS-17 ≥ 14 and especially in those with 1–3 failed psychiatric medication trials. In subjects reporting side effects burden at baseline, odds of having a FIBSER burden score ≤ 2 were higher in the PGx-guided group than in controls at 6 weeks and maintained at 12 weeks (68.5% vs 51.4%, $P = 0.0260$, OR = 2.06 [95%CI 1.09 – 3.89]). In MDD patients, PGx-guided selection of drug treatment resulted in significant clinical improvement of patient's response and burden of side effects but not on sustained response during the 12-week follow-up. Improvement was particularly relevant for subjects who received 1 to 3 previous psychiatric medication trials.

S1-002

PHARMACOGENETIC INTERVENTION IMPROVES THE SAFETY PROFILE OF ANTIPSYCHOTIC TREATMENTS

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El tratamiento principal de la esquizofrenia y trastornos relacionados son los medicamentos antipsicóticos. Sin embargo, 30–50% de los pacientes tratados no muestran una respuesta adecuada y / o desarrollan efectos secundarios graves y duraderos.[1] Existe una fuerte evidencia que apoya que los factores genéticos influyen en la cinética y la dinámica de los fármacos antipsicóticos y se ha demostrado que los polimorfismos funcionales del CYP influyen en el resultado del tratamiento antipsicótico en numerosos studios.[2] Hay varios kits farmacogenéticos disponibles para predecir la respuesta a los antipsicóticos, sin embargo, su uso en la práctica clínica es mínimo.[3] Nuestro grupo realizo un estudio multicéntrico cuyo objetivo era evaluar los beneficios clínicos del uso de una intervención farmacogenética. La información farmacogenética en los polimorfismos clave de los CYP , se utilizó para ajustar las dosis de AP en un grupo de pacientes que iniciaron o cambiaron el tratamiento con antipsicóticos (PharmG +, $N = 123$), y sus resultados se compararon con los de un grupo de pacientes tratado siguiendo las guías clínicas existente s (PharmG-, $N = 167$). Resultados: En los pacientes que se ajustaron las dosis de antipsicóticos en base a los polimorfismos clave de los CYPs (PharmG +) tuvieron una mayor reducción de los efectos secundarios a diferencia de aquellos pacientes tratados según práctica habitual (PharmG-) Nuestros resultados sugieren que las intervenciones farmacogenéticas pueden mejorar la seguridad de los tratamientos antipsicóticos al reducir los efectos secundarios asociados a la pauta antipsicótica. Esta intervención puede ser particularmente útil cuando se considera el tratamiento con un antipsicótico con solo una vía metabólica principal.

References:

[1] Spear BB., Heath-Chiozzi M., Huff J. Clinical application of pharmacogenetics. Trends Mol Med.2001;7:201–204.

[2] Leon J, Spina E. What is needed to incorporate clinical pharmacogenetic tests into the practice of psychopharmacotherapy? Expert Rev Clin Pharmacol. 2016;9(3):351–4.

SYMPOSIUM I: PHARMACOGENETIC IMPLEMENTATION IN THE SPANISH HEALTH SYSTEM PART II: TRANSPLANTATION

S1-003

PHARMACOGENETICS OF TRANSPLANTATION: CLINICAL IMPLICATIONS

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El escenario del trasplante de órganos es genéticamente complejo ya que en el mismo paciente conviven dos entidades genéticas diferentes: la propia del receptor del órgano, y la del donante, en el órgano donado. En el caso del trasplante hepático esto cobra especial relevancia ya que el hígado del donante será el órgano metabolizador por excelencia. En la terapia inmunosupresora del trasplante de órgano sólido el principal fármaco es el tacrólimus, para el que contamos con una guía de ajuste de dosis inicial del CPIC (Clinical Pharmacogenetics Implementation Consortium) basada principalmente en el SNP rs776746 de CYP3A5. Sin embargo, existen otros eventos clínicos que podrían beneficiarse de la farmacogenética según nuestros resultados: 1) en cuanto al nivel de tacrólimus en sangre, existe una triple interacción entre el genotipo del donante, del receptor y el tiempo, 2) la nefrotoxicidad asociada al fármaco puede predecirse en base a la combinación de rs776746 y del rs1045642 en ABCB1, lo que refleja tanto la metabolización de tacrólimus como su retención dentro de la célula renal. Por último, plantearemos la necesidad de un informe farmacogenético claro, sencillo, útil, integrador y rápido de evaluar por parte del médico para que realmente se pueda implantar una prescripción guiada farmacogenéticamente en la práctica clínica habitual.

SYMPOSIUM I: PHARMACOGENETIC IMPLEMENTATION IN THE SPANISH HEALTH SYSTEM PART III: CARDIOVASCULAR DISEASES

S1-004

PHARMACOGENETICS IN CARDIOVASCULAR DISEASES

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Las barreras existentes para la implementación de la farmacogenética en la práctica clínica son especialmente críticas en el área cardiovascular, a pesar de la existencia de biomarcadores validados con un alto nivel de evidencia en fármacos como acenocumarol, clopidogrel o simvastatina. Entre las estrategias que existen para superar estas barreras se encuentran: (1) Desarrollar de técnicas de genotipado que sean sencillas, rápidas y asumibles para el SNS, (2) Incrementar el conocimiento sobre la utilidad de la farmacogenética entre los clínicos, y (3) Definir adecuadamente la población diana, tiempo de respuesta e información a proporcionar al médico. En el Hospital Universitario La Paz hemos desarrollado dos arrays de SNP (PharmArray® y ClinPharmArray®) que incluyen 180 y 60 SNP, entre los que se encuentran aquellos relacionados con la respuesta a acenocumarol (CYP2C9, VKORC1 y CYP4F2) y clopidogrel (CYP2C19), y con la toxicidad a simvastatina (SLCO1B1). Para facilitar el conocimiento y aceptación de la farmacogenética entre los médicos desarrollamos sesiones clínicas, proyectos de investigación conjuntos y mantenemos contacto directo con el paciente (en la Consulta de Farmacogenética o a pie de cama en pacientes ingresados). El aspecto más crítico en la implementación de biomarcadores en la práctica clínica es definir la población diana y establecer el flujo de pacientes para que la información que reciba el médico prescriptor sea útil y llegue a tiempo. En nuestro caso pretendemos avanzar hacia un genotipado anticipado en poblaciones de riesgo.

S1-005

APPLICATION OF MASSIVE SEQUENCING IN CARDIOLOGY

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A lo largo de la última década, el desarrollo de nuevas técnicas de secuenciación masiva ha supuesto revolución en el campo de la Genética Clínica. Los estudios de asociación basados en la secuenciación de un pequeño número de genes o en la identificación de determinados polimorfismos, están siendo progresivamente sustituidos por técnicas NGS que ofrecen la posibilidad de explorar el espectro completo de variación genética en cada individuo. En el campo de las cardiopatías, las recomendaciones clínicas actuales restringen la utilización de técnicas de secuenciación masiva como herramienta diagnóstica en enfermedades con claro patrón de herencia Mendeliano, como las miocardiopatías hereditarias, las canalopatías y las dislipemias familiares. En la práctica clínica habitual, en función de la patología a estudiar se selecciona un determinado panel de genes para ser secuenciado mediante técnicas NGS. Este abordaje permite identificar variantes patogénicas susceptibles de ser utilizadas para diagnóstico genético en cascada y consejo genético en un porcentaje variable de pacientes en función de la patología en estudio (10–90%). Sin embargo, un elevado porcentaje de casos continúa sin diagnóstico molecular. En este escenario, se ha demostrado que los estudios de exoma y genoma completo pueden proporcionar información adicional de extraordinario valor tanto en el ámbito clínico como en la investigación. Más allá del diagnóstico genético de las cardiopatías, disponer de la secuencia completa del ADN plantea nuevas oportunidades. Entre ellas, puede plantearse un abordaje farmacogenómico dirigido a predecir la efectividad clínica o la susceptibilidad a arritmias ventriculares en respuesta a determinados fármacos.

SYMPOSIUM I: PHARMACOGENETIC IMPLEMENTATION IN THE SPANISH HEALTH SYSTEM PART IV: ONCOLOGY

S1-006

PHARMACOGENETIC MARKERS FOR TOXICITY PREDICTION IN ONCOLOGICAL TREATMENT

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Aunque lógicamente los mayores esfuerzos en la investigación del cáncer se centran en la curación del mismo, no podemos olvidar que la mayoría de tratamientos que se emplean producen efectos adversos severos y que éstos pueden afectar, tanto a la calidad de vida del paciente, como a la administración de la dosis correcta de fármaco, o a la pérdida de líneas de tratamiento, e incluso en casos extremos también pueden llegar a provocar la muerte del paciente. Hoy sabemos que la genética del paciente es el factor que más peso tiene sobre la variabilidad individual a los efectos adversos inducidos por un fármaco. Probablemente, uno de los campos más estudiados es el de la quimioterapia empleada en tumores sólidos, fundamentalmente colon y recto. Y, en este sentido, las fluoropirimidinas 5-fluorouracilo y su pro-fármaco capecitabina han sido ampliamente estudiadas, aunque los casos de éxito de aplicación de la farmacogenética son escasos. Tanto las fichas técnicas de los fármacos, como las agencias reguladoras o los grupos de expertos que establecen guías de dosificación han descrito biomarcadores que poco a poco se van implantando en la clínica habitual. Aun así, se hace necesaria la identificación de nuevos biomarcadores que nos permitan predecir de una manera mucho más precisa qué pacientes van a sufrir reacciones adversas severas a estos fármacos.

S1-007

ANALYSIS OF BIOMARKERS IN SOLID TUMORS USING NEXT GENERATION SEQUENCING GENE PANELS

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El tratamiento personalizado del cáncer, posible a través del análisis de biomarcadores con valor predictivo y/o pronóstico, ha permitido conseguir tasas de supervivencia inalcanzables con los tratamientos convencionales. El uso de paneles de genes analizables mediante NGS (*next generation sequencing*) permite racionalizar el uso de los recursos, a través del estudio de diferentes alteraciones en múltiples genes con un plazo de respuesta y costes razonables. Se han utilizado las plataformas de secuenciación *Oncomine Focus Assay* (n = 178) u *Oncomine Comprehensive Assay* (n = 108) (Thermo Fisher Scientific) para orientar el tratamiento de pacientes del Dpto. de Oncología Médica de la Clínica Universidad de Navarra. Estos paneles están dirigidos el análisis de 52 y 161 dianas respectivamente, genes accionables por tratamientos o participación en ensayos clínicos. La muestra utilizada es ADN y ARN extraído de citología o cortes de parafina, generándose dos librerías que permiten identificar mutaciones puntuales, indels y CNVs (librería de ADN) o traslocaciones (librería de ARN). En nuestra cohorte, >70% de los tejidos procedían de biopsias y 30% de citologías, en su mayoría de carcinomas de pulmón (otros diagnósticos frecuentes carcinomas de colon, ovario, colangiocarcinomas y tumores del SNC). Aproximadamente 74% de los casos analizados con el panel de 52 genes y 87% con el de 161 genes presentaban alguna alteración relevante clínicamente. El uso de paneles de genes analizables mediante NGS es una estrategia rentable, rápida (7 días laborables), clínicamente relevante, y útil para prácticamente cualquier tipo de muestra patológica para orientar el manejo terapéutico en pacientes oncológicos adultos.

SYMPOSIUM II: DEVELOPMENT OF QUALITY STANDARDS FOR PHARMACOGENETIC DIAGNOSIS IN SPAIN

S2-001

PROPOSAL OF A MODEL FOR IMPLEMENTATION OF PERSONALIZED MEDICINE IN HEALTH SYSTEMS IN EXTREMADURA [MEDEA]

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Si bien se ha proclamado la necesidad de la implementación de la Medicina Personalizada, existen barreras de implementación, entre ellas: (1) uso exclusivo de la genética; (2) falta de evaluación de la información generada por un programa útil para el prescriptor en la asistencia sanitaria- prescripción electrónica- que maneje toda la información útil además de la genética; (3) falta de implementación en la investigación para la selección de los participantes en los estudios; (4) Falta de adecuación a la Regulación española y europea; y (5) Evaluación del impacto, análisis de costo/eficiencia para los sistemas públicos de salud. La mayoría de las visiones, incluyen sobre todo el uso exclusivo de la genética, y/o como fenotipo la prescripción monoterapia en el contexto de un Ensayo Clínico o Proyecto de investigación, sin embargo se necesitan diseños como el propuesto en MEDEA, que centren el objetivo en el paciente, en situación real de tratamiento (politerapia), incluyendo no solo la información genética, sino otras fundamentales para la evaluación de la respuesta farmacológica (antecentes personales o familiares de respuesta a fármacos), y que evalúe no solo la asociación de un polimorfismo o varios con un fenotipo determinado, sino ademas su impacto en el Servicio de Salud, en resumen un Proyecto de Evaluación para la implantación de Tecnologías Sanitarias, que permita la toma de decisiones. El último objetivo de Programa de Implementación de Medicina Personalizada propuesto MEDEA será la disminución del daño generado al individuo, la sociedad, entre ella los Servicios de Salud, mediante la prescripción personalizada: (a) en base a factores: genéticos y otros clínicos y fisiológicos: fenotipos metabólicos, evaluación de interacción con el metabolismo endógenos y xenobióticos, ambiente, incluyendo la historia personal y familiar; (b) todo ello incluido en un programa informático que colabore con el prescriptor en la selección de la medicación adecuada (no existente en el mercado con toda esta información y validado clínicamente), (c) sobre la base de una tarjeta personalizada para el paciente. El Programa MEDEA del Servicio Extremeño de Salud desarrollará este Proyecto financiado por el Ministerio de Economía e Industria y el Servicio Extremeño de Salud (2018–2021) mediante Compra Pública Innovadora. Todo ello, en una estrategia de sostenibilidad para las poblaciones Iberoamericanas (400 millones), ya que poblaciones iberoamericanas y española compartimos información genética.

S2-002

IMPLEMENTATION OF QUALITY STANDARDS FOR PHARMACOGENETIC DIAGNOSIS IN SPAIN: HOW, WHEN AND WHY?

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La validez de cualquier prueba científica incluyendo el análisis de biomarcadores para la predicción de respuesta a un tratamiento requiere dos condiciones. La primera la validez fundacional de una metodología esto es que el biomarcador sea válido. Esto es que pueda ser medido con un test analítico con características de determinación bien establecidas y con un marco o cuerpo científico bien establecido y demostrado y que permita determinar el significado fisiológico, toxicológico, farmacológico o clínico del resultado del test. El segundo es la validez en la aplicación. Una vez determinado que el marcador es científicamente válido, ¿está siendo analizado de forma correcta por un laboratorio? Son muchos los aspectos que incluye una buena práctica de laboratorio pero un modelo de calidad (Quality assurance, QA) es siempre aconsejable. Esto requiere acreditación utilizando procedimientos nacionales aceptados para el análisis de biomarcadores predicitivos lo que que incluye controles de calidad (proficiency testing). En Europa una acreditación con ISO15189 (recomendado por EMA) o ISO17025. Sin duda la acreditación y el PT contribuye a mejorar la calidad y permite progresar en estándares. Hablaremos en esta presentación de los estándares necesarios en farmacogenómica y de la necesidad de implementación de sistemas que garanticen la calidad en los diagnósticos famacogenómicos.

S2-003

IMPLEMENTATION OF QUALITY STANDARDS FOR PHARMACOGENETIC DIAGNOSIS IN SPAIN: DESIGN OF THE FIRST PROFICIENCY TESTING

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En la actualidad, el diagnóstico farmacogenético supone una nueva especialización dentro de los laboratorios clínicos en España. Su implantación en el Sistema Sanitario constituye un pilar fundamental de la Medicina Personalizada de Precisión y exige la aplicación de unos estándares de calidad que garanticen la seguridad del paciente al que va dirigido. La implementación en el laboratorio de un sistema de calidad aplicado a la Farmacogenética requiere conocer los requisitos de gestión y técnicos de la norma. Entre ellos, revisaremos lo aspectos fundamentales de la cualificación del personal, instalaciones, equipos y procedimientos, así como la validación, el aseguramiento de la calidad, y los controles interno y externo. En este sentido, el control de calidad externo proporciona un marco de referencia para el desarrollo de nuestra actividad. Surge así la necesidad de la participación en ensayos de intercomparación o Proficiency testing (PT). En la elección de un PT es importante tener en cuenta que, éste, cumpla a su vez los requisitos que aplican a los ensayos de aptitud, con un diseño que incluya entre otros: la coordinación, los criterios para los participantes, la adecuada selección de las variantes génicas y la identificación de las posibles de fuentes de error. Debe cumplir, además, los requisitos de calidad en la producción, el almacenamiento y la distribución de las muestras y sobre todo permitir la evaluación de los informes, no solo los resultados, sino la correcta interpretación clínica y el empleo de una nomenclatura estandarizada, proporcionando finalmente el feedback adecuado a los participantes.

POSTER PRESENTATIONS & ORAL COMMUNICATIONS CANCER

P001
ETHANOL INCREASES SENSITIVITY OF HUMAN MYELOID U937 CELLS TO HYPERTHERMIA-INDUCED APOPTOSIS

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Hyperthermia is a therapeutic approach which has emerged as a potent cancer treatment combined with radiotherapy or chemotherapy and, therefore, the search of chemosensitizers is highly valuable to improve the antitumor properties of hyperthermia. Previous studies suggest that ethanol may increase sensitivity of cells to some chemotherapeutic agents. In the present study, the effect of low concentrations of ethanol (0.25%-1%) in combination with mild hyperthermia (43 °C for 30 min) was investigated. The human histiocytic lymphoma cell line U937 was selected since it is a widely used model for the study of hyperthermia-induced cell death. The results indicate that ethanol enhances cytotoxicity of hyperthermia, as revealed by the MTT assay and by the trypan blue exclusion procedure. No evidences of cytotoxicity were appreciated in the cells treated only with ethanol. In accordance with these results, the flow cytometric studies showed an increase in the percentage of apoptotic cells (2- to 4-fold) in the group treated with ethanol plus hyperthermia as compared to the cells exposed only to hyperthermia. The effect of ethanol was associated with an increase in the activity of initiator caspases -8 and -9 and also in the executioner caspase-3. Inhibition of caspases activities using the broad-spectrum inhibitor z-VAD-fmk blocked the effect of ethanol. A main role seems to play caspase-8 since its specific inhibitor zIETD-fmk but not the specific caspase-9 inhibitor zLEHD-fmk almost completely abolished apoptosis triggered by hyperthermia or hyperthermia plus ethanol. In conclusion, ethanol may be an effective strategy to augment sensitivity of the cells to hyperthermia-induced apoptosis.

Keywords: hyperthermia, ethanol, apoptosis, U937

P002
MELATONIN STIMULATES MELANOGENESIS VIA UP-REGULATION OF TYROSINASE AND TYROSINASE-RELATED PROTEIN 1 IN THE HUMAN MELANOMA CELL LINE SK-MEL-1

Perdomo J.[1], *Quintana C.*[1], *Rubio S.*[1], *González I.*[1,2], *Hernández I.*[1,2], *Loro J.F.*[2,3], *Estévez F.*[1,2], *Quintana J.*[1,2]

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Melatonin is an endogenous methoxyindole synthesized from tryptophan in most tissues that displays a wide range of well-documented biological functions. Although the pineal gland is the main tissue involved in the biosynthesis and secretion of melatonin, most tissues have the enzymatic machinery to synthesize this methoxyindole. Melatonin is produced and metabolized to 6-hydroxymelatonin, 5-methoxytriptamine and N^1-acetyl-N^2-formyl-5-methoxykynuramine in the human skin and also in melanoma cells. Melatonin was firstly isolated from bovine pineal gland and was shown to exhibit skin-lightening properties. In contrast, using the human melanoma cell line SK-MEL-1 as a model of study, we have previously demonstrated that melatonin stimulates the melanogenic capacity of the cells in a dose-dependent manner, an effect which was associated with the reduction in the proliferation. An increase in the melanin content and also in tyrosinase activity was already observed at 24 hr and maximal levels of both were detected at 72 hr. This effect was associated with the elevation in the expression of the melanocyte-specific enzymes involved in melanin biosynthesis, i.e. tyrosinase and tyrosinase-related protein 1, as revealed by immunoblotting studies. In contrast, changes in the cytosolic or nuclear amounts of microphthalmia-associated transcription factor were not detected. Because melatonin induced a fast increase in ROS levels, the oxidative stress could explain the phosphorylation (activation) of p38-mitogen activated protein kinase observed in SK-MEL-1. A major role, however, appears to play GSK3β since its specific inhibitor BIO blocked the increase of tyrosinase activity and melanin production. The effect of melatonin on melanogenesis seems not to be attributable to its metabolites.

Keywords: melatonin, tyrosinase, MITF, GSK3β, BIO, SK-MEL-1

P003
NOVEL NAPHTHOQUINONE-COUMARIN HYBRIDS AS INHIBITORS OF BCR-ABL-STAT5 SIGNALING PATHWAY IN CHRONIC MYELOGENOUS LEUKEMIA

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NPQ and coumarin represent promising scaffolds in medicinal chemistry for finding novel inhibitors of carcinogenic pathways. This is exemplified by the discovery of NPQ-coumarin hybrids as inhibitors of topoisomerase II [1]. BCR-ABL-STAT5 is an oncogenic signaling pathway in Human Chronic Myelogenous Leukemia (CML) and it represents a valid target for anti-CML drug design [2]. In this study, the effects of a novel naphthoquinone-coumarin conjugate NPQ-C6 were evaluated on human CML-derived K562 cells. Live-Cell Imaging analysis revealed that NPQ-C6 inhibited 2D ($IC50AUC = 1.4 \pm 0.6$ μM) growth of K562 cells. NPQ-C6 caused a dose- and time-dependent cell cycle arrest which was associated with increased levels of apoptotic markers (apoptotic nuclei, cleavage of caspase-3, -9, PARP and annexin V-positive cells) and increased γH2AX expression protein, a double-strand DNA break marker. NPQ-C6 showed multikinase modulatory effects through an early increased phosphorylation of JNK, P38-MAPK and AKT, and decreased phosphorylation of ERK1/2, BCR-

ABL and STAT5 and inhibited expression of oncoprotein c-MYC. Molecular modeling suggested to BCR-ABL and JAK2 proteins as potential targets for NPQ-C6. In summary, NPQ-C6 is a novel multikinase modulator that might be effective on BCR-ABL-STAT5 oncogenic pathway in BCR-ABL-STAT5 related malignancies.

Keywords: naphthoquinone-coumarin, BCR-ABL, STAT5, leukemia

References:

[1] Hueso-Falcon I, Amesty A, Anaissi-Afonso L, Lorenzo-Castrillejo I, Machin F, et al. Synthesis and biological evaluation of naphthoquinone-coumarin conjugates as topoisomerase II inhibitors. *Bioorg Med Chem Lett* 2017, 27: 484–48.

[2] Ren R (2005) Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer* 2005, 5: 172–183.

P004
MORPHINE PROMOTES OR COUNTERACTS CELL PROLIFERATION AND MIGRATION THROUGH DIFFERENTIAL ACTIVATION OF INTRACELLULAR SIGNALLING PATHWAYS IN TUMOR CELL LINES

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Opioids are widely used to treat acute, chronic, post-operative cancer pain. Mu opioid receptor (MOR) is expressed in several human malignancies[1] but opioid-mediated effects on uncontrolled cancer cell growth, invasion of normal tissues, tumor recurrence aren't still fully understood.[2] We aimed at characterizing morphine-mediated effects on cell proliferation and migration of different tumor cell lines expressing MOR, and at unraveling molecular mechanisms involved. HeLa cervix carcinoma displayed the highest MOR mRNA levels, followed by DAOY and UW228 medulloblastomas, U87-MG astrocytoma, MCF-7 breast carcinoma, HT-29 colon-carcinoma. MOR protein expression did not mirror that of mRNA, as MCF-7 cells displayed the highest expression (3903 $\pm$ 748.3 fmol/mg), followed by UW228 (2139 $\pm$ 361.9 fmol/mg), DAOY (1600 $\pm$ 427.7 fmol/mg), HeLa (702 $\pm$ 27.0 fmol/mg), U87-MG (604 $\pm$ 160 fmol/mg), HT-29 cells (180 $\pm$ 53 fmol/mg). Morphine did not alter DAOY medulloblastoma cell proliferation and migration, although MOR is highly expressed in these cells. Morphine significantly increased U87-MG astrocytoma cell proliferation and migration in a G-protein-dependent, PKC-, Src-, PI3K-mediated fashion. U87-MG cells lack of PTEN expression. Thus, enabling morphine to significantly activate AKT and PKC, leading to increased proliferation and migration. Similarly, morphine significantly promoted HeLa cervix carcinoma and UW228 medulloblastoma cell proliferation and migration in a G-protein-dependent, PKC-, Src-, PI3K-mediated way. Conversely, MCF-7 breast carcinoma cells express PTEN; in these cells morphine significantly reduced cell migration via activating PTEN and simultaneously inactivating AKT. Moreover, morphine increased MCF-7 cell proliferation only slightly, through G-protein-independent, arrestin-3-mediated signaling. These findings suggest that morphine may favor or counteract tumor cell proliferation and migration depending on PTEN expression and the specific intracellular signaling pathways activated.

Keywords: Opioid analgesics, tumor cell migration, tumor cell proliferation, PI3K/AKT, PTEN

References:

[1] Lennon FE, Mirzapoiazova T, Mambetsariev B, Poroyko VA, Salgia R, Moss J, Singleton PA, *PLoS One.* **2014**, 9(3):e91577.

[2] Bimonte S, Barbieri A, Rea D, Palma G, Luciano A, Cuomo A, Arra C, Izzo F, *Biomed Res Int.* **2015**, 2015:161508.

P005
ANTITUMORAL EFFECTS OF HISPANOLONE DERIVATIVES IN GLIOBLASTOMA CELLS

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Glioblastoma multiforme (GBM) constitutes the most frequent and aggressive primary brain tumor in adults. Even vast efforts have been made to develop effective treatments; the prognosis for the patients is extremely poor. Therefore, there is still an urgent need for novel and effective therapies for treating these tumors. Hispanolone derivatives have been shown to induce apoptosis in several human cancer cells. Nevertheless, the activity of these compounds against glioblastoma cells remains unclear. In the present study, we have investigated the effects of α-Hispanolol (α-H) on apoptosis, migration and invasion of human glioblastoma cells and analyzed the molecular targets underlying its mechanism of action. The results reveal that α-H showed significant cytotoxicity against human glioma cancer cell line U87 and U373 in a concentration- and time-dependent manner. This inhibitory effect was found to be higher in U87 cells and linked to induction of apoptosis as revealed the increase of sub-G1 population after cell cycle analysis and changes in cell morphology. Apoptosis was also confirmed by significant increases of Annexin-V positive cells, and caspase 8/3 activation. Moreover, we also found that α-H down-regulated the anti-apoptotic Bcl-2 and Bcl-xL proteins and up-regulated the pro-apoptotic Bax protein. Furthermore, α-H exhibited inhibitory effects on the migration and invasion of U87 cells in a concentration-dependent manner. Additional experiments showed that α-H treatment reduced the enzymatic activities and protein levels of matrix metalloproteinase (MMP)-2 and MMP-9. Taken together, these results provide important insights into the anticancer activities of α-H against glioblastoma.

Keywords: α-hispanolol, apoptosis, glioblastoma, caspases, migration, MMPs

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0008
ROLE OF EXOSOMAL CX43 AS NANOVEHICLES TO HALT METASTATIC MELANOMA

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Connexin43 (Cx43) a transmembrane protein involved in cell-cell communication and signalling, has been described as a tumor suppressor factor in melanoma, however its role in disease progression remains under debate.[1,2] Exosomes are cell-derived vesicles, which provide signals and "educate" neighboring or distant cells.[3] The presence of Cx43 in exosomes provides these particles with the capacity to exchange with cells small molecules and ions via gap junction

channels (GJs).[4] In this study we have investigated the role of Cx43 and exosomal Cx43 in metastatic melanoma. Exosomes were isolated from human melanoma cell lines and from the same cell lines but overexpressing Cx43 using a vector (M-Cx43 cells). Cx43 was only present in exosomes derived from the melanoma cells that overexpressed Cx43 (M-Cx43). When different melanoma cell lines were exposed to exosomes containing Cx43, these vesicles significantly decreased cell proliferation and blocked colonies growth. The effect of exosomal Cx43 was compared to the overexpression of the protein (using a vector) suggesting that these vesicles might act as a potent tumor suppressor during melanoma progression. Besides, the presence of Cx43 in these vesicles significantly increased the sensitivity of BRAF-mutant metastatic melanoma to drugs such as Dabrafenib and Trametinib. Our results indicate that exosomal particles containing Cx43 are potent drug vehicles to combat metastatic melanoma. Further understanding of the role of Cx43 in the exosomes will have implications for the development of new therapeutic strategies. For instance, we demonstrated their ability as drug carriers to release drugs and to combat metastatic melanoma when these vesicles contain Cx43.

Keywords: Connexin43, exosome, malignant melanoma

References:

[1] A Tittarelli, I Guerrero, F Tempio, M A Gleisner, I Avalos, S Sabanegh, C Ortíz, L Michea, M N López, A Mendoza-Naranjo and F Salazar-Onfray. British Journal of Cancer (2015) 113, 259–267.

[2] Mark J. Ableser, Silvia Penuela, Jack Lee, Qing Shao and Dale W. Laird. The Journal of Biological Chemistry (2014) VOL.289,NO.3, pp.1592–1603.

[3] Marco Tomasetti, Wan Lee, Lory Santarelli, Jiri Neuzil. Experimental & Molecular Medicine (2017) 49, e285.

0009

ALDH1A1 IS A NOVEL REGULATOR OF TUMOR ANGIOGENESIS IN BREAST CANCER

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Cancer progression and resistance to therapy is still a major cause of death in breast cancer patients. Angiogenesis (formation of new blood vessels) is associated with tumor growth and metastasis in patients with solid tumors.[1] Breast cancer with high MVD (microvascular density) are associated with tumor growth, invasion, and metastasis. Accumulating evidence suggests that cancer stem cells (CSCs) play pivotal role in metastasis process,[2] and aldehyde dehydrogenase 1 family member A1 (ALDH1A1) has been identified as a putative CSC marker in breast cancer. ALDH1A1 is highly expressed in breast cancer and its expression has been shown to correlate with metastasis development, resistance to therapy and poor clinical outcome.[3] High levels of ALDH activity coexist with cancer cells, indicating a role for ALDH in the detoxification mechanism, as for cytotoxic anti-cancer drugs suggesting a link with resistance. In this work we determined whether tumor ALDH1A1 was able to influence tumor angiogenesis. We have previously reported that activation of mitochondrial ALDH2 preserves mitochondrial functions and rescues pro-angiogenesis properties in endothelial cells.[4] Hence, we observed that ALDH1A1 expression in breast tumor cells correlated with a significant upregulation of proangiogenic factors. Co-culture with ALDH1A1 expressing tumor cells promoted endothelial cell (HUVEC) proliferation and migration. Conversely, downregulation of endogenous ALDH1A1 resulted in reduction of pro-angiogenic factors and HUVEC recruitment. In vivo ALDH1A1 overexpressing tumors display a higher microvessel density (MVD). These data demonstrate that ALDH1A1 expression is functional in tumor progression and underline its role in tumor angiogenesis.

Keywords: breast cancer cells, aldehyde dehydrogenase 1A1, angiogenesis, drug-resistance

Acknowledgements: AIRC (n. IG15443 to MZ) and MIUR-PRIN (n. 2015Y3C5KP to LM).

References:

[1] O. Gallo, E. Masini, L. Morbidelli, A. Franchi, I. Fini-Storchi, W.A. Vergari, M. Ziche, Role of nitric oxide in angiogenesis and tumor progression in head and neck cancer, J Natl Cancer Inst 90 (8) **(1998)** 587–96.

[2] Y. Liu, D.L. Lv, J.J. Duan, S.L. Xu, J.F. Zhang, X.J. Yang, X. Zhang, Y.H. Cui, X.W. Bian, S.C. Yu, ALDH1A1 expression correlates with clinicopathologic features and poor prognosis of breast cancer patients: a systematic review and meta-analysis, BMC Cancer 14 **(2014)** 444.

[3] E. Charafe-Jauffret, C. Ginestier, F. Iovino, C. Tarpin, M. Diebel, B. Esterni, G. Houvenaeghel, J.M. Extra, F. Bertucci, J. Jacquemier, L. Xerri, G. Dontu, G. Stassi, Y. Xiao, S.H. Barsky, D. Birnbaum, P. Viens, M.S. Wicha, Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer, Clin Cancer Res 16(1) **(2010)** 45–55.

[4] Solito R, Corti F, Chen CH, Mochly-Rosen D, Giachetti A, Ziche M, Donnini S. Mitochondrial aldehyde dehydrogenase-2 activation prevents β-amyloid-induced endothelial cell dysfunction and restores angiogenesis. J Cell Sci. **(2013)**;126(Pt 9):1952–1961.

CARDIOVASCULAR & SMOOTH MUSCLE

P006
UNICENTRIC RANDOMIZED TRIAL ON THE EFFECT OF REMOTE ISCHEMIC PRECONDITIONING IN PATIENTS WITH MYOCARDIAL INJURY AFTER OFF-PUMP CORONARY ARTERY BYPASS GRAFT

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Objective: Remote ischemic preconditioning (RIP) on cardiac surgery has given variable results in different studies. We designed a clinical trial to evaluate the effect of RIP on myocardial injury after off-pump coronary artery bypass grafting (CABG). We also studied clinical effects and the signaling molecules involved: Troponin 12 hours after surgery between two groups (RIP CABG vs Control) and to compare clinical events and protein quantification involved between the two groups.
Methods: Patients (67)* with indication to surgical myocardial revascularization off-pump were selected. Randomization (1:1)

*	RIP CABG (*n*: 31)	Control (*n*: 36)
Age (years)	66.2 (1.34)	62.7 (1.73)
Male	25 (80.7%)	33 (91.7%)
Diabetes Mellitus	16 (51.6%)	20 (55.6%)
Hypertension	22 (71 %)	24 (66.7%)
Smoking	4 (12.9%)	5 (13.9%)
Dyslipidemia	18 (58.1%)	20 (55.6%)
Logistic EuroSCORE	2.4 (1.2–3.6)	2.1 (1–3.2)

The RIP was performed with 3 cycles of 5 min ischemia and 5 min reperfusion in an upper using a blood pressure cuff inflated to 200 mmHg. We evaluated the troponin levels 6, 12, 24 hours after surgery and at discharge. The proteins quantification were obtained at basal and 6, 24 hours after surgery with by WB, renal failure and atrial fibrillation were evaluated.
Conclusion: PIR-GABG in the upper and the lower limbs simultaneously hasn't demonstrated reduce the myocardial injury and neither to have repercussion in the new onset of atrial fibrillation or in acute renal failure. Cardioprotective proteins tend to be expressed more in patients receiving remote ischemic preconditioning with a peak at 6 hours after surgery.

Results: (End point),Troponin levels (ng/ml)

Time group	RIP CABG (n: 31)	Control (n:36)	p value
Preoperative	0.04 (0.03)	0.03 (0.00)	0.84
6 h PO	0.67 (0.11)	0.80 (0.25)	0.33
12 h PO	1.67 (0.28)	2.13 (0.67)	0.28
24 h PO	2.08 (0.76)	2.08 (0.90)	0.5
At discharge	1.03 (0.54)	0.74 (0.32)	0.69

Clinical and laborator:1 death and 1 new onset AF in RIP group; Acute renal failure

ClCr (ml/min)	RIP CABG (n: 31)	Control (n:36)	p value
Preoperative	82.92 (3.56)	79.57 (2.75)	0.77
6 h PO	92.27 (3.38)	93.07 (2.98)	0.43
12 h PO	88.15 (3.64)	88.24 (2.56)	0.49
24 h PO	89.74 (4.26)	86.21 (2.90)	0.76
At discharge	83.48 (3.52)	83.47 (3.37)	0.50

Protein quantification

P007
EFFECTS OF CARVEDILOL ON MIGRATION, ANGIOGENESIS, AND ERK ACTIVATION DEPEND ON THE BLOOD VESSEL AND ENDOTHELIAL CELL TYPE

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Introduction: Carvedilol is a β- and α1-adrenoceptor blocker used in the treatment of cardiovascular diseases, that exhibits biased activity on β1-[1] and β2-adrenoceptors[2] through ERK-phosphorylation. Little is known about its effect on migration and angiogenesis and their relationship to ERK activation.
Aim: To investigate the effect of carvedilol on migration, angiogenesis and ERK-phosphorylation, *in vitro* and *ex vivo*.
Methods: Cell migration and capillary network formation on Matrigel were performed in human aortic ECs (HAoEC), cardiac microvascular ECs (HCMEC), coronary artery ECs (HCAEC) and rat aorta. Cells and tissue were incubated with 1 μM carvedilol or vehicle (DMSO 0.01%) for 8 h (migration), 18 h (ECs angiogenesis) and 6 days (aorta angiogenesis). To analyze ERK phosphorylation, these models were treated with carvedilol (1 μM, 15 min) and ERK and p-ERK expression were detected by western blot. Results were expressed as percentage *vs* untreated cells.
Results: In HAoEC, carvedilol significantly decreased migration (59.94 ± 6.43%; $n = 5$; $P < 0.01$) and angiogenesis (72.34% ± 5.60; $n = 4$; $P < 0.05$). The same effect was observed in HCMEC (61.71 ± 11.18%; $n = 4$; $P < 0.05$ and 84.96% ± 1.98; $n = 4$; $P < 0.01$, respectively). However, carvedilol did not modify neither migration ($n = 4$) nor angiogenesis ($n = 4$) in HCAEC. Similarly, carvedilol did not affect angiogenesis in rat aorta *(n = 5).* Surprisingly, carvedilol reduced p-ERK in HAoEC (63.26 ± 8.44%; $n = 4$; $P < 0.05$), HCMEC (77.75 ± 1.51%; $n = 3$; $P < 0.01$) and aorta (70.86% ± 6.95; $n = 5$; $P < 0.05$) but not in tail artery ($n = 3$).
Conclusion: Our findings provide evidence that carvedilol has an inhibitory role in migration and angiogenesis of some human ECs, but not in rat aorta angiogenesis. p-ERK signal does not seem to be determinant.
Funded by UVEG GR006.
Keywords: Carvedilol, endothelial cells, vessels, migration, angiogenesis, ERK activation, adrenoceptors
References:
[1] IM Kim, DG Tilley, J Chen, NC Salazar, EJ Whalen, JD Violin, HA Rockman, *Proc Natl Acad Sci U S A.,* **2008**, *105,* 14555–60.
[2] JW Wisler, SM DeWire, EJ Whalen, JD Violin, MT Drake, S Ahn, SK Shenoy, RJ Lefkowitz. *Proc Natl Acad Sci U S A.,* **2007**, *104*, 16657–62.

P008

MESENTERIC SYMPATHETIC NEUROTRANSMISSION IS MODULATED BY 5-HT$_{1D}$ RECEPTOR IN RAT

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The mesenteric vascular tone, which is mainly influenced by sympathetic, parasympathetic and non-adrenergic-non-cholinergic neurotransmission, regulates splanchnic blood volume.[1,2] It has been reported that endogenous neuromodulators, as 5-hydroxytryptamine (5-HT), can modify adrenergic activity, contributing to vascular homeostasis.[3] This study investigated the in vivo serotonergic influence, characterizing 5-HT receptors involved, in sympathetic innervation of mesenteric vasculature. Wistar rats were anaesthetised and prepared for the in situ autoperfused rat mesentery, monitoring systemic blood pressure (SBP), heart rate (HR) and mesenteric perfusion pressure (MPP).[4] Electrical stimulation of mesenteric sympathetic nerves resulted in frequency-dependent increases in MPP (9 ± 1.6, 25.7 ± 3.9 and 60.2 ± 5 mmHg for 2, 4 and 8 Hz, respectively), without altering SBP or HR. 5-HT (1–25 μg/kg), 5-carboxamidotryptamine (5-HT$_{1/7}$ agonist; 25 μg/kg) or L-694,247 (5-HT$_{1D}$ agonist; 1- 25 μg/kg) i.a. bolus inhibited vasopressor responses by mesenteric nerves electrical stimulation, unlike i.a. bolus of agonists 8-OH-DPAT (5-HT$_{1A}$), CGS-12066B (5-HT$_{1B}$), BRL54443 (5-HT$_{1e/1F}$), α-methyl-5-HT (5-HT$_2$), 1-PBG (5-HT$_3$), cisapride (5-HT$_4$) or AS-19 (5-HT$_7$) (25 μg/kg each). Interestingly, i.a. L-694, 247 (25 μg/kg) also reduced the exogenous norepinephrine-induced vasoconstrictions. Pretreatment with selective 5-HT$_{1D}$ receptor antagonist, LY310762 (1 mg/kg, i.v.), completely abolished L- 694, 247- and 5-HT-induced mesenteric sympathoinhibition. ELISA analysis confirmed 5-HT$_{1D}$ receptors expression in mesenteric artery. These findings suggest that serotonergic mechanisms-induced sympathoinhibition of mesenteric noradrenergic outflow is mediated by pre and/or postjunctional 5-HT$_{1D}$ receptors.

Keywords: 5-hydroxytryptamine, 5-HT$_{1D}$ receptor, sympathetic neurotransmission, autoperfused mesenteric vasculature, rat

References:

[1] K.L. Christensen, M.J. Mulvany, *J. Vasc. Res.*, **1993**, *30*, 73–79. (Helvetica 9 pt).

[2] E. Sastre, I. Márquez-Rodas, J. Blanco-Rivero, G. Balfagón, *Rev. Neurol.*, **2010**, *50*, 727–737.

[3] J.A. García-Pedraza, M. García, M.L. Martín, J. Gómez-Escudero, A.Rodríguez-Barbero, L. San Román, A. Morán, *Eur. J. Pharmacol.*, **2013**, *714,* 65–73.

[4] M.M. Fernández, A. Morán, M.L. Martín, L. San Roman, *Eur. J. Pharmacol.*, **2000**, *401*, 221–227.

P009

THE PHARMACOLOGICAL BLOCKADE OF INTERLEUKIN-1β PREVENTS ENDOTHELIAL SENESCENCE AND VASCULAR SMOOTH MUSCLE CELL INFLAMMATION

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Vascular complications are the main cause of mortality in type 2 diabetes mellitus (T2DM) patients. This vascular damage is associated with premature vascular ageing and enhanced inflammation, among other factors. (IL)-1β is overexpressed by the adipose tissue in obesity and T2DM. Therefore, the aim of this study was to elucidate the direct impact of the cytokine IL-1β on vascular smooth muscle cell inflammation and endothelial cell senescence and to pharmacological interference with the action of this cytokine. For this study, we used cell cultures of human aortic smooth muscle cells (HASMCs) and human umbilical vein endothelial cells (HUVECs). IL-1β (10 ng/mL; 18 h) induced NADPH oxidase and NF-κB activity, and inducible nitric oxide synthase expression (iNOS) in HASMCs. Moreover IL-1β (2.5 ng/mL; 18 h) induced senescence in HUVECs, determined by positive senescence-associated β-galactosidase staining (SA-β-gal+). These results were accompanied by an increase of total and telomeric DNA damage. In a high glucose environment (22 mM vs 5.5 mM) the pro-inflammatory signal activated by IL-1β was significantly exacerbated. In order to test the effects of IL-1β pharmacological blockade, we used anakinra (1 μg/mL), a biological IL-1 receptor antagonist, which prevented not only the pro-inflammatory action of IL-1β but also the exacerbation observed under a high glucose environment. IL-1β may have a direct role promoting T2DM-associated vascular damage by favouring vascular ageing and inflammation, which is potentiated by high glucose. In these terms, biological IL-1β blockers, such as anakinra, may become a promising tool to treat or delay vascular complications.

Keywords: IL-1β, endothelial senescence, inflammation

P010

CONSUMPTION OF CARDIOVASCULAR DRUGS IN A NURSING HOME IN LEÓN

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Cardiovascular diseases remain the main cause of death in the majority of countries. The recent decrease in cardiovascular mortality in high-income countries is associated with a rise in the numbers of patients living with cardiovascular disease and the wider use of preventive drugs. The purpose of this study was to provide preliminary evidence on the frequency of consumption of those drugs included in ATC group C (cardiovascular system) in nursing home residents in León. This was a observational, descriptive, and retrospective pharmacoepidemiological study of the use of ATC group C by 330 nursing residents in León. The mean age of the residents participating in the study was 86.5 ± 8.1 years old, 64.2% were women and 35.8% men. Cardiovascular diseases were the most prevalent in the center (87.5%; $P < 0.001$, χ^2 test) with higher prevalence in men (88.1%) and in ≥85 years old (90.9%). Drugs included in ATC group C were consumed by 83.6% of the residents, being their use higher in women (86.3%) than in men (78.8%) and in the group of 85 or more years old (85.2%). Considering ATC subgroups, diuretics (subgroup C03) were the most commonly used (61.2%), followed by subgroup C09 agents acting on the renin-angiotensin system (39.4%), subgroup C01 cardiac therapy (21.2%), subgroup C10 lipid modifying agents (20.6%), subgroup C08 calcium channel blockers (12.7%) and, subgroup C07 beta blocking agents (6.4%). The three most commonly used drugs were furosemide (43.6%), atorvastatin (14.9%) and, digoxin (10.9%).

Keywords: Drug consumption, cardiovascular drugs, nursing home

P011
THE ROLE OF HYDROGEN SULPHIDE SIGNALLING IN UTERUS OF DIABETIC MICE

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Diabetes is associated with reproductive impairment in women[1] with an impact on diminution in fertility or in an increase in fetal anomalies.[2–5] To date, the specific mechanism(s) behind these disorders remain largely unknown. Hydrogen sulfide (H_2S) pathway plays a key role in the regulation of uterus motility.[6] We have previously demonstrated that the tocolytic effect of sildenafil is mediated by an increase in H_2S production.[6] On this basis, we investigated the contribution of H_2S signalling and the role of PDE5 in a genetic model of diabetes, the non-obese diabetic (NOD) mice. NOD mice were classified in NODI (normoglycaemic group) and NODIII (hyperglycaemic group). We firstly analyzed the expression of cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), the enzymes involved in H_2S production in uterus. NODIII uterus expressed CBS and CSE in a similar strength of NODI mice but the basal production of H_2S was significantly higher in NODIII compared to control mice. In parallel, L-cysteine (the precursor of H_2S biosynthesis) reduced spontaneous uterus contractility in comparable manner in NODIII and NODI mice while sodium hydrogen sulfide, a H_2S donor, markedly reduced uterus motility in NODIII compared to NODI mice. In addition, PDE5 expression was modified in uterus of NODIII. This data was confirmed by the functional study showing a significant reduction in sildenafil-induced tocolytic effect in NODIII compared to control mice. In conclusion, our results suggest that the disruption of H_2S signalling in uterine tissue could contribute to the impairment of reproductive function in diabetic condition.

Keywords: Mouse uterus, Diabetes, NOD mice, H_2S, Sildenafil

References:

[1] L. Seethalakshmi, M. Menon, D. Diamond, *J Urol,* **1987**; 138, 190–4.
[2] M.J. Lucas, K.J. Leveno, M.L. Williams, et al., *Am J Obstet Gynecol*, **1989**; 161, 426–431.
[3] J.L. Mills, R.H. Knopp, J.L. Simpson, et al., *N Engl J Med,* **1988**; 318, 671–676.
[4] E. Miller, J.W. Hare, J.P. Cloherty, et al., *N Engl J Med*, **1981**; 304, 1331–1334.
[5] M.F. Greene, *Clin Perinatol*, **1993,** 20:533–547.
[6] E. Mitidieri E, T. Tramontano, E. Donnarumma, et al., *Pharmacol Res,* **2016**, 111:283–289.

P012
ANGIOTENSIN-(1-7) ATTENUATES ENDOTHELIAL CELL SENESCENCE VIA NRF2 ACTIVATION

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Background: Endothelial cell senescence is a mechanism contributing to vascular aging, which is also characterized by inflammation and endothelial dysfunction. We explored the capacity of angiotensin (Ang)-(1-7), an heptapeptide of the renin-angiotensin system (RAS), to mitigate human umbilical vein endothelial cells (HUVEC) senescence triggered by RAS-dependent and –independent adipokines, such as Ang-II or interleukin (IL)-1β upregulated in the context of metabolic diseases. In order to identify protective cellular mechanisms activated by Ang-(1-7), we tested nuclear factor (erythroid-derived-2)-like-2 (Nrf2)/heme oxygenase (HO)-1 axis, an antioxidant and anti-inflammatory pathway.

Methods: HUVECs were stimulated with Ang-II (100 nM) or IL-1β (2.5 ng/ml) for 18 h. The number of senescent cell was quantified by positive senescence-associated β-galactosidase (SA-β-gal$^+$) staining. DNA damage was analyzed by indirect immunofluorescence against histone H2AX. Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were quantified by flow cytometry, while leukocyte adhesion was determined using flow chamber assay. Nrf2 and HO-1 protein were quantified by Western blot.

Results: Ang-II and IL-1β enhanced the SA-β-gal$^+$ fraction, ICAM-1 and VCAM-1 expression as well as leukocyte adhesion. All these actions were blocked by the Mass receptor antagonist, A779 (1μM) pointing towards a Mas receptor-dependent mechanism Nrf2 and HO-1 protein levels were increased by Ang-(1-7). Indeed, the effects of Ang-(1-7) were mimicked by the Nrf2 activator sulforaphane (1μM) on the SA-β-gal$^+$ fraction, while the inhibitor of HO-1, Sn-protoporphyrin (1μM) blocked the action of Ang-(1-7).

Conclusion: Ang-(1-7) through the activation of Mas receptors and Nrf2 pathway, can act as a useful tool against endothelial senescence and vascular ageing.

Keywords: Senescence, angiotensin-(1-7), Nrf2

P013
SOLUBLE DIPEPTIDYL PEPTIDASE 4 INDUCES SENESCENCE IN HUMAN ENDOTHELIAL CELLS

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Soluble dipeptidyl peptidase 4 (sDPP4) has been identified as a novel adipokine potentially linking obesity to the metabolic syndrome. sDPP4 serum concentration is upregulated in obese patients and it has been demonstrated to directly contribute to the pathogenesis of atherosclerosis by the induction of vascular smooth muscle cell proliferation, upregulation of pro-inflammatory cytokines and microvascular endothelial dysfunction. Furthermore, obesity and type 2 diabetes mellitus have been considered progeric conditions due to the accelerated vascular ageing displayed by these patients, whose vasculature presents several ageing-related alterations. sDPP4 and other adipokines can exert important deleterious actions in the vascular wall. However, evidence that adipokines could play a role in endothelial senescence is still very limited. The aim of this study was to investigate the direct impact of sDPP4 in the context of endothelial senescence. Endothelial senescence was assessed by senescence associated-β-galactosidase staining and we analysed the expression of pro-senescent markers as the DNA damage indicator γH2AX and the cell cycle arrest protein p53, as well as pro-inflammatory markers NF-κB and IL-1β expression. We observed that sDPP4 induces endothelial senescence in human endothelial cells by a mechanism involving the G-coupled protein proteinase-activated receptor 2 (PAR2) and cyclooxygenase (COX). In conclusion, contrarily to other adipokines, sDPP4 pro-senescent effect does not involve NADPH oxidase activity. These data might bring insight into novel actions of sDPP4 in the vasculature, although still further investigation will be needed to elucidate the connection between sDPP4 derived pro-inflammatory and pro-senescent effects.

Keywords: dipeptidyl peptidase 4, endothelial senescence, vascular ageing, diabetes, adipokines

P014
BIFIDOBACTERIUM BREVE CECT7263 SUPPLEMENTATION PREVENTED MINERALOCORTICOID-INDUCED HYPERTENSION: ROLE OF SHORT-CHAIN FATTY ACIDS

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Probiotics affect the composition of the gut microbiota and their potential therapeutic role is currently a hot topic for research. Recent evidence has shown that probiotics play an important role in hypertension, but the mechanisms involved have not been elucidated yet. Mineralocorticoid-induced hypertension is associated with low gut acetate-producing bacteria. The aim of this study was to investigate whether changes in gut microbiota induced by probiotic treatment or supplementation with the short-chain fatty acids (SCFAs) prevent cardiovascular changes on a renin-independent hypertension model induced by deoxycorticosterone acetate (DOCA)-salt. We studied the effects of chronic treatment with the probiotic *Bifidobacterium breve* CECT7263 (BFM) or SCFAs, butyrate or acetate, on blood pressure, endothelial function, and oxidative status in DOCA-salt-induced hypertension. Animals were randomly divided into five groups: (n = 10): control, DOCA-salt, treated DOCA-salt-BFM (10^9 CFU day^{-1} by oral gavage), treated DOCA-salt-butyrate (0.5 mg kg^{-1} day^{-1}), treated DOCA-salt-acetate (10.7 mg kg^{-1} day^{-1}) both in drinking water, for 5 weeks. We found that both BFM and acetate treatments prevented the increase in systolic blood pressure (SBP) and heart and kidney hypertrophy induced by DOCA-salt. Moreover, they also improved the impaired nitric oxide-dependent vasodilatation induced by acetylcholine in aortic rings from DOCA-salt and reduced the rise in aortic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity found in DOCA-salt animals. None of these effects were observed in treated DOCA-salt-butyrate. In conclusion, BFM and acetate, which constitutes one of the main metabolites of the gut microbiota, prevented hypertension, endothelial dysfunction and vascular oxidative stress by inhibition of NADPH oxidase activity.

P015
CYP2C19 OR CYP3A5 GENOTYPING DO NOT PREDICT CLINICAL RESPONSE TO CLOPIDOGREL

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Clopidogrel has been, along with aspirin, a widely used anti-platelet therapeutic regime. Although generally well tolerated, its efficacy varies among individuals, being the main hypotheses that its bioavailability relies on its bioconversion to the active compound which, in turn, depends on the genetic background and/or interactions with other drugs.[1,2] To determine which factors influence response in our patients, 368 patients receiving combined anti-aggregation therapy with aspirin and clopidogrel were followed for one year to record 30 novel cardiovascular acute events. This clinical relapse was considered as a surrogate endpoint to measure therapeutic response under the influence of the CYP2C19 *2, *3 and *17 and CYP3A5 *3 alleles, as well as the effects of concomitant medication and the presence of known cardiovascular risk factors and comorbidity. We show that either single CYP2C19 or CYP3A5 genotyping, or combined, were not useful to predict clinical efficacy in this cohort. Rather than genetic testing, we have found that clinical observations such as suffering type 2 diabetes mellitus requiring insulin, having several vessels affected, and concurrent medication with calcium channel blockers, regardless of CYP3A5 genotype or drug class were, in that order, the strongest independent predictors of disease relapse.

Keywords: aspirin, clopidogrel, CYP2C19, CYP3A5, diabetes, calcium channel blockers

References:

[1] Siller-Matula JM, Trenk D, Krähenbühl S, Michelson AD, Delle-Karth G. *J Thromb Haemost*. **2014**, 12, 2–13.

[2] Jiang XL, Samant S, Lesko LJ, Schmidt S. *Clin Pharmacokinet.* **2015**, 54, 147–166.

P016
EFFECTS OF BLOOD PRESSURE REDUCTION BY LOSARTAN OR MYCOPHENOLATE MOFETIL IN GUT MICROBIOTA IN SPONTANEOUSLY HYPERTENSIVE RATS

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Hypertension is associated with gut microbiome dysbiosis. Blood pressure (BP) reduction by minocycline was able to rebalance the hypertension-related dysbiotic gut microbiota by reducing the Firmicutes/Bacteroidetes (F/B) ratio in angiotensin II-induced hypertension, and *Lactobacillus* reduced BP in spontaneously hypertensive rats (SHR). However, whether BP reduction by drugs without direct antibacterial effects changes gut microbiota composition is unclear. The aim of this study was to evaluate the effects of the angiotensin receptor blocker, losartan (Los), or the immunosuppressant mycophenolate mofetil (MMF) in gut microbiome in SHR. Twenty-weeks old Wistar Kyoto rats (WKY) and SHR were randomly assigned to four groups (n = 8): control WKY, control SHR, SHR treated with Los (20 mg/Kg/day) and SHR treated with MMF (20 mg/Kg/day). The treatments were given for 5 weeks. Both Los and MMF reduced systolic BP, improved endothelium-dependent relaxation to acetylcholine and vascular oxidative stress, but only MMF reduced the elevated CD4+CD45+ cells in mesenteric lymph nodes from SHR. The three-dimensional principal component analysis of the bacterial taxa showed perfect clustering among groups, with differences between both treated groups. In addition, the diversity and richness of gut microbiota were reduced in control SHR as compared to WKY, but were unaltered by treatment, whereas F/B ratio and acetate- and lactate-producing bacteria were restored after both treatments. In addition, the improvement of gut dysbiosis was linked to higher colonic integrity and lower plasma lipopolysaccharide levels. In conclusion, BP reduction improved gut dysbiosis in SHR, but changes in microbiota were different according to the intensity and/or type of treatment.

P017
PROTECTIVE EFFECTS OF PROBIOTICS AND SHORT-CHAIN FATTY ACIDS CONSUMPTION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Hypertension is associated with gut microbiome dysbiosis, characterized by low short-chain fatty acids (SCFAs), butyrate- or acetate-producing bacteria. Therefore, the modulation of gut microbiota by the administration of SCFAs-producing bacteria may lead to the development of novel antihypertensive therapies. The aim of this study was to evaluate the cardiovascular effects of probiotics *Lactobacillus fermentum* CECT5716 (LC40) or *Bifidobacterium breve* CECT7263 (BFM), and acetate and butyrate in spontaneously hypertensive rats (SHR). Ten five-weeks old Wistar Kyoto rats (WKY) and fifty SHR were randomly assigned to six groups (n = 10): a control WKY group and a control SHR group, a treated SHR-LC40 group, a treated SHR-BMF group, a treated SHR-butyrate group, and a treated SHR-acetate group. The probiotics were orally gavaged at the final concentration of 10^9 colony-forming units day^{-1}. SCFAs supplementation were given as 21.45 mg kg^{-1} day^{-1} acetate or 0.5 mg kg^{-1} day^{-1} butyrate in drinking water. The treatments were given for 13 weeks. Long-term administration of probiotics and butyrate prevented the rise in systolic blood pressure (SBP) and the cardiac hypertrophy. Both groups of probiotics and butyrate treatment improved the impaired aortic relaxation by acetylcholine in SHR. They also restored the increased oxidative stress by reducing NADPH oxidase activity. Acetate did not show protective effects in SHR. In conclusion, this study demonstrates a cardiovascular protective effect of probiotics in SHR by increasing nitric oxide bioavailability. The butyric acid, one of the main metabolites of the gut microbiota, might be involved in the beneficial effects of probiotic in genetic hypertension.

P018
METABOLIC PROFILING OF HEARTS FROM RATS TREATED WITH SERELAXIN

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Introduction: Serelaxin is under clinical trials with patients with acute heart failure to elucidate if it is capable to improve mortality and ameliorate the symptoms. The possible effects of serelaxin on cardiac metabolism have been unexplored. Our objective is to study its in vivo effects on the cardiac metabolic profile.
Methods: Male Sprague-Dawley rats (n = 10) were treated with 0.4 mg/kg/day serelaxin using subcutaneous osmotic minipumps. Metabolic profiling of atrial tissues was analysed using four UHPLC-MS based platforms: 1-fatty acyls, bile acids, steroids and lysoglycerophospholipids, 2-glycerolipids, glycerophospholipids, sterol lipids and sphingolipids, 3-aminoacids and 4-polar metabolites profiling, including Central Carbon Metabolism.
Results: Atrial tissues obtained from serelaxin treated rats showed significant changes in different metabolites. In amino acids, there is a significant decrease in taurine and a significant increase in aspartic acid, D(-)-2-Aminobutyric acid, asparagine and glutamine. In the carboxylic acids, there is a significant increase in malate. In glycerophospholipids, there is a significant decrease in phosphatidylethanolamine (PE): PE (18:2/18:2) and a significant increase in the levels of PE(16:0/22:6) and PE(18:0/22:6) and in phosphatidylcholines (PC): PC(15:0/20:4), PC(16:0/20:4), PC(15:0/22:6), PC(16:0/22:6), PC(18:0/22:4), PC(18:0/22:6), PC(18:1/22:6), PC(40:5), PC(40:8), PC(40:1), PC(38:5) and PC (O-38:4). In sphingolipids, there are a significant increase in ceramides (Cer): Cer(d18:1/23:0) and Cer(d43:1) and in sphingomyelins (SM): SM(42:1), SM(d18:0/16:0), SM(d18:0/22:0), SM(d18:1/16:0) and SM (38:1).
Conclusions: Serelaxin induces significant changes in the metabolome of rat cardiac tissue; increasing the levels of Cer and SM and affecting the PC/PE ratios. These molecules are highly bioactive compounds with profound effects on cardiac physiopathology.
Keywords: serelaxin, relaxin-2, heart, cardiac metabolome metabolism

O001
IS VISFATIN INVOLVED IN THE VASCULAR DYSFUNCTION ASSOCIATED WITH DIABESITY AND AGEING?

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Increased plasma levels of the pro-inflammatory adipokines IL-1β and visfatin/Nampt have been related to diabesity and vascular ageing. We explored whether exposure to these adipokines *ex vivo* or *in vivo* can produce vascular reactivity alterations. Murine mesenteric arteries were pre-incubated *ex vivo* with visfatin/Nampt (50 ng/mL) and/or its specific enzymatic inhibitor FK866 (10 μmol/L), or with IL-1β (5 ng/mL) and/or the IL-1-receptor antagonist anakinra (100 μg/mL). The impact of the NADPH oxidase inhibitor apocynin (10 μmol/L) and the thromboxane A2 receptor antagonist SQ-29, 548 (10 μmol/L) was also tested. Furthermore, mice were infused 7 days with osmotic minipumps containing visfatin/Nampt (100 ng/kg/day) and/or FK866 (2.4 mg/kg/day), as well as with IL-1β (12 mg/kg/day) and/or anakinra (100 mg/kg/day, 3 i.p. doses). Endothelium-dependent microvascular relaxation was studied in noradrenaline-pre-contracted segments (3 μM) exposed to cumulative concentrations of acetylcholine (10 nM – 10 μM). NF-κB activation in aortae was measured as p65 phosphorylation by Western blot. *Ex vivo* treatment with visfatin/Nampt impaired endothelial relaxations in isolated microvessels from control mice. This was prevented by FK866, apocynin and SQ-29, 548. Analogously, *in vivo* infusion of visfatin/Nampt induced defective relaxations, also prevented by FK866, apocynin, SQ-29, 548 and anakinra. Visfatin/Nampt infusion activated NF-κB in aortae via Nampt activity. Similarly, IL-1β produced endothelial dysfunction *in vivo,* which was prevented by anakinra. Visfatin/Nampt, besides being a biomarker, arises as vascular damage mediator by inducing endothelial dysfunction and inflammation, which are hallmarks of premature ageing associated to metabolic alterations. Thus, visfatin/Nampt arises as a potential pharmacological target to prevent those vascular complications.
Keywords: visfatin, IL-1β, endothelial dysfunction

O012
THE PROBIOTIC *LACTOBACILLUS FERMENTUM* PREVENTS DYSBIOSIS AND VASCULAR OXIDATIVE STRESS IN RATS WITH HYPERTENSION INDUCED BY CHRONIC NITRIC OXIDE BLOCKADE

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Lactobacillus fermentum CECT5716 (LC40) reduced blood pressure in spontaneously hypertensive rats. We tested whether the probiotic *Lactobacillus fermentum* CECT5716 (LC40) ameliorates hypertension in rats with chronic nitric oxide (NO) synthase inhibition. Rats were randomly divided into four different groups and treated for 4 weeks: a) vehicle (control), b) vehicle plus L-NAME (50 mg 100 mL^{-1} in drinking water), c) LC40 (10^9 colony-forming units/day by gavage), and d) LC40 plus L-NAME. L-NAME induced gut dysbiosis, characterized mainly by an increased *Fimicutes/Bacteroidetes* (F/B) ratio and reduced *Bifidobacterium* content, increased Th17 cells and reduced Treg in mesenteric lymph nodes (MLN), increased aortic Th17 infiltration and reactive oxygen species, reduced aortic endothelium-dependent relaxant response to acetylcholine, and hypertension. LC40 prevented gut dysbiosis, altered the Th17/Treg balance in MLN, vascular oxidative stress and inflammation, improved slightly endothelial dysfunction but did not inhibit the development of L-NAME-induced hypertension. In conclusion, chronic LC40 treatment, in this model of chronic inhibition of NO synthesis, reduced early events involved in atherosclerosis development, such as vascular oxidative stress and pro-inflammatory status, as a result of prevention of gut dysbiosis and immune changes in MLN, but not hypertension, confirming the critical role of NO in the antihypertensive effects of LC40 in genetic hypertension.

O013
APOE$^{-/-}$ MICE WITHOUT CCR3 RECEPTOR PRESENT HASTENED ATHEROSCLEROSIS

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Introduction and Objectives: Atherosclerosis is one of the most prevalent diseases in Western countries. Eotaxin-1 (CCL11) expression has been detected in human and mouse atherosclerotic aortas, however, its role in the atherosclerotic lesion development remain elusive. In this work, we have compared the lesion formation in apoE$^{-/-}$ mice (apoE$^{-/-}$CCR3^{++} mice) *vs.* those lacking eotaxin receptor (CCR3, apoE$^{-/-}$CCR3$^{-/-}$), both fed with an atherogenic diet.
Material/Methods: Two-month-old apoE$^{-/-}$CCR3$^{+/+}$ and apoE$^{-/-}$CCR3$^{-/-}$ mice were subjected or not to an atherogenic diet (0.75% cholesterol, 10.8% fat) for additional two months. Lesion formation, Collagen production, necrotic *core*, vascular smooth muscle cells (VSMC), macrophage and T lymphocyte content were determined within the lesion through immunohistochemistry and histology.
Results: Both mice types subjected to an atherogenic diet showed clear atherosclerotic lesion formation at the aortic sigmoid valve with enhanced collagen, necrotic *core*, VSMC proliferation, macrophage and T lymphocyte infiltration compared to those subjected to control diet. ApoE$^{-/-}$CCR3$^{-/-}$ mice subjected to an atherogenic diet showed significantly higher lesion formation, collagen content and macrophage and T lymphocyte infiltration within the lesion than apoE$^{-/-}$CCR3$^{+/+}$ mice. Eotaxin-1 expression within the lesion of apoE$^{-/-}$CCR3$^{+/+}$ mice in a hypercholesterolemic scenario was much greater than that detected in apoE$^{-/-}$CCR3$^{-/-}$ mice.
Conclusions: These results suggest that eotaxin-1 (CCL11)/CCR3 axis may exert a protective effect in the development of the atherosclerotic process.
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Keywords: Atherosclerosis, apolipoprotein E, eotaxin-1, CCR3

GASTROINTESTINAL

P020
DOES A HALOGENATED INDOLE DERIVATIVE PREVENT COLORECTAL CANCER ASSOCIATED TO ULCERATIVE COLITIS IN C57BL/6 MICE?

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The indole derivate 5-bromo-1-ethyl-3-[(E)-4-(furan-2-yl)-2-oxobut-3-enyl]-3-hydroxyindol-2-one (RZ) selected by molecular topology (MT) was studied as anti-inflammatory agent on ulcerative colitis (UC).[1] It was also shown to be an effective Akt and β-catenin inhibitor on cancer cell lines.[2] It is known that intestinal chronic inflammation is one of the factors that causes a favorable environment for the initiation and promotion of tumor growth, progression and metastasis. We present the effect of RZ in an experimental method of colorectal cancer (CRC) associated to UC in C57BL/6 mice. RZ was administered orally to the mice at 35 mg/kg by gavage (24 doses). Azoxymethane (AOM) (7.5 mg/kg, i.p., was given 7 days before the first cycle of dextran sulfate sodium (DSS) (2.5%). Two additional cycles of DSS at 1.5% were done. Each cycle consisted in 7 days of DSS followed by 14 days of relapse. Mice were sacrificed at day 63 by cervical dislocation and their colons were removed. 5-Aminosalicylic acid (75 mg/kg) was used as positive control.[3] Treatment with RZ reduced the severity of the chronic UC. This improvement was accompanied by a significative protection of the colon shortening as well as the histological features. Furthermore, RZ reduced the mortality, and the number and the size of tumors induced by AOM/DSS. RZ is a good anti-inflammatory agent although it was not completely effective as a chemopreventive in CRC. Our results corroborate MT as a powerful tool for identifying potential leads and new drugs for the treatment of UC.

Keywords: ulcerative colitis, colorectal cancer, dextran sulfate sodium, indole derivative

P021
KEY ROLE OF PALMITOYLETHANOLAMIDE IN EXPERIMENTAL COLON CARCINOGENESIS

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Palmitoylethanolamide (PEA) is both a dietary component and an endogenous mediator whose actions are mainly terminated by the enzyme N-Acylethanolamine-hydrolysing acid amidase (NAAA). Previous studies have shown that PEA exerts antiproliferative effects in cancer cells and attenuates intestinal inflammation *in vivo*.[1–4] Here, we have tested whether or not an elevation in PEA levels (either by its exogenous administration or by inhibition of its endogenous hydrolysis) may affect colon carcinogenesis. We have evaluated the *in vivo* (i.p.) effect of PEA (10 mg/kg) and of two NAAA inhibitors [i.e. AM9053 (20 mg/kg) and F96 (10 mg/kg)] in the azoxymethane (AOM) murine model of colon carcinogenesis. PEA (30 μM) and AM9053 (1–3 μM) were also tested on cell growth in colorectal cancer (Caco-2) and healthy colonic epithelial cells by using the BrdU incorporation. Finally, we quantified (by quantitative PCR) the NAAA mRNA expression as well as the expression of the key targets of PEA in colonic samples from colorectal cancer (CRC) patients. Exogenously-administered PEA and inhibitors of its enzymatic degradation reduced the numbers of tumors in the colon *in vivo* as well the proliferation of CRC - but not of healthy colonic - cells. In clinically-diagnosed CRC patients, we found a trend toward a reduction in NAAA expression and a significant decrease of the main downstream targets (i.e. PPARα, GPR55 and cannabinoid receptors) of PEA action. Collectively our results suggest that i) elevation of PEA levels counteracts experimental colon carcinogenesis and ii) adaptive changes of PEA targets occur in CRC patients.

Keywords: Palmitoylethanolamide (PEA), gastrointestinal disorders, colon cáncer

P022
A CRITICAL APPRAISAL OF THE PROPOSED CATEGORIZATION OF DRUG HEPATOTOXIC POTENTIAL LISTED IN LIVERTOX® WEBSITE

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Objective: Various commonly used drugs can cause idiosyncratic hepatotoxicity, but the potential of each drug is unclear A recent proposal (Bjornsson & Hooffnagle, Hepatology 2016) categorized drugs listed in LiverTox® (https://livertox.nih.gov/) as A, B (high hepatotoxicity potential) and C, D (less hepatotoxicity potential) based on number of published case reports. We aimed to test the classification accuracy when applied to the prospective Spanish DILI Registry.

Method: Culprit drugs in the Spanish DILI Registry were assigned to each of the categories A-D. Clinical, biochemical and outcome data were compared between the A/B and C/D groups.

Results: 567 DILI cases were classified as A/B and 87 DILI cases as C/D. No differences were observed in age or sex. Jaundice was more frequent in the A/B cases (70% vs 57%, $P = 0.02$), while hospitalization was similar in the two groups (61% vs 58%). A/B had shorter time to onset (median: 62 vs 97 days, $P = 0.05$). Hepatocellular damage predominated in both groups (52% vs 65%). Surprisingly, cases categorized with low hepatotoxicity potential presented higher severity with more ALF cases (7% vs 3%, $P = 0.02$).

Conclusions: Hepatotoxicity potential classification based on case report information can be misleading due to publication biases. The C/D category contains drugs with safety concerns, such as nefazodone and dronedarone. Hence, C/D drugs can cause severe DILI as seen in the current study. Drugs with sanitary regulatory measures should be classified separately. Furthermore, the classification is heavily dependent on constant updating of new case report publications.

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Keywords: drug-induced liver injury, hepatotoxicity

INFECTIOUS DISEASES

P023
PROTEOMIC DIFFERENTIAL PROFILE OF SENSITIZATION IN PATIENTS INFECTED WITH ROTAVIRUS VERSUS PATIENTS VACCINATED WITH ROTATEQ™

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Introduction: The rotavirus is a virus from Reoviridae family and has 11 segments of dsRNA in its genome that encode 12 proteins (VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6). This virus is the main cause of acute stomach flu in childhood and produce 1 death in 5 years old children each 20 seconds. The available vaccines against rotavirus have demonstrated to be effective and to have a big impact on disease.
Objective: Analyse the proteomic profile in serum of infected patients with rotavirus and vaccinated patients with RotaTeq™ vaccine, to determinate its immunological cellular response.
Methods: We used 2D electrophoresis and western blot for proteins separation from wild type rotavirus and its detection through antibodies- virus binding protein in a mixed of 10 serum samples of rotavirus infected patients and mixed 10 serum samples of vaccinated patients with RotaTeq™ vaccine.
Results: In infected patients we observed a homotypic profile of immune response, detecting the antibodies binding to VP1, VP6, VP7, NSP1, NSP2, NSP3 and NSP5 rotavirus proteins, all of them with post-translational modifications (PTM). In patients vaccinated, with heterotypic profile, we observed the same proteins but without PTM, we also observed two more rotavirus proteins VP2 and VP3, that were not found in infected patients.
Conclusion: We could identify differences in proteomic profile between infected patients and vaccinated patients and these differences were associated with post-translational modifications (PTM) of viral molecules. These PTM can affect both the activity and the potentiation of immune response.
Keywords: rotavirus, vaccines, immunization, proteomics, RotaTeq™.

P024
PHARMACOKINETICS OF CEFONICID AFTER INTRAVENOUS AND INTRAMUSCULAR ADMINISTRATION TO GOATS

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Introduction: Cephalosporins are a large group of β-lactam antimicrobial drugs that inhibit cell-wall synthesis. They are divided into first, second (cefonicid), third and fourth generation compounds.[1]. The aim of this study was to determine the specific pharmacokinetic profile of cefonicid in goats after intravenous (IV) and intramuscular (IM) administration.
Materials and Methods: A cross-over study (2 × 2 × 2) was carried out in six healthy Murciano-Granadina goats (2-3 years old). Each animal received single IV and IM injections of cefonicid at a dose of 10 mg/kg with at least 15-day washout period. Plasma concentrations of cefonicid were determined by a previously validated high performance liquid chromatography (HPLC) with ultraviolet detection. Blood samples were collected before and at predetermined times over a 12 h-period. Non-compartmental pharmacokinetic parameters were estimated using WinNonlin Professional™ (version 5.2).
Results and Discussion: The values of the main pharmacokinetic parameters are presented in the following table:

Parameters	IV	IM
λ_z	2.65 ± 0.42	0.79 ± 0.16
$t_{½\lambda z}$ (h)	0.27 ± 0.05	0.93 ± 0.21
V_z (L/kg)	0.22 ± 0.02	–
MRT (h)	0.35 ± 0.02	1.18 ± 0.09
CL (L/ h·kg)	0.58 ± 0.05	–
AUC_{Inf} (mg·h/L)	18.58 ± 2.58	20.69 ± 4.82
MAT (h)	–	0.83 ± 0.08
C_{max} (mg/L)	–	17.55 ± 1.50
t_{max} (h)	–	0.27 ± 0.08
F (%)	–	109.02 ± 9.41

Cefonicid administered intravenously showed a short elimination half-life similar to those reported for other cephalosporins in goats as cephalexin[2] and ceftriaxone.[3] Half-life and MRT were significantly longer after IM administration than those after IV administration. This fact could be due to a flip-flop phenomenon where absorption is the limiting step for drug elimination ($MAT > MRT_{IV}$).
Keywords: pharmacokinetics, cefonicid, goats, intramuscular, bioavailability.
References:
[1] R. Finch, D. Greenwood, R. Whitley, S.R. Norrby. Antibiotics and Chemotherapy. 9th ed. Sanders Elsevier, Philadelphia, **2010.**
[2] L. Ambros, V. Kreil, L. Tarragona, A. Montfrinotti, R. Hallu, M. Rebuelto**.** J. Vet. Pharmacol. Therap. **2010,** 34, 397-402.
[3] TK. Sar, TK. Mandal, SK. Das, AK. Chakraborty. Drug Metab. Lett. **2008**, 2, 23-28.

P025
CEPHALOSPORIN SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM COMMERCIAL RABBIT AND GOAT FARMS IN SPAIN

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Introduction: Cephalosporins are a large group of β-lactam antimicrobial drugs that inhibit cell-wall synthesis.[1] *Staphylococcus aureus* is one of the most important pathogenic agent in veterinary medicine.[2] This study was designed to evaluate the degree of in vitro activity of cefonicid (Cd), ceftiofur (Cr), cephalothin (Cl) and cephalexin (Cx) against ten *S. aureus* strains isolated from rabbits with staphylococcosis and from the milk of goats with mastitis.
Materials and Methods: Minimum inhibitory concentration (MIC) tests were performed according to the microdilution broth method as

Species	Antibiotics	CMI (mg/L) ≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	≥32
S. aureus	Cd					1	3	2	3			1
S. aureus	Cr					2	5	2			1	
S. aureus	Cl				7	1					1	1
S. aureus	Cx							3	3	3		1
E. coli ATCC 25922						Cr			Cd	Cl,Cx		
S. aureus ATCC 29213					Cl		Cr	Cd	Cx			

recommended by the Clinical and Laboratory Standards Institute.[3] The MIC was defined as the lowest concentration of antibiotic at which the bacterial growth was completely inhibited.

Results and Discussion: The MIC values of the selected antibiotics are shown in Table 1:

The MIC_{50} and MIC_{90} values were 2 and 4 mg/L for cefonicid, 1 and 2 mg/L for ceftiofur, 0.25 and 16 mg/L for cephalothin and 4 and 8 mg/L for cephalexin, respectively.

Keywords: *Staphylococcus aureus*, cephalosporin, goat, rabbit, mastitis.

References:

[1] R. Finch, D. Greenwood, R. Whitley, S.R. Norrby . Antibiotics and Chemotherapy. 9th ed. Sanders Elsevier, Philadelphia, **2010**.

[2] K. Hermans, P. De Herdt, L.A. Devriese, W. Hendrickx, C. Godard, F. Haesebroucj. Vet. Microbiol. **1999**, 67, 37-46.

[3] Clinical and Laboratory standards institute. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically; approved standard, eighth ed. NCCLS document M07–A8. Clinical and Laboratory Standards Institute, Pennsylvanis, USA. **2009.**

P026
HETEROLOGOUS EFFECT OF PCV13 ON ALLERGIC ASTHMA AND TYPE 1 DIABETES MELLITUS

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Background: Allergic asthma (AA) and type 1 diabetes mellitus (DM) share an exacerbates auto-aggression response of the immune system, produced by a deregulation of the immune response effector. Conjugated 13-valent pneumococcal vaccine (Prevenar13®, VCN13, Pfizer) has demonstrated a beneficial immunomodulatory effect in murine models of allergic asthma, through promotion of regulatory T cell subpopulation, achieving the suppression of the clinical symptoms and inflammatory response that characterize the disease.

Objective: To evaluate a possible heterologous immunoregulatory effect of vaccination with PCV13 in a paediatric population with a well-defined immunological basis: type 1-diabetes mellitus and allergic asthma.

Method: A phase IV single-centre randomized clinical trials was carried out to compare results of vaccination with PCV13 with asthmatic (n = 20) and diabetic (n = 26) patients with results obtained from vaccination in an age-matched healthy population (n = 17). Evaluation of changes in proportion of effector immune sub-populations: IFN-γ, IL-17 and IL-4 secretor-CD3+ T cells after PCV13 vaccination were analysed considering the geometric mean and the standard deviation for all groups in each visit.

Results: Significant differences in immune sub-populations were found only in diabetes group in the percentage of IFN-γ secretor CD3+ T cell, where a decline was observed in follow-up visit 2, regarding recruitment visit (P-value = 0.0083). No significant differences were observed in the rest of parameters and groups.

Conclusion: A slight immunoregulatory heterologous effect in diabetes paediatric patients was shown after PCV13 vaccination. Persistence of this effect over time and clinical impact on the pathology needs to be elucidated.

Table Geometric mean and standard deviation for all groups in each visit.

	Diabetes Mellitus 1 (DM)			Allergic Asthma (AA)			Healthy Population		
	%IFN-γ	%IL-17	%IL-4	%IFN-γ	%IL-17	%IL-4	%IFN-γ	%IL-17	%IL-4
Basal visit	22,36% ± 7,30%	8,24%±3,32%	0,61% ± 0,49%	18,12% ± 6,69%	8,14% ± 3,23%	0,95% ± 0,60%	13,70% ± 5,50%	6,15% ± 2,84%	0,84% ± 0,65%
Visit 1	20,12% ± 6,49%	9,25% ± 4,04%	0,87% ± 0,67%	16,39% ± 4,53%	8,83% ± 3,40%	0,26% ± 0,84%	12,58% ± 3,89%	6,00% ± 1,45%	0,81% ± 0,55%
Visit 2	16,91% ± 6,22%	7,95% ± 2,92%	0,83% ± 0,55%	14,36% ± 2,48%	8,08% ± 3,64%	1,15% ± 0,78%	14,48% ± 5,06%	8,00% ± 2,96%	0,89% ± 0,41%

Keywords: vaccination, immunology, cytokines.

INFLAMMATION

P027
ROLE OF THE MITOCHONDRIAL NA+/CA2+ EXCHANGER IN NLRP3 INFLAMMASOME ACTIVATION

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The pathophysiology of multiple neuroinflammatory diseases involve the activation of NLRP3 inflammasome.[1] Several studies propose mitochondria as key elements in the activation of this inflammatory signalling pathway[2]; however, the exact mechanisms operating in this process are poorly understood. Previous results have shown that inhibition of the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) by the benzothiazepine CGP37157 exerts a protective effect in several *in vitro* models of neurodegeneration.[3] In this context, our aim is to study the role of NCLX in NLRP3 inflammasome activation. We have used the compound ITH12575, a synthetic derivative of CGP37157, and we have studied its effect in glial mouse primary cultures and in the mouse macrophage cell line J774 A.1 exposed to NLRP3-activation conditions. Stimulation of glial cultures with lipopolysaccharide (LPS) 1 μg/ml during 3'5 hours followed by ATP 5 mM 30 min induced NLRP3 inflammasome activation and IL-1β release. ITH12575 reduced the release of this cytokine in a concentration-dependent manner (1, 3 and 10 μM). NCLX inhibition also reduced oxidative stress parameters (ROS and RNS) caused by LPS. LPS treatment of J774 A.1 macrophages for 24 hours induced the stabilization the hypoxia-inducible factor 1-alpha (HIF-1α) and pro-IL-1β expression, an effect that was notably potentiated under hypoxic conditions (1% O_2). Inhibition of NCLX by ITH12575 reduced HIF-1α stabilization and pro-IL-1β expression, suggesting a possible mechanism by which mitochondria can be participating in NLRP3 inflammasome activation. From these results we can conclude that inhibition of NCLX by ITH12575 reduces IL-1β release in glial cultures by inhibiting NLRP3 inflammasome.

Keywords: NLRP3 inflammasome, NCLX, mitochondria, inflammation, ROS.

References:

[1] F. Awad, E.Assrawi, C. Louvrier, C. Jumeau, S. Georgin-Lavialle, G. Grateau, S. Amselem, I. Guirgea, S-A. Karabina. *Pharmacology & Therapeutics.* **2018.** https://doi.org/10.1016/j.pharmthera.2018.02.011.

[2] R. Zhou, A.S. Yazdi, P. Menu, J. Tschopp. *Nature.* **2011.** *469*, 221–225.

[3] F.J. Martínez-Sanz, R. Lajarín-Cuesta, A.J. Moreno-Ortega, L. González-Lafuente J.C. Fernández-Morales R. López-Arribas, M.F. Cano-Abad, C. de los Ríos. *ACS Chemical Neuroscience.* **2015**. *6*, 1626–1636.

P028
GRACILINS AS LEAD COMPOUNDS FOR ANTI-INFLAMMATORY EFFECTS

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Gracilins are diterpenes derivative, with diacetoxyhexahydrodifuro structure, isolated from the marine sponge *Spongionella gracilis*. Gracilins have tyrosine kinase inhibitory properties and antioxidant and neuroprotective capacity. It has been reported that some gracilins showed binding affinities to cyclophilin A (Cyp A) and cyclophilin D (Cyp D). Cyclophilins are a group of proteins with peptidyl prolyl *cis-trans* isomerase (PPIase) activity which bind strongly to cyclosporine A (CsA), a potent immunosuppressant. CsA-Cyp A complex inhibits calcineurin phosphatase activity leading to has an immunosuppressant effect. Thereby, due to its ability to join the CsA, gracilins are good candidates to study for their anti-inflammatory and immunosuppressant capacity. The aim of this work was to study the potential anti-inflammatory activity of the norditerpene sponge-derived gracilin L and two synthetic analogs, Mika 17 and 19, on a cellular model of inflammation. In this way microglia, BV-2 cell line was used. Cellular viability was determined by MTT test and the compounds did not show cytotoxicity effect at concentrations lower than 1 μM. To carry out the experiments the cells were pre-treated with the compounds for 1 h and stimulated with lipopolysaccharide (LPS) for 24 h to determine reactive oxygen species (ROS) production and the release of NO, IL-6 and TNF-α. CsA was used as control of effect in all experiments. *Spongionella* compounds decreased intracellular ROS production induced by LPS as well as the release of NO, IL-6 and TNF-α to the media culture. For this reason, natural and synthetic gracilins could be interesting for developing anti-inflammatory drugs.

Keywords: inflammation, gracilins, ROS, NO, IL-6, TNF-α.

P029
INHIBITORY EFFECTS OF OLEUROPEIN AND ITS PERACETYLATED DERIVATIVE ON HUMAN OSTEOCLASTOGENESIS

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During inflammation, several growth factors and cytokines are increased inducing osteoclast (OC) differentiation and activation. Chronic inflammation is a condition that initiates systemic bone loss. Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterised by active synovitis and associated with early periarticular bone loss. Oleuropein (OL) is an olive oil secoiridoid with antioxidant and anti-inflammatory properties. The present study was designed to investigate the effects of OL and its peracetylated derivative (Per-OL) on osteoclast differentiation induced by the macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor κ-B (RANK) ligand (RANKL) in freshly isolated human circulating monocytes. Tartrate-resistant acid phosphatase (TRAP) staining was used as a marker of OC activity. Cell viability was determined using MTT assay. OC-related gene expression and cytokines were assessed by RT-qPCR and ELISA, respectively. OL and Per-OL inhibited osteoclastogenesis by means of a reduction of the TRAP activity and a downregulation of OC marker gene expression (TRAP, RANK, OSCAR and CATHK), while promoted the transcriptional activation of OPG gene in the monocyte-derived OCs. OL and Per-OL also induced a downregulation of MMP-9 gene expression and an upregulation in the expression of HO-1 gen. Additionally, OL and Per-OL decreased the release of osteoclastogenic cytokines (TNF-α, IL-1β and IL-6) and increased the release of IL-10 (anti-osteoclastogenic cytokine) in the medium of the monocyte-derived OCs. Overall, these

results suggest that OL and its peracetylated derivative are effective inhibitors of M-CSF/RANKL-induced osteoclastogenesis in human monocytes *in vitro* and may be potent therapeutic agents for bone-related diseases such as RA.
Keywords: VOO, Oleuropein, Osteoclasts, RANKL.

P030
OLEUROPEIN AND ITS NEW ACYLATED DERIVATIVES REDUCE LPS-INDUCED INFLAMMATORY RESPONSE IN MURINE PERITONEAL MACROPHAGES

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Background: Oleuropein (OL) is a secoiridoid with antioxidant and anti-inflammatory properties. The present study was designed to investigate the potential anti-inflammatory activity of three new acyl derivatives from OL with better pharmacodynamic/pharmacokinetic profile, in an *ex vivo* model of murine peritoneal macrophages stimulated with LPS compared with OL, and clarify the potential molecular mechanisms involved.
Methods: Isolated murine peritoneal macrophages were treated with OL or its derivatives in the presence or absence of LPS. Cell viability was determined using Sulforhodamine B assay. Nitric oxide production was analyzed by Griess method. Production of cytokines was evaluated by ELISA. Pro-inflammatory enzymes and signalling pathways were determined by Western blot.
Results: OL derivatives reduced significantly nitrites levels and Th1, Th2 and Th17 inflammatory cytokines levels and COX-2/ iNOS overexpression. Besides, OL derivatives demonstrated to be able to inhibit janus kinase/signal transducer and activator of transcription pSTAT3, and (ERK, JNK and p38) mitogen-activated protein kinases (MAPKs) activation and as well as increased haem-oxygenase 1 (HO-1) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) expressions showing better results than the observed in the natural compound.
Conclusions: These new OL derivatives exhibited better anti-inflammatory effects that OL; that seems to be related to the modification of the chemical structure of the original compound. The mechanisms underlying these protective effects could be related to the activation of the antioxidant Nrf2/HO-1 pathway and inhibition of both JAK-STAT and MAPKs signalling pathways. Therefore, these new synthetic derivatives may constitute an interesting alternative in the management of inflammatory related pathologies.
Keywords: COX2, HO-1, macrophages, MAPKs, Nrf2, oleuropein.

P031
PERACETYLATED-HYDROXYTYROSOL, A NEW HYDROXYTYROSOL DERIVATE, REGULATES NON-CANONICAL INFLAMMASOME SIGNALLING PATHWAY IN LPS-STIMULATED MURINE PERITONEAL MACROPHAGES

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Background: Hydroxytyrosol (HTy) is an olive polyphenol with interesting effects as well as very good available safety/toxicity. The present study was designed to characterize the role of the inflammasome-signalling pathway in the anti-inflammatory response of a new HTy derivative: peracetylated hydroxytyrosol (Per-HTy) compared with its parent, on lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages.
Methods: Isolated murine peritoneal macrophages were treated with HTy or Per-HTy in the presence or absence of LPS (5 μg/mL) for 18 h. Cell viability was determined using sulforhodamine B (SRB) assay. Supernatants were collected, and then it were used to determined cytokine production of interleukin (IL)-1β, using Enzyme-linked immunosorbent assay (ELISA) kits. IL-18 expression and inflammasome pathway were evaluated by Western blotting.
Results: Our finding demonstrated a potent reduction on IL-1β and IL-18 pro-inflammatory cytokines levels, after treatment with HTy or Per-HTy when compared with LPS-DMSO treated control cells. In term of canonical via, HTy and HTy-derivate did not significantly decreased NLRP-3, ASC, procaspase-1 and caspase-1. However, the treatment of peritoneal macrophages with Per-HTy, but not HTy, produced a significant down-regulation of non-canonical inflammasome-signalling pathway by decreased pro-, partially cleaved and cleaved caspase-11 enzymes.
Conclusions: This study establishes, at first time, that Per-HTy could improve LPS-induced inflammatory response on murine peritoneal macrophages via non-canonical inflammasome-signalling pathway by decreasing pro-inflammatory IL-1β and IL-18 cytokine levels as consequence of regulation of pro-, partially cleaved and cleaved caspase-11 enzymes.
Keywords: caspase-1, caspase-11, inflammasome, inflammation, macrophages, NLRP-3.

P032
NEW PHARMACOLOGICAL TOOLS TO BLOCK TLR4-ASSOCIATED INFLAMMATION IN OA CHONDROCYTES

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The rising preponderance of rheumatic diseases in our society has caused a dramatic impact on social welfare. Osteoarthritis (OA), the most prevalent rheumatic disease, is defined by joint-space narrowing due to progressive cartilage degradation. No treatment has yet proved to be efficient enough despite the growing knowledge in OA pathophysiology. Damage-associated molecular patterns (DAMPS) activate chondrocyte inflammatory responses through innate immune receptors such as Toll-like receptor 4 (TLR4). There are currently no available drugs aimed to block TLR4-mediated inflammatory responses. Nonetheless, there are already known drugs being employed in other indications that could have this activity; namely amitriptyline, naloxone, and thalidomide. The anti-inflammatory effect of these drugs upon TLR4 activation was determined in human primary OA chondrocytes. The co-stimulation of these cells with the TLR4 agonist LPS [100 ng/ml] and amitriptyline [1 μM], reduced the mRNA expression of pro-inflammatory factors LCN2 (90%), IL-6 (95%) and MCP-1 (87%). The pre-stimulation of naloxone [100 μM] with LPS also reduced the mRNA expression of LCN2 (53%), IL-6 (78%) and MCP-1 (79%). Similar results LCN2 (63%), IL-6 (74%), and MCP-1 (78%) were obtained upon the pre-stimulation of these cells with thalidomide [500 μM]. Methyl-thiazolyl-tetrazolium (MTT) reagent confirmed that the drugs did not significantly affect chondrocyte viability. Amitriptyline, naloxone, and thalidomide have already passed the toxicology and safety tests for their clinical use and could be ready for its repurposing in the management of TLR4 innate immune responses in OA patients.
Keywords: TLR4, OA.

P033
ANTI-INFLAMMATORY PROFILE OF 3,4,6-TRIMETHOXY-6'-TRIFLUOROMETHYLCHALCONE BY INHIBITION OF INFLAMMASOME PATHWAY

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Inflammasomes are intracellular sensors activated by pathogen-associated molecular patterns such as LPS, inducing IL-1β and IL-18 maturation by Caspase-1 activation.[1] Inflammasome activity has been linked to many diseases including arthritis. Previous studies demonstrated the potential anti-inflammatory profile of 3,4,6-trimethoxy-6'-trifluoromethylchalcone (CH) in the mouse air pouch model.[2] In the present study, we have evaluated the mechanism of action of CH in mouse macrophages in order to determine its regulation on caspase-1 activation. Peritoneal macrophages were isolated 4 days after thioglycollate injection into the peritoneal cavity.[3] Cells were pre-treated with CH (1–10 μM, 30 min), and stimulated with LPS (1 μg/ml, 4 h) and ATP (5 mM, 30 min). Cell supernatants were used to determine IL-1β, IL-18, CXCL-1 and TNF-α by ELISA, and active p20-subunit of caspase-1 by Western Blotting. The effect of CH on ROS release was determined in the RAW 264.7 cell line, after stimulation with TPA and detection by luminol-chemiluminescence signal. Nrf-2 translocation was determined by immunocytochemistry. Results demonstrated that IL-1β (12 067 ± 3785 pg/ml) and IL-18 (1401 ± 300 pg/ml) levels in control supernatants were significantly decreased after CH treatment (IL-1β: 10 μM 8806 ± 3715 pg/ml, 1 μM 7852 ± 4065 pg/ml; IL-18: 10 μM 670 ± 94 pg/ml, 1 μM 920 ± 260 pg/ml). Besides, the increase in the expression of activated p20 subunit from control cells was reduced by 68% after CH treatment at 1 μM. Furthermore, CH produced a significant concentration-dependent inhibition of ROS generation, which maybe related with the nuclear translocation of Nrf2 induced by CH. In conclusion, CH decreases cytokine levels and down-regulates caspase-1 activation. These results demonstrate that inhibition of inflammasome activation by CH could be a potential mechanism of its anti-inflammatory effect.

Keywords: : inflammasome, caspase-1, macrophages.

P034
CHARACTERIZATION OF THE SYSTEMIC INFLAMMATORY STATE IN METABOLIC SYNDROME: ROLE OF NEUTROPHILS, EOSINOPHILS AND CX3CL1/CX3CR1 AXIS

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Introduction and Objectives: Metabolic syndrome (MS) is characterized by several physiological alterations leading to a systemic inflammatory state. Previous results from our group detected an increased activation state of platelets, monocytes and lymphocytes in MS patients, as well as enhanced CX_3CR1 expression in these populations. Therefore, in the present study we have now explored the impact of MS on neutrophils and eosinophils, their CX_3CR1 expression and the levels of circulating cytokines and adipokines.
Material/Methods: Whole blood samples from 18 MS patients and 21 age-matched controls were analysed by flow cytometry to determine the percentage of circulating neutrophils and eosinophils, neutrophil- and eosinophil-platelet aggregates, CX_3CR1 expression and their activation state (CD11b expression). Additionally, the plasma levels of some cytokines and adipokines were measured by ELISA.
Results: Increased percentages of circulating eosinophils and eosinophil-platelet aggregates were detected in MS patients compared with age-matched controls. Higher percentage of CX_3CR1-expressing cells in MS patients than in the control group was also found when platelets were bound to them together with increased percentage of activated neutrophils and eosinophils. Finally, enhanced levels of TNFα, IL-8 (CXCL8) and leptin and decreased levels of adiponectin were detected in MS patients.
Conclusions: These results suggest that MS promotes neutrophil and eosinophil activation which may be the consequence of enhanced circulating levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory adipokines.
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Keywords: metabolic syndrome, cardiovascular, inflammation, fractalkine.

P035
TOWARDS EXTRACELLULAR VESICLE THERAPEUTICS: ANNEXIN-A1 AS A POTENTIAL CHONDROPROTECTIVE MICROVESICLE EFFECTOR IN OSTEOARTHRITIS

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Adipose tissue-derived mesenchymal stem cell (ASC) extracellular vesicles (EV) have shown promising immunomodulatory properties in chronic inflammatory pathologies such as osteoarthritis (OA).[1] However, EV isolation and characterization has not been standardized yet. In this work we have compared several isolation procedures to better identify microvesicle (MV) proteins with potential chondroprotective effects. We obtained ASC from abdominoplasty-derived fat, and comparatively isolated MV from their conditioned medium by single-step precipitation, ultracentrifugation or exclusion chromatography. Concentration and size were evaluated by RPS in freshly isolated MV and after several freeze-thaw cycles. Then, we treated OA chondrocytes, isolated from knee articular cartilage of advanced OA patients and stimulated with IL-1β, with different MV concentrations to calculate the MIC_{50} value for the inflammatory markers IL-6 and TNFα. Our results indicate that ultracentrifugation provides the greatest MV recovery. Successive freeze-thaw cycles significantly reduce MV concentration. The anti-inflammatory and membrane-repairing annexins are considered ubiquitous EV markers.[2,3] We detected an increased annexin-A1 expression in MV-treated OA chondrocytes. Similarly, MV treatment upregulated collagen II in chondrocytes stimulated with IL-1β for 72 h compared to control. To determine the contribution of annexin-A1 to this effect, we blocked this MV protein a specific antibody, which significantly prevented the enhancement in collagen II expression induced by MV. Cell-free approaches can have promising advantages in therapeutics. In this context, we have developed a robust protocol to isolate ASC MV and test their effects. With this method we have also identified annexin-A1 as a potential chondroprotective agent in OA.

Keywords: EV, inflammation, osteoarthritis, mesenchymal stem cell.
References:
[1] M. Tofiño-Vian, MI. Guillén and MJ Alcaraz. *Biochem Pharmacol.*. **2018**, *Feb 7; S0006-2952(18)30061-3.*
[2] S. Mathivanan, H. Ji and RJ. *J. Proteomics.* **2010**, *Sep 10;73 (10):1907–20.*
[3] MH. Sheikh and E. Solito. *Int J Mol Sci.* **2018**, *Mar 31;19(4).*

P036
CD16 MACROPHAGES ACT AS A SOURCE OF PROFIBROTIC FACTORS PROMOTING INTESTINAL FIBROSIS

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Background: Fibrosis is a common complication of Crohn's disease and it is related to dysregulated tissular repair following inflammation. Macrophages play a central role in both mucosal repair and fibrosis and these cells constitute an important source of pro-fibrotic factors.[1] $CD16^+$ macrophages are increased in fibrotic tissue of Crohn's disease patients[2] and we aim to analyse the relevance of CD16 positive macrophages as a source of fibrotic mediators in murine intestinal fibrosis.
Methods: Colon resections from donor mice were transplanted subcutaneously into the neck of recipient mice. After 7 days, intestinal grafts were obtained. An adjacent segment of the colon from each donor mouse was kept to be used as autologous control tissue.[3] The expression of macrophage markers, pro-fibrotic markers and Wnt ligands and receptors was analyzed by qPCR.
Results: Fibrotic tissue compared with control tissue revealed: a) increased profibrotic markers Vim (4.6 ± 1.1 vs 1.0 ± 0.1), α-Sma (13.0 ± 1.7 vs 1.1 ± 0.2) and Col1a1 (69.4 ± 17.3 vs 1.7 ± 0.7); b) increased macrophage markers F4/80 (7.9 ± 2.2 vs 1.1 ± 0.1), CD16 (23.8 ± 3.5 vs 1.3 ± 0.3), CD206 (10.2 ± 4.6 vs 1.3 ± 0.4) and CD86 (15.8 ± 2.9 vs 1.1 ± 0.2); c) increased expression of Tgf-β (2.1 ± 0.2 vs 1.0 ± 0.0). Moreover, fibrotic tissue showed: a) a positive and significant correlation between CD16 and Wnt6 and between CD16 and Tgf-β and b) a positive correlation between Wnt6 and Fzd10.
Conclusion: CD16 positive macrophages are increased in murine intestinal fibrosis and these cells may act as a source of fibrotic factors such as Tgf-β and Wnt6.
Keywords: Wnt ligands, intestinal fibrosis, macrophages.

P037
ANTIRESORPTIVE AND CHONDROPROTECTIVE EFFECTS OF PTHRP (107-111) IN A CHRONIC MODEL OF ARTHRITIS IN MICE

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PTHrP derived peptides are used for the treatment of several bone resorption diseases due to its anabolic properties. Furthermore, one of them, osteostatin (PTHrP (107-111)), has shown anti-inflammatory properties *in vitro*.[1] The aim of the present study was to assess whether osteostatin is able to reduce inflammation and bone resorption in Collagen Induced Arthritis (CIA) in DBA1/J mice. After the onset of the disease we administered 40 μg/kg/day, 80 μg/kg/day and 120 μg/kg/day s.c. of osteostatin for 13 days. On day 14, mice were sacrificed and blood was collected for Luminex ® assay in order to measure bone metabolites, such as DKK-1 and Sclerostin (SOST). Paws were sectioned for X-rays analysis and for tissue sections and safranin, TRAP and haematoxylin & eosin stainings. Results showed a general dose-dependent improvement of arthritis macroscopic score on day 14 between treated and non-treated mice. Bone resorption metabolites levels were reduce by treatment with osteostatin, as well as TRAP staining. X-rays showed an improvement in bone density in treated mice. Furthermore, safranin staining revealed an increase of chondrocyte survival in ankle joint and a maintenance of cartilage integrity when mice were treated with osteostatin. Nevertheless, haematoxylin & eosin staining did not show any change in cell infiltration. These results demonstrate that osteostatin is able to reduce not only bone resorption, but also joint inflammation and cartilage erosion in a model of arthritis. Hence, osteostatin could be a candidate for rheumatoid arthritis treatment.
Keywords: Arthritis, bone, osteostatin, in vivo, CIA.
References:
[1] Platas J, Guillén MI, Gomar F, Castejón MA, Esbrit P, Alcaraz MJ. J Gerontol A Biol Sci Med Sci. 2016;0(0):1–8.

P038
ADENOSINE A_{2B} RECEPTOR PLAYS AN ESSENTIAL PROTECTIVE ROLE IN PSORIASIS

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Adenosine ameliorates psoriasis through the interaction with its cell-surface receptors (A1, A2A, A2B and A3).[1] Previously, we have demonstrated the anti-inflammatory and antiproliferative properties of selective activation of A2B receptor in human keratinocytes.[2] In the present study we determine the effect of A2BR agonists and antagonists in the mouse skin hyperplasia model induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in order to assess the role of this adenosine receptor in psoriasis-like skin. The A2BR agonist BAY60-6583 (BAY) (10 μg/site), the A2BR antagonist PSB-1115 (50 μg/site), and both together were applied on the shaved backs of female Swiss mice 30 minutes before TPA (2 nmol/site) for three consecutive days. The evolution of skin lesions were visually scored, using a scale of 0-4. The next day, animals were sacrificed and 1 cm2 punch biopsies were collected, weighed and either homogenized to measure inflammatory mediators or processed for histological analysis. Topical application of BAY (10 μg/site) diminishes the severity of skin lesions induced by TPA, producing a significant reduction of edema (110.2 ± 5.7 mg vs 134 ± 7.8 mg in TPA-treated mice) and IL-1β release (464.1 ± 21.6 pg/ml vs 761.1 ± 62.2 pg/ml in TPA-treated mice). It is interesting to note that treatment with PSB not only reversed the beneficial effect of BAY on the skin, but also worsened the lesions and the inflammatory response. In fact, there was a remarkable increase in levels of IL-1β (1909 ± 125.2 pg/ml vs 761.1 ± 62.2 pg/ml in TPA-treated mice). All these results in a "in vivo" study suggest the beneficial role of A2B receptor in regulating epidermal integrity and inflammatory response on skin.
Keywords: adenosine, A_{2B} receptor, BAY60-6583, PSB1115, skin.
References:
[1] BN. Cronstein, M. Sitkovsky. Nat Rev Rheumatol., **2017**, *12*, 41.51.
[2] RM. Andrés, MC. Terencio, J. Arasa. JID., **2017**, *137*, 123–31.

P039
SUCNR1 AS A NEW PHARMACOLOGICAL TARGET FOR INTESTINAL FIBROSIS

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Background: Intestinal fibrosis is a common complication associated with Crohn`s Disease (CD) which cannot be reverted with any drug and forces repeated surgery. Succinate is accumulated in inflammatory pathologies and activates hepatic fibroblasts through its receptor SUCNR1. We aim to analyze the role of SUCNR1 in intestinal fibrosis.
Methods: Intestinal resections from CD and colon-carcinoma patients were obtained. SUCNR1 and α-sma were analyzed by qPCR and immunostaining. Primary intestinal fibroblasts from human, wild-type (WT) and SUCNR1-/- (KO) mice were isolated and treated with succinate (0, 0.1, 0.5, 1.5 mM) for 24 hours. Intestinal fibrosis was induced *in vivo* using the heterotopic transplant model. The expression of profibrotic markers was analyzed by qPCR and collagen layer was analyzed with Sirius Red staining.
Results: SUCNR1 is expressed in epithelial and $\alpha\text{-sma}^+$ cells of intestinal resections from CD patients and correlates positively with α-sma ($r = 0.759$, $P < 0.0001$, $n = 24$) and colA1 expression ($r = 0.82$, $P < 0.001$, $n = 24$). In primary fibroblasts, the expression of SUCNR1 was significantly higher in CD-fibroblasts (13.54 ± 4.6) vs control-fibroblasts (2.07 ± 0.86). Succinate induced the expression of ColA1, α-sma, Tgfb, and TIMP1 in a dose-response manner. This profibrotic effect was also observed in WT-fibroblasts and it was completely reverted in KO-fibroblasts. The murine model of intestinal fibrosis revealed in KO- vs WT-colons a reduction in: a) thickness of collagen layer; b) expression of colA1, α-sma and vimentin (19.7 ± 10.1 vs 69.8 ± 26.6, 0.9 ± 0.1 vs 2.5 ± 0.5, 1.7 ± 0 vs 12.4 ± 0.3, respectively).
Conclusion: SUCNR1 is increased in CD-fibroblasts, its activation induces a pro-fibrotic effect and mediates murine intestinal fibrosis, which points to SUCNR1 as a new pharmacological target for CD.
Keywords: SUCNR1, succinate, intestinal fibrosis.

P040
TLR4 IS INVOLVED IN S1P/$S1P_1$ PATHWAY IN THE LUNG AND CONTRIBUTES TO S1P-INDUCED ALLERGIC INFLAMMATION

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Background: Sphingosine-1-phosphate (S1P) levels significantly increase in BAL of asthmatic patients following segmental allergen challenge and this increase well correlates with pulmonary inflammation.[1] Compelling evidence suggest that Toll-like Receptor 4 (TLR4) is required to develop allergic responses. Many association studies have reported that TLR polymorphisms predispose to allergic disease.[2]
Aim: To investigate the role of TLR4 in S1P-dependent asthma-like disease in mice.
Methods: BALB\c or C3H/HeJ (TLR-4 defective) female mice received on days 0 and 7 subcutaneous injection of S1P (10 ng) or lipopolysaccharide LPS (0.1 μg, intranasal instillation) or the association LPS+S1P. In another set of experiments BALB/c mice received the purified rabbit anti-TLR4 (10 μg) 30 min prior to S1P administration. Mice were sacrificed at days 10 and 21; their bronchi and lungs were used for functional and molecular studies.
Results: S1P administration promotes in BALB\c mice asthma like features such as bronchial hyperreactivity and lung inflammation. All these S1P-mediated effects were absent in TLR4 defective mice or reversed by treatment with anti-TLR4 antibody. Confocal analysis of pulmonary sections and immunoprecipitation studies showed a colocalization of S1P1 and TLR4 within lung following S1P challenge.
Conclusion: Our data suggest a novel receptor cooperation in which functional interaction of $S1P_1$ and TLR-4 results in an enhanced allergic inflammatory response in the lung, paving the way for the biological role of S1P highly detected in the BAL of asthmatic patients.
Keywords: asthma, sphingosine-1-phosphate, TLR4, hyperreactivity.
References:
[1] Ammit AJ, Hastie AT, Edsall LC. Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma. *FASEB J* (2001) 15:1212–4.
[2] Hammad H, Chieppa M, Perros F. House dust mite allergen induces asthma via toll-like receptor 4 triggering of airway structural cells. *Nat Med* (2009) 15:410–6.

P041
POSTPRANDIAL AND LONG-TERM EFFECTS OF DIETARY FATTY ACIDS ON MICROGLIA M1/M2 POLARIZATION

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Microglia are the primary cells that exert immune function in the central nervous system. It is now well recognized that microglia have functional plasticity and dual phenotypes, proinflammatory M1 and anti-inflammatory M2 phenotypes. Inhibiting the M1 phenotype while stimulating the M2 has been suggested as a potential therapeutic approach for the treatment of neuroinflammatory diseases. Our hypothesis is that the type of dietary fatty acids into human postprandial triglyceride-rich lipoproteins (TRLs) could modulate the plasticity of microglia. We isolated TRLs at the postprandial peak from blood samples of healthy volunteers after the ingestion of a meal rich in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) or MUFAs plus omega-3 long-chain polyunsaturated fatty acids (PUFAs). Autologous monocytes isolated at fasting were first induced to differentiate into microglia. We observed that postprandial TRL-MUFAs enhance competence to monocytes to differentiate and polarize into M2 microglia. Postprandial TRL-SFAs made polarised microglia prone to an M1 phenotype. In addition, in contrast to dietary SFAs, dietary MUFAs primed for a reduced pro-inflammatory profile in the brain of mice fed with the different fatty acids-enriched diets. Our study underlines a role of postprandial TRLs as a metabolic entity in regulating the plasticity of microglia and also brings an understanding of the mechanisms by which dietary fatty acids are environmental factors fostering the innate immune responsiveness in humans. These exciting findings open opportunities for developing nutraceutical strategies with olive oil as the principal source of MUFAs, notably oleic acid, to prevent development and progression of neuroinflammation-related diseases.
Keywords: olive oil, microglia, neuroinflammation, lipoproteins, polarization.
References:
[1] N. Author1, N. Author2, N. Author3, *J. Our. Nal.*, **Year**, *Vol.*, page–page. (Helvetica 9 pt).
[2] N. Author1, N. Author2. Book title. ed. n°, Publisher, Publisher City. Year.

P042
ROLE OF SPHINGOSINE KINASE/SPHINGOSINE-1-PHOSPHATE (SPK/S1P) IN AIRWAY HYPER-RESPONSIVENESS INDUCED BY CIGARETTE SMOKE

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Background: Airway hyper-responsiveness (AHR) is recognized as an important predictor of progression of airway obstruction especially in continuing smokers and a prognostic factor in chronic obstructive pulmonary disease (COPD) patients. However, little it is known about the mechanisms of AHR in patients with COPD.
Aim: To investigate the role of SPK/S1P pathway in smoke-induced airway hyper-responsiveness.
Methods: Mice were exposed to either room air or to the smoke of three cigarettes/day, 5 days/week for 9, 10 and 11 months (Virginia filter cigarettes, 12 mg of tar and 0.9 mg of nicotine). Bronchi and pulmonary tissues were used for functional and molecular studies.
Results: S1P challenge of bronchi harvested from non-smoker mice does not cause any direct effect on bronchial tone, but significantly enhances carbachol-induced contractions. Conversely, in mice chronically exposed to cigarette smoke for 9, 10 and 11 months S1P/SPK pathway triggers airway hyper-responsiveness. Indeed, S1P causes a dose dependent contraction of isolated bronchi. Similarly in the whole lung system S1P increased airway resistance only in mice chronically exposed to cigarette smoke. The action on airways of S1P is coupled to an enhanced expression of SPK_2 as well as of $S1P_2$ and $S1P_3$ receptors in both airways epithelium and pulmonary macrophages. In these experiments the key role for SPK/S1P in airway hyper-responsiveness has been confirmed by pharmacological modulation of SPKs and S1P antagonists.
Conclusions: SPK/S1P pathway drives development of airway hyper-responsiveness in mice chronically exposed to cigarette smoke. These results open new windows for therapeutic strategies in treatment of AHR in patients with COPD.
Keywords: smoke, airway, sphingosine-1-phosphate, COPD.

P043
SEX DIMORPHISM IN MURINE ASTHMA-LIKE FEATURES DURING ALLERGEN SENSITIZATION

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Background: Asthma is a clinically heterogeneous disorder characterized by airway hyperreactivity, chronic inflammation and mucus production. Asthma has a higher incidence in adult females and the prevalence and severity of asthma coincide with key phases in the reproductive life stage of women.[1,2]
Aim: To assess a sex dimorphism in murine asthma-like features during allergen sensitization.
Methods: Both female and male BALB/c and C57BL/6 mice (8–9 weeks old) were sensitized with subcutaneous injection of ovalbumin (100 μg) complexed with alum (13 mg/mL) or vehicle (saline). Mice were sacrificed at different time points and bronchi and lung tissues were used for functional and molecular studies.
Results: Non-sensitized female mice showed an higher bronchial reactivity when compared to males independently from the strain and sensitization. These differences were correlated with differences in airway structure and IgE plasma level in favor of females. Following OVA sensitization female BALB/c mice, but not C57Bl/6, showed increased LT levels already after 3 days from sensitization. The increase of LT levels in female BALB/c mice well correlated to higher reactivity to carbachol and increased pulmonary inflammation.
Conclusion: Our findings reveal a sex dimorphism in airway physiology IgE dependent and a sex-dependent LT production as a basic mechanism of sex dimorphism in allergic asthma.
Keywords: asthma, hyperreactivity, inflammation, LT.
References:
[1] Zein,J.G. & Erzurum, S. C. Asthma is different in women. Curr Allergy Asthma Rep.15, 28 (2015).
[2] Melgert, B. N., et al. Female mice are more susceptible to the development of allergic airway inflammation than male mice. Clin Exp Allergy. 35 (2005).

P044
IDENTIFICATION OF SMALL MOLECULES TARGETING THE IL-17 INFLAMMATORY PATHWAY BY LABEL-FREE ASSAYS

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Interleukin 17 (IL-17) is a proinflammatory cytokine that not only plays a pivotal role in host defence against extracellular pathogens, but also acts as an immune checkpoint in several autoimmune diseases. As a result, the IL-17 family has become an attractive pharmacological target for inflammatory autoimmune diseases such as psoriasis,[1,2] for which therapeutic antibodies targeting IL-17 have been approved for clinical use.[3] Given the high cost of manufacture biologicals, a novel approach using small molecules may further improve the cost-effectiveness of therapeutics. Recently, a class of macrocyclic compounds was discovered, indicating that the IL-17 pathway druggability might be feasible.[4] We aim to identify novel pharmacological modulators for the IL-17 proinflammatory pathway with potential therapeutic utility in psoriasis. For this purpose, we developed a biophysical label-free assay based on Dynamic Mass Redistribution technology (DMR) that allows the detection of the interaction between proteins and small molecules. A protocol for the production of recombinant human IL-17A and the extracellular domain (ECD) of IL-17RA in mammalian cells was set up. We determined the optimum pH and concentration of the IL-17RA ECD for its immobilization, and found a dose-response increase with IL-17A exposure. 67 ligands from our chemical library of 60000 compounds were selected by virtual screening using docking and molecular dynamics studies against IL-17 available structures. We carried out a primary screening with our label-free assay and identified 2 molecules as potential binders. Hit validation by biochemical and cellular assays is ongoing to confirm their properties as novel IL-17 inflammatory pathway modulators.
Keywords: inflammation, IL-17, label-free, psoriasis.
References:
[1] A. Beringer, M. Noack, P. Miossec, *Trends Mol Med.*, **2016**, *22*, 230–241.
[2] N. Amatya, AV. Garg, SL. Gaffen, *Trends Immunol.,* **2017**, *38*, 310–322.
[3] E. Sbidian, A. Chaimani, G. Do, C. Hua, C. Mazaud, C. Droitcourt, et al, Cochrane Database Syst Rev., **2017**, *12*, CD011535.
[4] S. Liu, LA. Dakin, L. Xing, JM. Withka, PV. Sahasrabudhe, W. Li, et al, *Sci Rep.*, **2016**, *6*, 30859.

P045
A ROLE FOR ECTO-5'-NUCLEOTIDASE (CD73) IN AIRWAYS OF SENSITIZED MICE

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It is well known that adenosine is an endogenous signalling nucleoside involved in the pathogenesis of bronchial asthma.[1] CD73 is the key enzyme in the generation of extracellular adenosine.[2] It has been demonstrated that the number of CD4+T cells and CD73+Treg cells is higher in moderate-severe asthmatics patients than in patients with intermittent to mild asthma.[3] However, the role of CD73 in allergic asthma remains unclear. Here, we evaluated the contribution of CD73 in the main features of allergic sensitization, an important pathophysiological aspect of asthma. Experiments were performed on C57BL/6 mice of both sex. Groups of animals were sensitized by subcutaneous injection of ovalbumin (OVA) 100 mg/kg at day 1 and day 8 and sacrificed 14 and 21 days thereafter. Bronchi obtained from naïve (non-sensitized) female mice showed increased reactivity to carbachol compared to bronchi obtained from naïve male mice; this gender difference was abolished by incubation with the CD73 inhibitor, adenosine 5'-(α, β-methylene) diphosphate (APCP, 100 μM). Mice sensitization increased bronchial reactivity to carbachol in vitro and also increased CD73 bronchial expression, CD73 activity and adenosine levels in plasma. Furthermore, plasma IgE and pulmonary IL-4 and LTC4 levels were significantly high in OVA sensitized mice of both sex. Our results suggest that CD73 takes part in determining bronchial reactivity. In addition, we demonstrate changes in CD73 bronchial expression and plasma activity in allergic mice.

Keywords: asthma, bronchial reactivity, CD73, adenosine.

References:

[1] Cicala C, Ialenti A, *Eur. J. Pharmacol.*, **2013**, *714*, 522–5.
[2] Zimmermann H, *Naunyn Schmiedebergs Arch.Pharmacol.*, **2000**, *362*, 299–309.
[3] Wang LL, Tang HP, Shi GC, Wan HY, Tang W, Hou XX, Pan LN, Shi BY, Tao LQ, *Mol. Med. Rep.*, **2013**, 8, 1432–8.

P046
INCREASED PLATELET AND LEUKOCYTE ACTIVATION ARE ASSOCIATED TO SYSTEMIC INFLAMMATION IN PRIMARY HYPERCHOLESTEROLEMIA

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Introduction and Objectives: Primary hypercholesterolemia (PH) is an autosomal dominant monogenic disease characterized by severely elevated low density lipoprotein (LDL) levels that may contribute to the development of premature cardiovascular disease (CVD). In this study we have evaluated the activation state of different immune players in PH.

Methods: Whole blood from 22 PH patients and 21 age-matched controls was analysed by flow cytometry to determine platelet activation (P-selectin$^+$ and PAC-1$^+$), leukocyte activation (CD11b$^+$ and CD69$^+$) and the percentage of circulating platelet-leukocyte aggregates. Circulating inflammatory markers were also determined by ELISA.

Results: PH patients presented greater numbers of activated platelets (P-selectin$^+$ and PAC-1$^+$) than age-matched controls. Interestingly, the percentage of circulating eosinophils and type 3 monocytes, was significantly increased in patients with PH. This pathological state was linked to higher percentages of circulating platelet-granulocyte and platelet-monocyte (types 1 and 3) aggregates and enhanced activation state of different leukocyte. Additionally, PH patients had higher plasma levels of CXCL4, sP-selectin, CXCL8 and CCL2 than control volunteers. However, no differences between groups were found in circulating CCL5.

Conclusion: PH patients have an increased activation state of platelets and leukocytes compared with age-matched control together with increased circulating levels of different chemokines and soluble adhesion molecules. These inflammatory markers may predict the likelihood of suffering further cardiovascular events in these patients.

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Keywords: primary hypercholesterolemia, leukocyte subsets, flow cytometry, ELISA.

P047
A NOVEL DUAL PPARα AND PPARγ AGONIST INHIBIT TNFα-INDUCED MONONUCLEAR CELL RECRUITMENT

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Introduction and Objectives: Nine semisynthetic derivatives were prepared from two natural compounds **1** and **2** which displayed dual PPARα/γ activity. SAR studies revealed that substituents at the C-6 position (in chromane moiety) decreased PPARα/γ activity and methoxy groups increased cytotoxicity. Substitutions at C-9' (in lateral chain) shifted the activity towards PPARγ activation. Molecular modeling studies with the most active compounds (**1** and **8**) provided useful hints about differential binding interactions with PPARα or PPARγ. Since these compounds may exert anti-inflammatory activity, the effects of some compounds were tested on TNFα-induced leukocyte adhesion.

Material/Methods: Compound **1** was analysed by parallel-plate flow chamber assay to evaluate leukocyte adhesion to TNFα-stimulated endothelium. Flow cytometry was employed to determine ICAM-1, VCAM-1 and fractalkine (CX_3CL1) expression and p38MAPK and NF-κB activation. ICAM-1, VCAM-1 and fractalkine (CX_3CL1) expression were also determined by immunofluorescence. Immunoprecipitation assays were used to evaluate RXRα/PPARγ interactions

Results: Compound **1** concentration-dependently reduced TNFα-induced endothelial mononuclear cell adhesion via RXRα/PPARγ interaction. This effect was mediated through down-regulation of TNFα–induced endothelial ICAM-1, VCAM-1 and fractalkine (CX_3CL1) expression and via inhibition of p38MAPK and NF-κB activation.

Conclusions: Compound **1** may constitute a good lead molecule for the synthesis of novel dual PPARα/γ agonists with low toxicity and high effectiveness in the treatment of PPAR-related metabolic disorders preventing the development of the associated cardiovascular disease.

Funding: This work was supported by grants SAF2014-57845R, CP15/00150 and PI15/00082 from the Spanish Ministry of Economy and Competiveness, Carlos III Health Institute and the European Regional Development Fund.

Keywords: chromane derivatives, peroxisome proliferator-activated receptor (PPAR), mononuclear cell adhesion.

P048
PHARMACOLOGICAL MODULATION OF CARDIAC QT INTERVAL PROLONGATION THROUGH ALR2 SELECTIVE INHIBITION

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Prolongation of cardiac QT interval is one of the most dangerous consequence of hyperglycemia[1] and is partly sensitive to antioxidant drugs acting on the nitric oxide bioavailability, glycosylated products, accumulation of reactive oxygen species and impairment of ionic pumps.[2,3] Here we show that the selective inhibition of the endogenous aldose reductase 2 (ALR2) activity, involved in the oxidative damage in the heart following diabetes,[4] by the newly synthetized benzofuroxane derivative 5(6)-(benzo[*d*]thiazol-2-ylmethoxy)benzofuroxane (BF-5 m) (0.01–0.05–0.1 μM), dose-dependently results in cardioprotection from the electrical instability. This cardioprotection is exerted by reduction of the long cardiac QT interval and the decrease of the coronary perfusion pressure (CPP) in isolated rat heart perfused with high glucose (33 μM). BF-5 m also promotes increase of the expression and activity of endogenous antioxidant pathways and free radical scavengers such as SIRT1 and MnSOD into the heart following exposure to high glucose. This result is also confirmed by the increase in the expression of the Forkhead transcription factor 1 (FOXO-1), a direct downstream product of SIRT1 activity involved in gluconeogenesis, glycogenolysis, and adipogenesis.[5] To confirm these results, the expression of the potassium channel KCNE1 and KCNQ1, involved in QT prolongation, in H9C2 cardiomyocytes cultured in high glucose (33 μM) and pretreated with BF-5 m (0.01 – 0.025 – 0.5 μM). Also the expression levels of mir-1, down-regulating KCNE1, have been analyzed.

References:

[1] D. M. Nathan, "Diabetes: advances in diagnosis and treatment," *The Journal of the AmericanMedical Association*, vol. 314, no. 10, pp. 1052–1062, 2015.

[2] A. Ceriello, L. Quagliaro, M. D'Amico et al., "Acute hyperglycemia induces nitrotyrosine formation and apoptosis in perfused heart from rat," *Diabetes*, vol. 51, no. 4, pp. 1076–1082, 2002.

[3] C. Di Filippo, M. D'Amico, R.Marfella, L. Berrino, D. Giugliano, and F. Rossi, "Endothelin-1 receptor antagonists reduce cardiac electrical instability induced by high glucose in rats," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 366, no. 3, pp. 193–197, 2002.

[4] N.-H. Son, R. Ananthakrishnan, S. Yu et al., "Cardiomyocyte aldose reductase causes heart failure and impairs recovery from ischemia," *PLoS ONE*, vol. 7, no. 9,Article ID e46549, 2012.

[5] J. Nakae, T. Kitamura, Y. Kitamura, W. H. Biggs III, K. C. Arden, and D. Accili, "The forkhead transcription factor Foxo1 regulates adipocyte differentiation," *Developmental Cell*, vol. 4, no. 1, pp. 119–129, 2003.

P049
MODULATION OF DIABETIC RETINOPATHY BY MELANOCORTIN MC1 AND MC5 RECEPTOR SUBTYPES ACTIVATION IN A MOUSE MODEL OF STZ-INDUCED DIABETIC RETINOPATHY AND RETINAL CELLS CULTURE

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Diabetic Retinopathy (DR) is the vascular and neural injury of the retina caused by metabolic disorders in diabetes. In the retinal vascular endothelial cells a protective effect can be exerted by α - melanocyte stimulating hormone (α-MSH), a modulator of immune-response that binds to 5 subtypes of G protein–coupled melanocortin receptors (MC1R-MC5R). A model of streptozotocin-induced diabetic retinopathy (DR) in mice was used in order to characterize the melanocortin receptor subtypes involved in this protection. Intravitreal injections of melanocortin receptor agonists/antagonists, fluorescein angiography (FAG), RT-PCR, western blotting, immunohistochemistry, and ELISA were performed. 12 weeks after the induction of diabetes. FAG showed microvascular changes typical of DR in 80% of the mice. These microvascular changes were also evident after 16 weeks of diabetes. Interestingly, intravitreal injections of the MC5 agonist PG-901 at 7.32 ± 2.28 nM showed a significant retina protection, with no alterations in size, shape and/or course of the retina vessels (Rossi et al., 2016). In contrast, intravitreal injection of the MC5 melanocortin receptor antagonist PG20N at 130 nM (Grieco et al., 2008) worsened the signs of DR captured by FAG. In addition to this structural data we further aimed at investigating the functionality and integrity of retinal photoreceptors. For this reason, we investigated in primary cell cultures isolated from retinas of C57BL/6 mice, the hypothesis where the photoreceptors integrity would be preserved by MC5 receptor agonist PG-901 in condition of high levels of glucose (25 mM) that simulate the same pathological conditions of diabetic retinopathy (Baptista et al., 2015). The cells were first characterized through labeling with rhodopsin (opsin) and recovering, that show the presence of the photoreceptors in retinal cells. The results claim for high staining of these two markers in the cells treated with the MC5 agonist PG-901 with respect to the cells without treatment, and thus with high glucose only. Moreover, considering that photoreceptor membranes are particularly rich in polyunsaturated fatty acids and extremely vulnerable to the oxidative damage induced by free radicals (Varano M, 2007), we observed that an agonism at MC5 receptors promotes increased levels of GPx and MnSOD from retinal cells. In conclusion, the activation of the melanocortin MC5 receptor subtype reduces the retinal damage in a mouse model of STZ-induced retinopathy and in a high glucose-cultured retinal cells it preserves photoreceptors.

References:

Grieco et al., J Med Chem.2008 51, 2701–7.
Rossi et al., Med Inflammation 2016.
Baptista FI et al . Neurosci Lett. **2015:** 56–61.
Varano M. ISSN 1122-2557- NPT, Anno XVII – n 3/ 2007.

P050
DIFFERENTIAL PROTECTIVE EFFECTS OF BIOLOGICS AND METHOTREXATE ON VASCULAR INFLAMMATION AND THROMBOTIC RISK IN A MODEL OF PSORIASIS

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Psoriasis is commonly associated with an increased risk of cardiovascular disease[1,2] beyond that due to traditional risk factors,[3–5] but the mechanisms responsible for this are unclear. The aim of this study is to evaluate the effect of psoriasis on vascular inflammation (leukocyte/endothelium interactions) and on the formation of thrombi, and to assess the effects of drugs from the major pharmacological families used to treat psoriasis on both vascular features. We evaluated leukocyte-endothelium interactions using intravital microscopy in a murine model of psoriasis by topical application of imiquimod. For the study of thrombosis, we used the well-known ferric chloride model. Clinical concentrations of anti-TNFα (adalimumab, etanercept, infliximab), anti-IL-17 (secukinumab), anti-IL-12/23 (ustekinumab) and methotrexate were administered to determine their effect on these cardiovascular

parameters. Imiquimod-induced psoriasis prompts a significant increase in leucocyte adhesion to the vascular wall -a sign of vascular alteration- and increases susceptibility to thrombosis. Furthermore, single administration of clinical concentrations of anti-TNFα and anti-IL-17 drugs prevent leukocyte recruitment by the endothelium and protect against the onset of thrombosis. Pre-treatment with single doses of anti-IL-12/23 or methotrexate had no effect on either of these parameters. Our findings endorse the idea that treatment of psoriasis with some of the currently prescribed biological drugs can have a positive impact on concomitant manifestations of vascular inflammation. They also fuel the ongoing debate about the appropriateness of an earlier initiation of treatment with selected biologics in psoriatic patients at a high risk of developing cardiovascular disease.

Keywords: psoriasis, thrombosis, biological drugs, adalimumab, methotrexate, cardiovascular.

References:

[1] N.N. Mehta, R.S. Azfar, D.B. Shin, A.L. Neimann, A.B. Troxel, J.M. Gelfand, *Eur. Heart J.,* **2010**, 31, 1000–1006.
[2] N.N. Mehta, Y. Yu, R. Pinnelas, *et al*., *Am. J. Med.*, **2011**,124, 775.e1–6.
[3] O. Ahlehoff, G.H. Gislason, M. Charlot, *et al*., *J. Intern. Med.*, **2011**, 270, 147–157.
[4] O. Ahlehoff, L. Skov, G. Gislason, *et al*., *J. Intern. Med.*, **2013**, 273, 197–204.
[5] J.M. Gelfand, A.L. Neimann, D.B. Shin, X. Wang, D.J. Margolis, A.B. Troxel, *JAMA*, **2006**, 296, 1735.

P051
BUTYRATE MODULATES GUT-BRAIN AXIS IN ANTIBIOTIC-INDUCED INTESTINAL INJURY ASSOCIATED TO PARKINSON'S DISEASE IN MICE

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Early involvement of gut is observed in Parkinson's Disease (PD) and symptoms, such as gastrointestinal dysmotility, and digestive disorders, may precede motor ones.[1] A recent study demonstrated the link between gut microbiota alteration in PD patients and a reduction of bacteria producing short chain fatty acids (SCFAs).[2] Among these, butyrate has been shown to improve motor functions when administered in PD animals; moreover, it exerts beneficial effects in the gut, reducing inflammation, improving gut integrity, visceral sensitivity and intestinal motility. Here, we investigated whether antibiotic-induced gut alterations are involved in worsening PD symptoms and the effects of sodium butyrate (**BuNa**) in modulating brain and gut bidirectional interplay. In order to induce gut microbiota dysbiosis, mice were treated with ceftriaxone (CFX, 8 g/kg, per os) for 5 days[3]; afterwards mice were injected with 6-hydroxydopamine (6-OHDA, 4 µg/2 µl) in the right striatum.[4] Mice challenged with 6-OHDA or with CFX and 6-OHDA were treated with BuNa (100 mg/kg os) once daily for 14 days. Here, we demonstrated the worsening effects of CFX administration in PD pattern and BuNa capability in improving motor deficit in both 6-OHDA and 6-OHDA+CFX mice. These behavioural motor effects were related to BuNa-induced reduction of inflammatory, oxidative and apoptotic parameters at striatal level. Moreover, we demonstrated that BuNa improved colonic inflammation and integrity altered by both CFX and/or 6-OHDA, which mirrored the reduction of serum inflammatory mediators. These findings addressed gut alterations as risk factor for PD and BuNa therapeutic potential in limiting PD progression.

Keywords: Parkinson's disease, gut-brain axis, short chain fatty acids.

References:

[1] Mulak and Bonaz, *World J Gastroenterol*., **2015**, 21(37), 10609–20.
[2] M.M. Unger, J. Spiegel, K.U. Dillmann et al., *Parkinsonism Relat Disord*., **2016**, 32, 66–72.
[3] Z. Ling, X. Liu, Y. Cheng et al., *Biomed Res Int*., **2015**, 2015, 582048.
[4] C. Avagliano, R. Russo, C. De Caro et al., *Pharmacol Res*., **2016**, 113 (Pt A), 276–289.

O006
SODIUM-GLUCOSE COTRANSPORTER-2 INHIBITION REDUCES ANGIOTENSIN-II INDUCED ABDOMINAL AORTIC ANEURYSM IN APOLIPOPROTEIN E-KNOCKOUT MICE

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Objective: Abdominal aortic aneurysm (AAA) is a vascular disorder characterized by chronic inflammation of the aortic wall.[1] Inhibition of sodium-glucose cotransporter-2 (SGLT-2) has beneficial outcomes on the treatment of some metabolic and cardiovascular diseases as diabetes and atherosclerosis[2]; however, the potential direct effect of SGLT-2 inhibition on AAA remains unknown. This study evaluates the effect of oral treatment with the SGLT-2 inhibitor, empagliflozin (1 mg/kg/day and 3 mg/kg/day), on AAA induced by angiotensin-II (Ang-II) infusion for 28 days in apoE$^{-/-}$ mice.

Approach and Results: Oral treatment with the SGLT-2 inhibitor, empagliflozin reduced Ang-II–induced AAA formation in apoE$^{-/-}$ mice. Immunohistochemistry and reverse transcription polymerase chain reaction analysis demonstrated a significant increase in macrophage infiltration, neovessel formation, matrix metalloproteinase-2 and matrix metalloproteinase-9, chemokine (CCL2 [(C-C motif) ligand 2], CCL5 [(C-C motif) ligand 5]) and vascular endothelial growth factor (VEGF) expression in suprarenal aortic walls of apoE−/− mice infused with Ang-II, and all were significantly reduced by cotreatment with empagliflozin. Immunostaining for smooth muscle α-actin and stains for elastin fiber (Van Gieson) show the degradation of the lamina in the suprarenal aortas of Ang-II infused ApoE -/- and this degradation was significantly reduced by cotreatment with empagliflozin.

Conclusion: SGLT-2 inhibition reduces dissecting AAA formation induced by Ang-II in apoE−/− mice and may constitute a novel therapeutic strategy to prevent AAA progression. This work was support by grants from the Spanish Carlos III Health Institute from the Carlos III Health Institute and the Spanish Ministry of Economy and Competeveness [PI15/00082][SAF2014-57845-R], and the European Regional Development Fund (FEDER).

Keywords: abdominal aortic aneurysm, inflammation, cardiovascular.

References:

[1] Emeto TI et al, Curr Drug Targets, 2014, *15(*9):860–73.
[2] Tang H et al., Am J Cardiol., 2016, *118* (11):1774–1780.

ISCHAEMIA & REPERFUSION

P052
SAFETY AND EFFICACY OF RIVAROXABAN AFTER INFRA-INGUINAL SURGICAL REVASCULARIZATION

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Background: The anticoagulants can potentially improve patency rate and limb salvage, but its long-term use is associated to significant complications such as bleeding and increased mortality. New oral anticoagulants such as rivaroxaban can represent an alternative to the standard vitamin K-antagonists.
Objective: To evaluate the efficacy failure (bypass occlusion and major amputation) and serious adverse events (major bleeding and mortality) of rivaroxaban compared to acenocoumarol after infra-inguinal lower limb surgical revascularization.
Material and methods: Retrospective observational study of patients with peripheral arterial disease submitted to lower limb infra-inguinal bypass revascularization, who were anticoagulated (acenocoumarol or rivaroxaban) after hospital discharge. If patients presented revascularization due to other pathology than peripheral arterial disease, coagulation disorder, stroke or acute myocardial infarction in <30 days, glomerular filtration rate <15 mL/min or hemodialysis were excluded.
Results: 109 patients were included, with a mean age of 64.8 years. 40 (36.7%) patients were treated with rivaroxaban and 69 (63.3%) patients with acenocoumarol. After 1-year follow-up, patients under rivaroxaban and acenocoumarol presented comparable amputation rates (12.5 % vs. 10.1%, $P = 0.756$), bypass occlusion (22.5% vs. 24.6 %, $P = 0.769$), and mortality rate (10% vs. 8.7%, $P = 0.756$). Major bleeding occurred in 13.8% of patients. In patients with moderate renal dysfunction the risk of bleeding was higher with acenocumarol compared to rivaroxaban (45.5% vs. 0%, $P = 0.028$).
Conclusion: Rivaroxaban showed equivalent efficacy to acenocoumarol after infra-inguinal bypass revascularization, presenting similar occlusion, and major amputation and mortality rates. However, in patients with moderate renal dysfunction with decreased incidence of major bleeding.
References:

1. Sarac TP, Huber TS, Back MR, et all. Warfarin improves the outcome of infrainguinal vein bypass grafting at high risk for failure. J Vasc Surg. 1998 Sep;28(3):446–57.
2. Tangelder M, Lawson J, Algra A, Eikelboom B. Systematic review of randomized controlled trials of aspirin and oral anticoagulants in prevention of graft occlusion and ischemic events after infrainguinal bypass surgery. J Vasc Surg 1999;30:701–9.
3. Girolami B, Bernardi E, Prins M, et al. Antiplatelet therapy and other interventions after revascularization procedures in patients with peripheral arterial disease: a meta-analysis. Eur J Vasc Endovasc Surg 2000;19:370–80.
4. Dorffler-Melly J, Buller H, Koopman M, et al. Antithrombotic agents for preventing thrombosis after peripheral bypass surgery. Cochrane Database Syst Rev 2003;CD000536.
5. Norgren, L. et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). Journal of Vascular Surgery , Volume 45 , Issue 1 , S5–S.
6. Schulman S, Kearon C; Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost. 2005 Apr;3(4):692–4.
7. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002; 324:71.

P053
NLRP3 INFLAMMASOME INHIBITION REDUCES INFARCT VOLUME, BLOOD-BRAIN-BARRIER BREAKDOWN AND INFLAMMATION IN CEREBRAL ISCHEMIA

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Cerebral ischemia is the third cause of death and the main cause of adult disability worldwide. Currently the only pharmacological treatment for acute ischemic stroke is intravenous tissue plasminogen activator (tPA). However, only 3% of patients benefit from tPA administration, due to its limited therapeutic window and the risk of intracerebral hemorrhage. Inflammation in ischemic injury is crucially mediated by NLRP3 a key component of immune system. In this study, we investigated the role of NLRP3 in post-ischemic inflammation, using MCC950, a potent inhibitor of NLRP3 inflammasome. For that purpose, we used transient middle cerebral artery occlusion (tMCAO) during 1 hour in mice as a model of cerebral ischemia. Administration of MCC950 1 h after reperfusion reduced infarct volume in a dose-dependent manner (1, 3, 10 mg/kg; 53.23%, 50.57%, 107.87%, respectively). As a clinical outcome parameter, MCC950 at 3 mg/kg improved neuro-motor function and reduced expression of different pro-inflammatory cytokines (IL-1β and TNF-α) and NLRP3 inflammasome component. We observed that tMCAO produced BBB disruption that was improved in animals treated with MCC950 3 mg/kg. In MCC950-treated animals, we observed a functional recovery of endothelial proteins that forms the tight junctions of BBB (VE-cadherin, Cd31, ZO-1). From these results we can conclude that i) inhibition of NLRP3 inflammasome with MCC950 significantly reduces infarct volume and improve neuro-motor function, and ii) MCC950 preserves BBB integrity through stabilization of the tight junctions. Hence, the inhibition of NLRP3 may be a promising target in cerebral ischemia.
Keywords: cerebral ischemia, NLRP3 inflammasome, blood-brain barrier

METABOLISM

P054
DECREASED GRK2 EXPRESSION IN B-LYMPHOCYTES FROM INSULIN-RESISTANT OBESE PATIENTS

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Introduction: G protein-coupled receptor kinase 2 (GRK2) plays a pivotal role as modulator of several cardiovascular and metabolic regulatory pathways, as GPCR and insulin signalling cascades, NO bioavailability and mitochondrial function.[1] GRK2 is expressed in peripheral blood mononuclear cells (PBMC) and an increased expression/activity was observed in PBMC from obese insulin resistant patients respect to not insulin-resistant lean controls.[1,2] However, the expression of GRK2 in obese patients not insulin resistant has not been analyzed, nor GRK2 expression in different circulating mononuclear cell types.

Aim: To determine in different populations of PBMC from obese patients, insulin-resistant or not, the changes in the expression of GRK2.

Methods: Patients were consecutively recruited in the Hospital Clínico Univesitario de Valencia (Spain). GRK2 expression in T-lymphocytes ($CD45^+CD3^+$), B-lymphocytes ($CD45^+CD19^+$), neutrophils ($CD45^+SSC\text{-}A^{high}$) and monocytes ($CD45^+SSC\text{-}A^{low}$) from 23 insulin-resistant (HOMA-IR index >3.2) and 7 not insulin resistant obese controls, was analyzed using a FACSVerseBD.

Results: GRK2 expression in monocytes (81.8 ± 2.4%) was significantly higher than T-lymphocytes (51.4 ± 3.7%; $P < 0.001$), B-lymphocytes (53.9 ± 5.3%; $P < 0.001$) and neutrophils (63.6 ± 3.4%; $P < 0.01$) ($n = 30$). Fraction of B-lymphocytes that express GRK2 was lower in insulin-resistant patients (46.59 ± 5.87%) *vs* controls (77.88 ± 6.55; $P = 0.0099$). No changes were found in other cell populations. In all patients, a negative correlation was found between the fraction of B-lymphocytes that express GRK2 and the HOMA-IR index ($P = 0.0033$ Spearman) or the plasmatic insulin levels ($P = 0.0039$ Spearman).

Conclusions: GRK2 expression in B-lymphocytes shows potential as a therapeutic target related to insulin-resistance in obese patients

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Keywords: GRK2, lymphocytes, obesity, insulin resistance

References:

[1] Mayor F Jr., Cruces-Sande M, Arcones AC, Vila-Bedmar R, Briones AM, Salaices M, Murga C. *Cell Signal*. 2018, *41*, 25–32

[2] García-Guerra L, Nieto-Vázquez I, Vila-Bedmar R, Jurado-Pueyo M, Zalba G, Díez J, Murga C, Fernández-Veledo S, Mayor F Jr, Lorenzo M. *Diabetes*. 2010, *59*(10), 2407–17

P055
BLUEBERRY JUICE ATTENUATES LIVER INJURY PROGRESSION IN A RAT MODEL OF DIET-INDUCED PREDIABETES

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Blueberries bioactive compounds have recognized benefits on metabolic disorders[1,2]; however, the effects of blueberry juice (BJ) against progression from prediabetes to diabetes are completely unknown. This study aimed to evaluate the impact of BJ on the evolution of metabolic dysregulation, with a major focus on hepatic tissue, using a prediabetic animal model. Prediabetic male Wistar rats (8 weeks old) were developed by ingestion of an high-sucrose diet (HSu, 35%) for 9 weeks (W9).[3] In order to aggravate the prediabetic state, animals were submitted to a high-fat diet (HF, 60% calories from fat) for further 14 weeks (HSuHF) (W23). BJ were given orally at 9 weeks after HSu diet consumption, as a preventive strategy (between W9 to W23) (according to Figure 1). At each time-point, glycemic, insulinemic and lipidic profiles were assessed; markers of redox status were analysed on serum and liver tissue. Hepatic characterization in terms of morphology, echogenicity, fibrosis/steatosis and mitochondrial function were evaluated by ultrasonography, histochemical staining techniques and bioenergetics assays, respectively. HsuHF rats displayed metabolic dysregulation as well as hepatic focal steatosis and liver mitochondrial bioenergetics deficits. Notably, BJ treatment (HsuHF/BJ group) improved glucose tolerance, insulin sensitivity and hypertriglyceridemia along with an amelioration on hepatic histomorphological and functional features as seen by improved steatosis and mitochondrial function.

Overall, this work provides novel evidence on BJ ability to prevent the aggravation of prediabetes induced by Hsu/HF diet with notable benefits on hepatic tissue, although the precise mechanisms should be further explored.

Keywords: Prediabetes, liver injury, blueberries, preventive strategy

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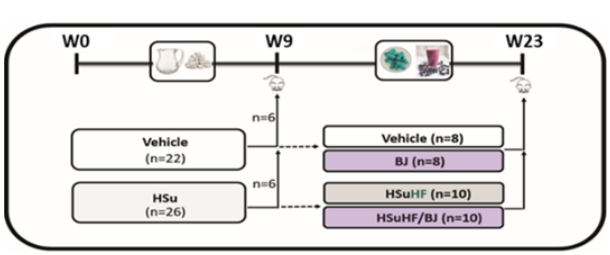

Figure 1 Diagram of experimental groups and protocol design. **BJ**: Blueberry juice (25 g/kg-BW/day); **HSu**: High-sucrose (35% of

sucrose solution); **HF**: High-fat diet (60% calories from fat); : Animals sacrifice / blood and tissues collection; Blueberry fruit (Vaccinium corymbosum L.) – Liberty variety (supplied by COAPE - Mangualde).

References:

[1] *Norberto S. et al* J. Funct. Foods, 2013, 5, 1518–1528.
[2] Vendrame S. et al *J. Nutr. Biochem*., 2013, 24, 1508–1512.
[3] Nunes S. et al. *Cardiovasc. Diabetol*., 2013, 12, 44.

P056
EFFECT OF PALMITOYLETHANOLAMIDE (PEA) ON MODULATION OF MITOCHONDRIAL FUNCTION IN IN VIVO AND IN VITRO MODELS OF INSULIN RESISTANCE AND OBESITY

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Mitochondrion is the driving force of cellular metabolism, where energy is released, oxygen is consumed, and water and carbon dioxide are produced.[1] Our previous data demonstrated the capability of PEA, a PPAR-α agonist; in modulating feeding behavior, food intake and body weight in ovariectomized an mild obese rats.[2] Here, we evaluate PEA effect on energy metabolism and mitochondria function in an *in vitro* and *in vivo* models of insulin resistance and obesity. After ten weeks of feeding with standard diet (STD) or diet-induced obesity (DIO), a subset of DIO mice were treated with PEA (30 mg/kg/day, os) (DIO-PEA) for eight weeks. At the end of the treatment, the animals were caged into a calorimeter: PEA enhanced energy metabolism, promoting an increase in lipid catabolism (RQ≈0.7). Consistently, histological liver section from DIO-PEA mice showed a lower lipid content than DIO group, as confirmed by Red Oil ORO staining. In isolated liver mitochondria, PEA induced CPT1 activity, a downstream shuttle of long chain-fatty acid-Acyl CoA, increased oxygen consumption rate and reduced mitochondria efficiency, measured polarographically by a Clark-type electrode. Furthermore, PEA balanced the redox state, inducing aconitase and SOD activity. To address PEA effectiveness on cell respiration, MitoStress assay was performed in HepG2 cells by Seahorse analyzer. Cell PEA stimulation (1 μM) recovered palmitic acid-induced mitochondrial dysfunction,[3] reducing mitochondrial efficiency and enhancing proton leak. Altogether, these data indicated that PEA promotes metabolic flexibility, glucose and lipid metabolism in *in vitro* and *in vivo* models of metabolic impairment induced by fatty acid overload.

Keywords: Mitochondria, bioenergetics, metabolic impairment, obesity, PPAR-α agonist

References:

[1] C. Koliaki, M. Roden, *Annu Rev Nutr*, 2016, *Vol.36*, 337–367.
[2] G. Mattace Raso, A. Santoro, *Endocrinology*, 2014 *Vol.155*,1291–301.
[3] M Ishii, A. Maeda et al, *Arch Biochem Biophys,* 2015, *Vol.566,* 26–35.

O002
TARGETING METABOLIC AND ENDOTHELIAL FUNCTIONS: THE ROLE OF ALDH2

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Endothelial cell (EC) metabolism only recently entered the field of angiogenesis as an important player with numerous questions remaining to be addressed.[1] ECs generate most of their energy anaerobically, but they have an extensive mitochondrial network and consume oxygen. As cells are exposed to oxidative stressors, mitochondria are capable of drawing upon a 'reserve capacity'. Aldehyde dehydrogenases (ALDHs) are a family of $NADP^+$-dependent enzymes that catalyze the oxidation of aldehydes. ALDH2 is responsible for the metabolism of acetaldehyde and other toxic aldehydes, as 4-hydroxynoneal (4-HNE). In conditions in which lipid peroxidation products and reactive oxygen species (ROS) are accumulated, endothelial cells become dysfunctional significantly contributing to the progression of vascular-dependent diseases. We recently demonstrated that the detrimental effects of Aβ peptides are associated with accumulation of 4-HNE.[2] We aimed to investigate the role of ALDH2 on HUVECs bioenergetic properties and whereby inhibition of ALDH2 affects endothelial functions. To this end we defined mitochondrial function using extracellular flux analysis to determine rates of O_2 consumption in HUVECs silenced for ALDH2 (siALDH2). Next we determined the involvement of ALDH2 activity on endothelial functions, such as cell proliferation and migration in siALDH2-HUVECs. Preliminary results suggest that ALDH2 silencing reduces mitochondrial respiration and reserve capacity. ALDH2 silencing correlates with modification of cell morphology associated with impairment of endothelial functions. These results document the relevance of ALDH2 to the progression of endothelial dysfunction in vascular diseases and highlight that molecules targeting this enzyme might have a potential clinical application.

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Keywords: Aldehyde dehydrogenase 2, endothelium, metabolism, mitochondrial dysfunction

References:

[1] M. Potente and P. Carmeliet, The link between angiogenesis and endothelial metabolism, *Annu. Rev. Physiol*. 2017, 79:43–66.
[2] R. Solito, F. Corti, C. Chen, D. Mochly-Rosen, A. Giachetti, M. Ziche, S. Donnini, Mitochondrial aldehyde dehydrogenase-2 activation prevents b-amyloid-induced endothelial cell dysfunction and restores angiogenesis. *J. Cell Sci*. **2013**; 26:1952–1961.

NATURAL PRODUCTS

P057
EFFECTS OF QUERCETIN AND RUTIN ON THE IL-6 RELEASE

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It is widely known nowadays the important role of inflammation events on the cardiovascular diseases. Many studies support that different levels of inflammation appear in every step involved in the arteriosclerotic process; therefore this process has some inflammation event characteristics such as cytokines expression and chemotactic factors. Previous experiments carried out with selected flavonoids showed those products inhibited the release of some pro-inflammatory cytokines like IL-1β and TNF-α. In these assays we tested with the same products, the inhibition of IL-6, another pro-inflammatory cytokine. Material/Methods: Whole blood aliquots extracted from 10 healthy volunteers were incubated with quercetin and rutin, both to 0.5 mM and 1 mM concentration. Lipopolysaccharide (LPS) was added for inducing pro-inflammatory cytokines release. Samples were centrifuged and supernatant was collected in order to measure the IL-6 secretion from monocytes using specific immunoassay techniques. Results: LPS (10 mg/ml) induced a significant increase on the IL-6 release (532.2 pg/ml±3.17) compared to control values (3.9 pg/ml±0.01). This production was inhibited by quercetin at 0.5 mM and 1 mM concentrations, but in never case by rutin. IL-6 inhibition mean for quercetin 0.5 mM was 62.9 pg/ml±0.98 and for quercetin 1 mM was 24.8± 0.53. Conclusion: The effect of both flavonoids is completely different. It could be due to the differences of their chemical structures. However these studies suggest quercetin could have a potential therapeutic use in the inflammatory processes of cardiovascular disease.

Keywords: quercetin, rutin, IL-6, pro-inflammatory cytokines

P058
ANNURCA POLYPHENOLIC EXTRACT RELAXES HUMAN CORPUS CAVERNOSUM VIA NITRIC OXIDE AND HYDROGEN SULFIDE

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Among fruits, apples have historically shown medicinal properties. "Annurca" is the only apple cultivar native to Southern Italy and forms about 60% of Campania region and 5% of national apple production.[1] Recently, a nutraceutical product (AppleMetS®, AMS) containing an Annurca polyphenolic extract has been formulated.[2] The data reported in a clinical trial assert that AppleMet formulation can effectively correct lipid profile in humans resulting suitable for the treatment of mildly hypercholesterolemia.[2] Here, we have investigated the possible vasoactive effect of the AMS in human corpus cavernosum (HCC) strips. A curve-concentration effect of AMS (10 μg/ml-2000 μg/ml) was performed on stable tone of phenylephrine. The AMS relaxed in endothelium-dependent manner HCC strips and this effect was significantly reduced by L-NAME (eNOS inhibitor) or wortmannin (inhibitor of eNOS phosphorylation, p-eNOS). Interestingly, the relaxant effect of AMS was also reduced by blocking cysthationine-β-synthase (CBS) and cysthationine-γ-lyase (CSE), both enzymes deputed to hydrogen sulfide (H_2S) biosynthesis. H_2S, together with nitric oxide, is a gasotransmitter that relaxes HCC contributing to the penile erection.[3] Since, H_2S phosphorylates eNOS[4] we have incubated the fresh tissue with AMS at (0.5–1000–1500 μg/ml) in order to evaluate the eNOS phosphorylation. The incubation caused a trend of increase in eNOS/peNOS ratio. Basing on these data, we can hypothesized that AMS elicits a transient increase in H_2S production that in turn phosphorylates eNOS causing relaxation of human tissue. Therefore, the AMS may ameliorate the vascular function in HCC suggesting a general improvement in the vasculature within the body.

Keywords: human corpus cavernosum, annurca polyphenolic extract, nitric oxide, hydrogen sulfide

References:

[1] B. D'Abrosca, A. Fiorentino, P. Monaco, *Food Chemistry*, **2006**, 98, 285–290.
[2] GC. Tenore, D. Caruso, G. Buonomo, *J Med Food,* **2017**, 20 (3):288–30.
[3] R. d'Emmanuele di Villa Bianca, R. Sorrentino, P. Maffia, *Proc Natl Acad Sci U S A,* **2009**, 106(11):4513–8.
[4] C. Coletta, A. Papapetropoulos, K. Erdelyi, *Proc Natl Acad Sci U S A,* **2012**, 109 (23):9161–65.

P059
DETERMINATION OF THE CELL VIABILITY IN HUMAN PROSTATE CANCER PC-3 CELL LINE BY *SIDERITIS HYSSOPIFOLIA*

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Introduction: *Sideritis hyssopifolia* has been shown to possess antioxidant properties, a key factor in reducing chronic diseases like cancer. In this study, the effects of the ether, methanol and chloroform extracts obtained from the aerial parts of *Sideritis hyssopifolia*, on cell viability in human prostate cancer PC-3 cells, using MTT assay have been evaluated.

Material and Methods: PC-3 cells were seeded (10^4cells/well) in 100 μL of culture medium. After 24 h, the cells were treated with 200 μL of each extract dissolved in culture medium and incubated at 37 °C for 24, 48 and 72 h. Control- treated (0.1% DMSO) cell and control-untreated cell were incubated with culture medium. At the end of treatment the medium was removed, cells were washed with PBS, and of were added 100 μL MTT solution (5 mg/mL). After 3 h incubation, the formazan crystals were solubilized in 50 μL of SDS (20%) and the plates were incubated overnight at room temperature. The absorbance was measured at 560 nm.

Results: The results were expressed as the percentage of viable cells in comparison with the control cells, for which the cell viability was 100%. The percentage of cell viability tested at 24, 48 and 72 h were for ether extract: 70.284, 50.249 and 20.382; for methanol extract: 72.438, 55.333 and 26.443 and for chloroform extract: 67.566, 65.419 and 14.242, respectively.

Conclusions: PC-3 cells exhibited a time-dependent inhibition of the cell viability. The highest inhibition of the cell viability was observed for chloroform extract at 72 h.

Keywords: *Sideritis hyssopifolia*, PC-3, viability cell, MTT assay

P060
EFFECT OF A MILK-BASED FRUIT BEVERAGE ENRICHED WITH PLANT STEROLS AND GALACTO-OLIGOSACCHARIDES IN A MURINE COLITIS MODEL INDUCED BY DEXTRAN

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In this research we have evaluated the effect of an milk-based fruit (MBF) beverage enriched in phytosterols (PS, 1 g/100 mL) and fortified with galacto-oligosaccharides (GOS, 2 g/100 mL) on the prevention/induction/remission in murine experimental model of chronic colitis induced with dextran sodium sulfate (DSS).[1] MFB was administrated to mice orally (0.2 mL, p.o) every day by gavage to achieve a PS and GOS doses of 35 and 90 mg/kg, respectively. Female mice (C57BL/6) received four cycles of DSS (2% w/v). The animals were assigned randomly to one of four treatment groups: control (drinking water), control + MBF (drinking water, + 0.2 mL MBF), DSS group (DSS in drinking water) DSS + MBF group (DSS in drinking water + 0.2 mL of MBF). Severity of colitis was assessed using Disease Activity Index (DAI), checking body weight, stool consistency and gross rectal bleeding three times a week. Mice were sacrificed at day 56 by cervical dislocation, and their colons were removed. Colon lengths, histology studies and myeloperoxidase (MPO) activity were determined. Oral gavage of MFB to healthy animals do not showed any toxic effects. However, administered to colitic animals treated with DSS, MFB did not prevent colitis onset nor reduced the severity of the disease, even worsened clinical and histological remission. Although some reports indicate the beneficial effects of bifidobacteria-fermented milk containing GOS or the efficacy of PS in inflammatory bowel disease,[2–4] our results do not support a positive effect of this combination in ulcerative colitis induced by DSS.

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Keywords: milk-based fruit, plant sterols, galacto-oligosaccharides, mouse, chronic ulcerative colitis

References:

[1] Giner et al, *J. Nat. Prod*., **2013**, 76, 1113–1120.
[2] Kim et al, *Mol. Nutr. Food Res*., **2014**, *59*, 936–972.
[3] Ishikawa et al, *Digestion*, **2011**, *84*, 128–133.
[4] Aldini et al, *PLoS ONE*, **2014**, *9*, e108112.

P061
OLIVE OIL PRESERVES INTESTINAL BARRIER FUNCTION AND INTRAEPITHELIAL LYMPHOCYTES IN HIGH-FAT DIET-INDUCED OBESE MICE

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Obesity causes intestinal barrier dysfunction and immune cell alterations. Intraepithelial lymphocytes (IELs) play important roles in intestinal mucosal immunity. Although olive oil is known to modulate the functions of immune cells, there has been no information about how it affects immunological functions of IELs and mucosal barrier integrity. The aim of this work was to investigate the possible effect of olive oil on IEL subsets and intestinal barrier function in high-fat diet-induced obese mice. Thirty-six C57BL/6 mice were divided into one of three diets: (I) control diet (low fat diet, LFD), (II) cow's milk cream (saturated fatty acid-enriched high-fat diet, SFA-HFD), and refined olive oil (monounsaturated fatty acid-enriched HFD, MUFA-HFD). After 8 weeks of diet, intestinal epithelium was isolated. IELs and mucosal barrier integrity were analysed by FACS, RT-qPCR, WB, and ELISA. SFA-HFD decreased intestinal claudin-I protein expression, increased inflammatory cytokine protein and gene expression, and promoted intestinal immune cells recruitment. Olive oil increased claudin-I protein expression, reduced intestinal inflammatory and immune profile. Moreover, olive oil preserves natural IEL alterations in contrast to SFA-HFD, which lead to increases induced IELs. These exciting findings open new opportunities for developing novel nutritional strategies with olive oil as the principal dietary source of oleic acid to prevent development and progression of obesity-related inflammation. However, further investigations are required to elucidate the molecular mechanism of olive oil on IELs.

Keywords: olive oil, gut, intraepithelial lymphocyte, obesity

P062
CENTELLA ASIATICA AND BEESWAX AGAINST OSTEOARTHRITIS PAIN: EFFICACY OF INTRA-ARTICULAR INJECTIONS OF NATURAL MOLECULAR COMPLEXES

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The intra-articular injection of corticosteroids and hyaluronic acid displays only short-term benefits (pain and stiffness reduction) in patients with osteoarthritis (OA). This study was aimed to evaluate the efficacy of intra-articular injections of a natural complexes of phenols, tannins and polysaccharides obtained from *Centella asiatica* and beeswax in an OA animal model. OA was induced by injecting monosodium iodoacetate (MIA, 2 mg/25 μl) into-tibiotarsal joint space. One week after injury, 20 μl of *Centella asiatica* hydroalcoholic fraction 2 mg/ml, beeswax hydroalcoholic fraction 2 mg/ml alone or their mixture were intrarticularly injected. Behavioural measurements and histological evaluation were performed 14, 30 and 60 days after injection. The effect was compared with that induced by triamcinolone acetonide 100 μg/20 μL. On days 14 and 30, rats treated with *Centella asiatica* showed a significant prevention of pain threshold alterations (Paw-Pressure test), reduction of postural unbalance (Incapacitance test) and improvement of motor coordination and activity (Beam-Balance test and Animex test, respectively) with respect to MIA + vehicle group. Beeswax, the mixture *Centella asiatica*+ beeswax and triamcinolone acetonide significantly reduced pain as well as postural unbalance but with a lower efficacy than *Centella asiatica*. Histological analysis of the tibio-tarsal joint showed a significant reduction of tissue damage in *Centella asiatica*-treated animals. The effect is comparable to that obtained with triamcinolone acetonide. The mixture *Centella asiatica* + beeswax induced a less intense effect whereas beeswax fraction showed a lower efficacy. In conclusion, the intra-articular administration of *Centella asiatica* hydroalcoholic fraction appears a suitable treatment for OA.

Keywords: *Centella asiatica*, beeswax, intra-articular injection, osteoarthritis, articular pain

P063
THE USE OF HERBAL MEDICINES DURING BREASTFEEDING: RESULTS FROM THE HERBAL SUPPLEMENTS IN BREASTFEEDING INVESTIGATION (HABIT)

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Aims: Use of complementary and alternative drugs (CADs) during breastfeeding is commonly increasing, mainly due to their presumed higher safety compared to traditional medications. Indeed, CADs can cause serious adverse effects, and high-quality evidence supporting their use during lactation is limited. In Italy, specific investigations on the attitude of lactating women towards CADs are lacking. The Herbal supplements in Breastfeeding InvesTigation (HaBIT) aimed to explore the attitudes and knowledge on CADs among lactating women.
Methods: A web-based survey was conducted over a six-years period among lactating women resident in Tuscany (Italy). Data on lactating behavior, CADs use during pregnancy or breastfeeding, and women knowledge about CADs' efficacy and safety were collected.
Results: 388 lactating women answered the questionnaire; the majority of them were primiparae with high education level. Two-hundred and four women declared to have been CADs users during breastfeeding. Moreover, the 61 and 48% of subjects reported CADs use also before and during pregnancy. A significant proportion of subjects were unable to correctly identify the type of CADs they were using. The 73% of women were convinced that CADs had higher or comparable safety than conventional medications; nevertheless, 65% of women admitted to have no scientific information about the potential risks of CADs, and 14 CADs users reported to have experienced side effects.
Conclusions: These results claim the necessity that healthcare providers amplify their role to increase nursing women' awareness about CADs. Further research is needed to support the evidence base of non-pharmaceutical approaches for symptom control during breastfeeding.
Keywords: herbal medicine, pregnancy, breastfeeding, complementary medicine, adverse drug reactions
*Contributed equally.

P064
SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF NOVEL HISPANOLONE DERIVATIVES VIA INHIBITION OF NF-κB SIGNALING AND/OR INFLAMMASOME PATHWAYS

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Inflammation is a crucial host response triggered by invading pathogens and injured tissue. Recent studies have highlighted the critical role of NLRP3 inflammasome in the inflammatory response. Diterpenes are bioactive natural products with great therapeutic potential and are considered very promising starting points for the development of new therapeutic agents. In this study, a series of novel hispanolone derivatives (N1-N17) were synthesized and evaluated for potential anti-inflammatory activity in J774A.1 macrophages, focusing on their potential as inhibitors of classical inflammatory pathways (NF-κB) and/or inflammasome activation. Macrophages were activated with lipopolysaccharide (LPS) alone or LPS + nigericin in the absence and presence of diterpenes. Compounds N2 and N5 were cytotoxic as revealed the MTT assay . Inhibitory effects of the non-toxic compounds on nitric oxide (NO) were evaluated and derivatives N1, N11 and N12 were selected. N1 and N12 showed the most potent anti-inflammatory effects with significant inhibition of NOS-2 and COX-2 gene expression at the transcriptional level. Inflammatory cytokines (IL-6, TNF-α, IL-18, IL-12, IL-10, KC and IP-10) were downregulated in the presence of the compounds. Moreover, the phosphorylation of ERK1/2, p38 and JNK were reduced by pre-incubation with these compounds. Non-toxic hispanolone derivatives were also evaluated on the inflammasome pathway. Significant inhibition of IL-1β and caspase-1 expression were observed in the presence of compounds N9, N10 and N16. In conclusion, preliminary results show the promising anti-inflammatory effects of some hispanolone derivatives acting on a dual level: inhibiting classical inflammation signaling or downregulating the inflammasome pathway.
Keywords: diterpenes, NF-κB, inflammasome, macrophages
Acknowledgement: We thank the financial support from the Spanish MINECO (SAF2015-65113-C2-1-R).

O003
GPETAFLR, A NOVEL BIOACTIVE PEPTIDE FROM *LUPINUS ANGUSTIFOLIUS* L. PROTEIN HYDROLYSATE, REDUCES OSTEOCLASTOGENESIS

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Highlights:
- GPETAFLR reduced TRAP activity and the expression of osteoclast marker genes.
- GPETAFLR increased the release of anti-osteoclastogenic cytokines (IL-4 and IL-10).
- *Lupinus angustifolius* L. may prevent osteoclast-related diseases.

The effect of GPETAFLR, a novel peptide isolated from *Lupinus angustifolius* L. protein hydrolysate (LPH), on osteoclast differentiation and maturation was investigated. Human osteoclasts generated from circulating monocytes were used to analyse the effects of GPETAFLR (50–100 μg/mL) on osteoclastogenesis using TRAP reaction, RT-qPCR, and ELISA procedures. LPS enhanced TRAP activity and the expression of osteoclast marker genes (TRAP, OSCAR, RANK, and CATHK) while downregulated the expression of OPG gene in human monocyte-derived osteoclasts. These effects were reduced with GPETAFLR. Moreover, LPS increased the release of osteoclastogenic cytokines (TNF-α, IL-1β, and IL-6) meanwhile GPETAFLR increased the release of anti-osteoclastogenic cytokines (IL-4 and IL-10) in the medium of human monocyte-derived osteoclasts. For the first time, we show that plant peptides from lupine protein hydrolysates have anti-osteoclastogenic activity. These exciting findings open opportunities for developing nutritional strategies with *Lupinus angustifolius* L. as dietary source of plant proteins, notably GPETAFLR, to prevent development and progression of osteoclast-related diseases.
Keywords: Peptide, protein hydrolysate, Lupine, osteoclast, osteoporosis

O004
APORPHINE, 1-BENZYLISOQUINOLINE AND PHENANTHRENE ALKALOIDS AS D_2-DOPAMINERGIC LIGANDS

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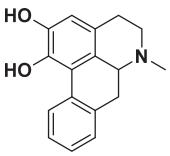
Introduction and Objectives: Among isoquinoline (IQ) alkaloids, aporphines and 1-benzyl-IQ have attracted considerable attention because of their numerous biological activities, including dopamine receptor (DR) agonism (antiparkinsonian)[1] and antitumor,[2] as well as anti-diabetic, anti-obesity and anti-hyperlipidemic effects by inhibition of the peroxisome proliferator-activated receptor (PPAR)γ.[3] Phenanthrenes, biogenetically derived from aporphines, represent a small group with antioxidant, antiplasmodial, antimicrobial[4] and cytotoxic effects as well as their inhibitory action on platelet aggregation and leukocyte-endothelial cells interactions.[5] DR, mainly the D_2R-like, are implicated in several central nervous system disorders including Parkinson's disease and psychosis (schizophrenia), among others. The aim of this study was to synthetize series of aporphines and 1-benzyl-IQ and phenanthrenes, as potential D_2R ligands.

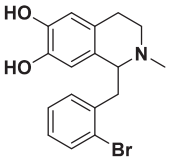
Material/Methods: IQ nucleus was obtained by Bischler-Napieralski cyclodehydration, aporphine by direct arylation of a brominated benzyl-IQ, and phenanthrene system by Hofmann degradation. Compounds were tested *in vitro* for their affinity towards DR by radioligand binding assays in rat striatum.[6] and then, the most active compounds at human D_2R stably transfected in CHO-K1 cells.

Results: Aporphine **1** and 1-benzyl-IQ **2** were able to displace selective radioligands into nanomolar range in rat striatum and hD_2R.

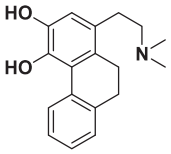
Conclusions: Aporphines and 1-benzyl-IQ could be potential candidates in the treatment of dopaminergic disorders. Funding: This study



Aporphine (1)



Benzyl-IQ (2)



Phenanthrene (1)

was supported by grants SAF2014-57845-R, SAF2017-89714-R and CP15/00150 from the Spanish Ministry of Economy and Competiveness and Carlos III Institute of Health (ISCIII), co-funded by the European Regional Development Fund. Nuria Cabedo holds a Miguel Servet-I contract funded by the ISCIII, co-funded by the European Social Fund.

Keywords: Aporphines, phenanthrenes, 1-benzylisoquinolines, dopamine, synthesis

References:

[1] A. Li, H. Guo, X. Luo, J. Sheng, S. Yang, Y. Yin, J. Zhou, J. Zhou, *FASEB J.,* 2006, *20*, 1263–1265.

[2] C.-M. Liu, C.-L. Kao, H.-M. Wu, W.-J. Li, C.-T. Huang, H.-T. Li, C.-Y. Chen, *Molecules*, **2014**, *19*, 17829–17838.

[3] N. Ikarashi, M. Tajima, K. Suzuki, T. Toda, K. Ito, W. Ochiai, K. Sugiyama, *Phytother. Res.*, **2012**, *26*, 91–100.

[4] A. A. Nasrullah, A. Zahari, J. Mohamad, K. Awang, *Molecules*, **2013**, *18*, 8009–8017.

[5] R. Estellés, J. López-Martín, L. Milian, J. E. O'Connor, M. Martínez-Losa, M. Cerdá-Nicolás, E. M. Anam, M. D. Ivorra, A. C. Issekuts, J. Cortijo, J. E. Morcillo, M. A. Blázquez, M. J. Sanz, *Br. J. Pharmacol.,* **2003**, *140*, 1057–1067.

[6] L. Moreno, N. Cabedo, M. D. Ivorra, M. J. Sanz, A. López Castel, M. C. Álvarez, D. Cortes, *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 4824–4827.

NEUROPHARMACOLOGY

P065
NOVEL IMIDAZOLINE I2 RECEPTOR LIGANDS FOR COGNITIVE DECLINE TREATMENT

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A common symptom in age-related neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), is a progressive cognitive decline. Moreover, cognitive deficits are also a cross-diagnostic symptom of many neuropsychiatry disorders, such as depression, schizophrenia, among others. Overall, brain disorders are an increasing burden on the health systems and the society as a whole because no truly effective pharmacological treatments are currently available. Imidazoline I2 receptors (I2-IR), which have been directly shown to be altered in the brain of patients with cognitive deficits, will be considered as a new pharmacological target. MCR5 and MCR9, selective I2-IR ligands were administered to SAMP8 mice (5 mg/kg/day added to drinking water). The SAMP8 treated groups showed an improvement in cognitive and short and long learning capabilities measured through Novel Object recognition test (NORT) depicted a beneficial effect on hippocampal memory processes. Moreover, I2-IR ligands treatment prevents synaptophysin loss in SAMP8 mice. H_2O_2 and IL1-β Tnfα expression were also diminished in brain tissue of treated mice. The increase in gene expression for Adam10 and Neprilysin, and s-APPα protein levels confirm a potentiation of non-amyloidogenic pathway. In addition, a significant reduction in tau phosphorylation in Ser404 was determined in treated SAMP8. In conclusion, altogether these results demonstrate the neuroprotective role for these new I2-IR ligands being promising therapeutic agents in brain disorders and age-related neurodegenerative diseases. To our knowledge, this work is the first experimental evidence demonstrating the possibility to use I2-IR as a pharmacological target for cognitive impairment.

This study was supported by Ministerio de Economía y Competitividad of Spain SAF2016-33307 and SAF2014-55903-R. C.G.F., F.V., C.E. and M.P. belong to 2017SGR106 (AGAUR, Catalonia). J.A.G.-S. is a member emeritus of the Institut d'Estudis Catalans (Barcelona, Catalonia).

Keywords: Imidazoline I2 Receptor, SAMP8, oxidative stress, amyloid, tau, inflammation

References:

[1] N. Author1, N. Author2, N. Author3, *J. Our. Nal.*, Year, *Vol.*, page–page. (Helvetica 9 pt).

[2] N. Author1, N. Author2. Book title. ed. n°, Publisher, Publisher City. Year.

P066
STUDY OF 3-BENZYLIDENPHTHALIDES WITH POTENTIAL ACTIVITY IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most important progressive and irreversible neurodegenerative disorder of unknown aetiology. The current available treatment of PD is only symptomatic, and it is not able to stop the progression of this disease.[1] In this study we have evaluated changes in locomotor activity of previously (18 h) reserpinized mice (1.25 mg/kg, i.p.) treated with levodopa + benserazide (LD+BZ 100:25 mg/kg, i.p.) and one of four new molecules –B-020, B-090, B-1380, MeBF– (10 mg/kg, i.p.) with benzylidenphthalide structure (Figure 1) that have exhibited a potent *in vitro* MAO-B inhibition (IC_{50} B-020: 62.26 nM; IC_{50} B-090: 0.65 nM; IC_{50} B-1380: 5.20 nM; IC_{50} MeBF: 8.0 nM), or reference drug (selegiline 10 mg/kg, i.p.). Changes in locomotor activity were evaluated using a video computerized system (Noldus Ethovision, Wageningen, Netherland). Additionally, the theoretical physicochemical properties of the new molecules were evaluated with the Molinspiration program (http://www.molinspiration.com/).

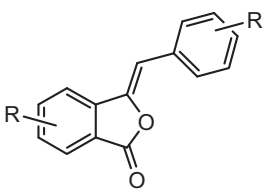

Figure 1 General structure of 3-benzylidenphthalides.

These new molecules do not violate Lipinski's rule of five although we have observed differences compared to selegiline. These molecules have higher molecular weight, logP and TPSA (more than ten times higher) than the selegiline but similar volume. Locomotor activity was increased in mice treated with LD+BZ and selegiline (10966.56 cm/h ± 993.30, $p < 0.01$) vs. vehicle treated animals (1288.21 cm/h ± 250.78). Unfortunately, the new molecules did not increase locomotor activity significantly (B-020: 2257.61 cm/h ± 392.86; B-090: 2596.02 cm/h ± 648.71; B-1380: 2262.58 cm/h ± 594.21; MeBF: 1618.54 cm/h ± 249.10).

Keywords: Parkinson's disease, MAO Inhibition, locomotor activity

References:

[1] Lang A.E., Espay A.J. Disease modification in Parkinson's Disease: Current approaches, challenges, and future considerations. *Mov Disord.* 2018, https://doi.org/10.1002/mds.27360.

P067
EFFECTS OF DAD3R BLOCKADE ON DA TRANSPORTER (DAT) AND DA D3 RECEPTOR (DAD3R) EXPRESSION IN THE MOUSE NUCLEUS ACCUMBENS (NAC) AFTER COCAINE PRIMING-INDUCED REINSTATEMENT OF COCAINE SEEKING BEHAVIOUR

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Cocaine is a commonly abused stimulant that enhances DA neurotransmission through its ability to block DAT, which is responsible for the reuptake of extracellular DA into presynaptic terminals. On the other hand, relapse is a major issue in drug addiction treatment, and importantly, DAD3R subtype has attracted attention as a potential target for controlling the effects of stimuli that induce reinstate extinguished drug-seeking behaviour.[1] Recently, it has been proposed that DAT activity may be under the control of DAD3 autoreceptors that would modulate cocaine effects.[2]

Aims: The present study investigated whether SB-277011-A, a selective DAD3R antagonist, attenuated conditioned place preference (CPP) to cocaine elicited by priming injections of the drug in, as well as the changes in DAT and DAD3R in the NAc of C57 male mice.

Methods: Groups of mice were conditioned with cocaine (25 mg/kg i.p. for 4 days). Animals received drug suppression by CPP paradigm twice a week for, approximately, eight weeks. Then, we evaluated the effects of SB-277011 (24 and 48 mg/kg i.p.) administration on DAT and DAD3R expression in the NAc after CPP reactivation induced by cocaine priming (12.5 mg/kg i.p.).

Results: Cocaine priming-induced reinstatement was accompanied by an increase of DAD3R expression in the NAc, which was blocked by administration of SB-277011-A. Additionally, cocaine priming induced a decrease of DAT levels that was not modify by SB-277011-A pretreatment.

Conclusions: Present data suggest that alterations in DAD3R and DAT expression may be involved in cocaine reinstatement.

Keywords: Cocaine addiction, reinstatement, dopamine D3 receptor (DAD3R), DAT, NAc

Supported by grant SAF 2013-49076-P (MINECO, Spain).

References:

[1] P. Sokoloff, B. Le Foll, *Eur. J. Neurosci.,* **2017**, *45*, 2–19.

[2] M.M. McGinnis, C.A. Siciliano, S.R. Jones, *J. Neurochem.,* **2016**, *138*, 821–829.

P068
SCREENING OF NEW SELECTIVE MAO-B INHIBITORS: POTENTIAL THERAPEUTIC USE OF COUMARIN DERIVATIVES IN PARKINSON'S DISEASE

Rodríguez-Enríquez F.[1], Quezada E.[2], Matos M.J.[2], Costas-Lago M.C.[3], Torres-Terán I.[1], Besada P.[3], Terán C.[3], Uriarte E.[2], Viña D.[1], Fontenla J.Á.[4]

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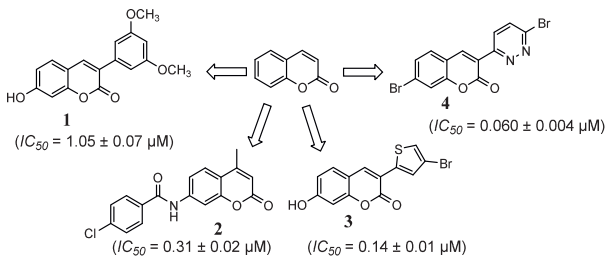
Parkinson's disease (PD) is the second most prevalent neurodegenerative disease. Current dopamine-replacement strategies provide symptomatic relief, however, their effectiveness wear off over time and their prolonged use leads to disabling side-effects in PD patients. Besides, selegiline and rasagiline, two known irreversible MAOI-B, Recently the EMA has approved the reversible safinamide.[1] The activity of MAO-B increases with age. The hydrogen peroxide released by the action of MAOs produces neurotoxic free radicals and other intermediate metabolites in the metabolism of dopamine that favour neurotoxicity and death by apoptosis.[2] Due to the limited number of MAOIs-B in clinical practice and their importance, many research efforts continue to be directed towards the development of new drugs with this mechanism of action. In recent years, our group has evaluated more than a thousand new coumarin derivatives, confirming that this suitably substituted structure is an adequate synthon for obtaining new reversible MAOIs-B, which in some cases also have neuroprotective properties.[3]



In this work, we present the results of MAOI-B activity *in vitro* and *in vivo*, in hypokinetic mice treated with reserpine, of four selected coumarin derivatives. Molecules **3** and **4** (100 mg/Kg) exhibited *in vivo* effects similar to those of selegiline (10 mg/Kg) in all parameters studied (locomotor activity, speed, % total movement).

Keywords: Parkinson's disease, MAO-B inhibitors, coumarin

References:

[1] L. Dezsi, L. Vecsei, *CNS Neurol Disord Drug Targets*., **2017**, 16, 425–30.

[2] D. Aarslandi, B. Creese, K. R. Chaudhuri, *Nat Rev Neurol*., **2017**, 13, 217–31.

[3] M. J. Matos, D. Viña, S. Vázquez-Rodríguez, E. Uriarte, L. Santana, *Curr Top Med Chem*., **2012**, 12, 2210–39.

P069
A SUPERPOTENT BIVALENT LIGAND FOR DOPAMINE D_2 RECEPTOR HOMODIMERS

Casadó-Anguera V.[1], Pulido D.[2], López L.[3], Moreno E.[1], Ferré S.[4], Cordomí A.[3], Cortés A.[1], Pardo L.[3], Royo M.[2], Casadó V.[1]

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Dopamine plays a key role in numerous CNS processes including motor control and response to reward-associated stimuli. Dopamine acts on specific receptors belonging to the G protein-coupled receptor (GPCR) family, which are categorized in two groups: D_1-like (D_1R and D_5R) and D_2-like (D_2R, D_3R and D_4R). It has been demonstrated that many of the GPCRs are associated as homodimers, heterodimers and higher order oligomers *in vivo*, which are important for signal transduction and cross-talk between GPCR pathways. Recently, it has been described the existence of several heterotetrameric complexes, such as the constituted by $A_{2A}R$ and D_2R homodimers, which heteromerize through the transmembrane domain (TM) 5. In this study, we have demonstrated the formation of D_2R homodimers through TM6 interface. Using computer modeling, we have predicted the distance between the orthosteric binding pockets of the two protomers in the homodimer and synthetized spiperone-based bivalent ligands. By

radioligand binding assays, we have determined the affinity of the monomeric and dimeric ligands, obtaining a significant better affinity of the bivalent ones. Furthermore, this differential affinity was abolished by the treatment with D_2R TM6 peptide, which disrupts de homodimer. These results demonstrate that our bivalent ligands are able to bind simultaneously to both receptors within the homodimer. Our bivalent ligands set the basis for the synthesis of agonist- or agonist/antagonist-based bivalent ligands useful for disease-treatments. Furthermore, they open the possibility of using them as a homodimer detection tool in tissues and also for the design and synthesis of tetravalent ligands to detect D_2R heterooligomers.

Keywords: GPCR, bivalent ligand, dopamine receptor, homodimer

P070
VALIDATION OF INFLAMMATORY BIOMARKERS AND PHARMACOLOGICAL TARGETS IN TRAUMATIC BRAIN INJURY

Farré-Alins V.[1,2,3], *Palomino-Antolín A.*[1,2,3], *Narros P.*[1,2,3], *Rosa J.M.*[1,2,3], *Carabias C.S.*[4], *Alegre M.S.*[4], *Lagares A.*[4], *Hernández-García B.J.*[5], *Egea J.*[1,2,3]

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Traumatic brain injury (TBI) is a frequent serious problem and its consequences are unknown. In this context, neuroinflammation that takes place after TBI plays a key role in the development of secondary lesions, which can become chronic.[1] Thus, the main aim of this study is to validate inflammation-related biomarkers in TBI patients. Furthermore, we analyze *in vitro* and in the mice model "Closed Head Injury" (CHI) the inflammatory routes that participate in TBI in order to detect potential pharmacological targets, focusing on NLRP3 inflammasome inhibition. In TBI patients, we have shown that SAA1 protein increases dramatically in serum at 24 hours after hospital admission, reaching a pick at 72 h. At 1 week SAA1 levels decrease to similar values than 24 h subjects. Moreover, we have detected a correlation between SAA1 serum levels and the severity of the lesion. The potential beneficial effect of inflammasome inhibition was evaluated in the CHI model.[2] We assessed the neurological functions of mice 1 h and 24 h after TBI using Neurological Severity Score (NSS) scale, and the treated groups were injected intraperitoneally with MCC950 (3 or 10 mg/kg) immediately following the 1-hour test. In non-treated animals, we obtained a score of 7 after 1 h, while after 24 h it decreased to 5. Both dosages decreased NSS at 24 h, although it was only significant at 3 mg/kg (3.8). Furthermore, MCC950 reduced brain edema as well as blood brain barrier impairment. Therefore, inflammation could be a potential target to treat detrimental effects of traumatic brain injury.

Keywords: traumatic brain injury, inflammation, biomarkers, SAA1, inflammasome

References:

[1] Simon DW, McGeachy MJ, Bayır H, *Nat. Rev. Neurol*., 2017, *13*, 171–191.

[2] Fierl MA, Stahel PF, Beuachamp, KM, *Nat. Protoc.*, 2009, *4*, 1328–1337.

P071
IS THERE A ROLE FOR ASTROCYTES IN THE REGULATION OF VENTRAL TEGMENTAL AREA DOPAMINE NEURON ACTIVITY AND IN BINGE-LIKE ALCOHOL DRINKING?

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Dopamine (DA) neurons originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens are crucially involved in the neurobiological mechanisms underlying reward and drug seeking behavior. Emerging evidence suggests that astrocytes can play an important and underestimated role in the development and maintenance of drug abuse by actively influencing many aspects of neuronal function. Astrocytes respond to different neurotransmitters and/or drugs with intracellular Ca^{2+} elevations and the release of gliotransmitters that ultimately modulate neuronal activity and impact behavior. Whether a similar cross-talk between astrocytes and neurons is present in the VTA, and whether this specific interaction contributes to the development of addictive behaviors, is a still unexplored issue. Here we took advantage of a combination of tools, including inositol 1,4,5-trisphosphate receptor type 2 (IP3R2) knockout mice (in which astrocytic Ca^{2+} surges are absent), *in vivo* single-unit electrophysiological recordings, and a model of binge-like alcohol drinking to address the following questions. Are astrocytes involved in the regulation of VTA DA neuron activity? Do astrocytes play a role in binge-like alcohol drinking? While preliminary, our results indicate that removal of IP3R2, the predominant source of physiological Ca^{2+} elevations in astrocytes, does not significantly affect basal electrophysiological properties of VTA DA neurons and binge-like alcohol drinking in mice. We are currently investigating whether a more sophisticated approach, namely selective impairment of gliotransmission within the VTA, would allow to uncover astrocyte role, if any, in the modulation of mesolimbic DA circuitry and alcohol drinking.

Keywords: astrocytes, dopamine neurons, ventral tegmental area, alcohol

P072
NEW STREPTOMYCES COMPOUNDS MODULATE NEUROINFLAMMATION IN VITRO

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Neuroinflammation has been related to neurodegenerative disorders as Alzheimer's or Parkinson's diseases. Microglia are the main immune cells in the central nervous system and provide beneficial functions to the brain, as tissue repair and host defence. However, chronic and dysregulated activation of microglia produces the accumulation of proinflammatory molecules as cytokines, reactive oxygen species (ROS) and nitric oxide (NO), which can lead to the death of surrounding neurons. Marine actinobacteria are an important source of bioactive secondary metabolites with anti-inflammatory, antioxidant or antibacterial activities. Two new glycosylated angucyclinones were obtained from a culture of *Streptomyces* sp. Their anti-inflammatory potential was evaluated in an *in vitro* model of neuroinflammation with the murine microglial cell line BV2. Compounds were able to reduce the levels of proinflammatory molecules (interleukin 1-β, tumor necrosis factor-α, NO and ROS) and to increase the release of interleukin-10, an anti-inflammatory cytokine. The translocation to the nucleus of NF-κB, master

regulator of the inflammatory response, and Nrf2, a key transcription factor that regulates antioxidant and anti-inflammatory enzymes, was also assessed. Both compounds reduced the nuclear expression of NF-κB-p65 subunit and increased the translocation of Nrf2 to the nucleus. In order to determine the effect of the compounds in the survival of neuronal cells, a trans-well co-culture was established with BV2 cells and SH-SY5Y neuroblastoma cells. The addition of compounds to activated microglia increased the viability of SH-SY5Y cells. Our results indicate that these new *Streptomyces* compounds can be considered as potential candidates for the treatment of neuroinflammation.

Keywords: *Streptomyces*, BV2, neuroinflammation

P073
STRAIN-DEPENDENT PERFORMANCE IN NALOXONE-INDUCED CONDITIONED PLACE AVERSION: ROLE OF HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL AXIS

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The intense associative memories that develop between drug-paired contextual cues and the drug withdrawal associated aversive feeling have been suggested to contribute to the high rate of relapse. Our study was aimed to elucidate the implication of hypothalamic-pituitary-adrenocortical (HPA) axis activity in the expression and extinction of aversive memory in two inbred mouse strains, Swiss and C57BL/6(B6) mice. The animals were rendered dependent on morphine by i.p. injection of increasing doses of morphine (10–60 mg/kg). Negative state associated with naloxone (1 mg/kg s.c.)-precipitated morphine withdrawal was examined by using conditioned place aversion (CPA) paradigm. B6 mice present a higher aversion score than Swiss mice together with a delay in the period of aversive memory extinction. These results suggest a stronger withdrawal syndrome in B6 mice. The greater sensitivity of this strain for addiction could be related to its greater capacity to perform learning that leads to associate naloxone and contextual cues, recovering better the aversive memory and extending the extinction period. The differences observed between Swiss and B6 mice suggest that treatment of addictive disorders should take into account an individual predisposition to associate the aversive learning with the context.

Keywords: drug withdrawal, aversive memory, CPA, extinction, strain

P074
EVALUATION OF PALMITOYLETHANOLAMIDE THERAPEUTIC EFFECT IN A MOUSE MODEL OF AUTISM SPECTRUM DISORDER

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in language, social interactions and severe stereotyped behaviours,[1] whose variety or severity is related to genetic and/or environmental factors.[2] Lipid signals play an important role in modulating behaviour, metabolism, pain and inflammation.[3] Among endogenous lipids, palmitoylethanolamide (PEA) has been extensively studied for its anti-inflammatory and neuroprotective effects mediated by activation of the nuclear peroxisome proliferator activated receptor (PPAR)-alpha.[4] Noteworthy, the genetic inactivation of this receptor resembles the behavioral phenotype consistent with ASD and the administration of a synthetic PPAR-alpha agonist improves behavioural traits in BTBR T+tf/J (BTBR) mouse model of ASD.[5] Based on this background, the aim of the study was to investigate the possible efficacy of PEA in BTBR mice and to shed light on the mechanisms underlying PEA effects. Our results showed that PEA reverted behavioral phenotype of BTBR mice in a dose-dependent manner, and this effect was contingent to PPAR-alpha activation, since PEA failed in exerting its effect in PPAR-alpha null mice or in mice pre-treated with PPAR-alpha antagonist. Moreover, the effect of PEA was related to a modulation of the expression of pro-inflammatory cytokines at central level, together with an involvement of neuroprotective factors. In conclusion, functional and molecular findings demonstrated a therapeutic potential of PEA in limiting ASD symptoms, suggesting bioactive lipids as new candidates for the treatment of ASD, able to improve the quality of life for ASD patients.

Keywords: autism, PEA, behavior, inflammation, neuropharmacology

References:

[1] M.C. Lai, M.V. Lombardo, S. Baron-Cohen. *Lancet*. **2014**, *383 (9920)*, 896–910.
[2] C. Cristiano, A. Lama, F. Lembo, M.P. Mollica, A. Calignano, G. Mattace Raso. *Front Physiol.* **2018**, *9*, 184.
[3] D. Piomelli, O. Sasso. *Nature neuroscience*. **2014,** *17(2)*, 164–174.
[4] J. Lo Verme, J. Fu, G. Astarita, G. La Rana, R. Russo, A. Calignano, D. Piomelli. *Mol Pharmacol*. **2005**, *67(1)*, 15–9.
[5] G. D'Agostino, C. Cristiano, D.J. Lyons, R. Citraro, E. Russo, C. Avagliano, R. Russo, G. Mattace Raso, R. Meli, G. De Sarro, L.K. Heisler LK, A. Calignano. *Mol Metab.* **2015**, *4(7)*, 528–36.

P075
PRENATAL EXPOSURE TO Δ9-TETRAHYDROCANNABINOL AFFECTS VENTRAL TEGMENTAL AREA DOPAMINE NEURONS AND ASSOCIATED BEHAVIOURS

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Cannabis is the most commonly abused illicit substance among pregnant women. Prenatal exposure to its major psychoactive ingredient, Δ^9-tetrahydrocannabinol (Δ^9-THC), has negative impact on cognitive and affective processing. Indeed, Δ^9-THC is a partial agonist at the type 1 cannabinoid receptor, a key component of the endocannabinoid system, which is involved in the central nervous system development. Despite cannabis' potential to interfere with neurodevelopment, investigations about its long-term effects following in utero exposure are limited, especially during preadolescence, a highly susceptible period before the onset of psychiatric disorders. Here, we investigated how prenatal exposure to Δ^9-THC (pTHC) affects brain regions involved in psychotic/affective disorders, such as the ventral tegmental area (VTA), in preadolescent rats. In this study, Sprague-Dawley rat dams were administered Δ^9-THC (2 mg/kg s.c.), or its vehicle, once per day from gestational day 5 to 20. Male offspring were used for experiments. Patch-clamp recordings from VTA dopamine cells showed that pTHC induces an intrinsic plasticity associated with an increased postsynaptic response to excitatory stimuli, as well as a reduced GABA release. Correlated confocal/STochastic Optical Reconstruction Microscopy (STORM) revealed significantly altered structural plasticity of presynaptic active zone at excitatory and inhibitory terminals impinging onto VTA dopamine neurons. Moreover, behavioural experiments unveiled increased susceptibility to acute effects of Δ^9-THC. Our results show that prenatal exposure to Δ^9-THC is associated with molecular and cellular alterations in the VTA and with increased vulnerability to Δ^9-THC-induced psychosis during preadolescence, which might lead to psychiatric disorders later in life.

P076
EFFECT OF A LONG-TERM TREATMENT WITH METFORMIN IN DYSTROPHIC *MDX* MICE: A RECONSIDERATION OF ITS THERAPEUTIC INTEREST IN DUCHENNE MUSCULAR DYSTROPHY

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The pharmacological stimulation of AMP-activated protein kinase (AMPK) via metabolic enhancers has been proposed as potential therapeutic strategy for Duchenne muscular dystrophy (DMD). Metformin, a widely-prescribed anti-hyperglycemic drug which activates AMPK via mitochondria, has been recently tested in DMD patients in synergy with NO-precursors, with encouraging results.[1] However, preclinical data supporting the use of metformin in DMD are still poor, and its actions on skeletal muscle appear controversial. Therefore, we investigated the effects of a long-term (20-week) treatment with metformin (200 mg/kg/day, orally) in the exercised *mdx* mouse model, characterized by a severe mechanical-metabolic maladaptation.[2,3,4] Metformin significantly ameliorated histopathology in *mdx* gastrocnemius muscle, in parallel reducing TGF-β1 with a recovery score (r.s) of 106%; this was accompanied by a decreased plasma matrix-metalloproteinase-9 (r.s.43%). Moreover, metformin significantly increased *mdx* diaphragm twitch (r.s.44%) and tetanic (r.s.36%) tension ex vivo, in spite of minor effects on in vivo weakness. However, no clear protective actions on dystrophic muscle metabolism were observed, as shown by the poor metformin effect on AMPK activation measured by western blot, on the expression of mechanical-metabolic response genes analyzed by qPCR, and by the lack of myofiber-type-shift assessed by SDH staining in tibialis anterior muscle. Similar results were obtained in sedentary *mdx* mice. The lack of metabolic effects could be, at least partly, due to metformin inability to increase *mdx* muscle levels of L-arginine, L-citrulline and taurine, found by HPLC. Our findings encourage to explore metabolism-independent mechanisms of action to differently repurpose metformin in DMD, supporting its therapeutic combination with NO-sources.

Keywords: metformin, AMP-activated protein kinase, Duchenne muscular dystrophy, *mdx* mouse, skeletal muscle, chronic exercise

References:

[1] P. Hafner et al. *PLoS One*, **2016**, *11(1)*: e0147634.
[2] G.M. Camerino et al. *Hum Mol Genet*, **2014**, *23(21)*: 5720–32.
[3] R.F. Capogrosso et al. *J Appl Physiol (1985)*, **2017**, *122(4)*: 828–43.
[4] T. Gamberi et al. *J Proteomics*, **2018**, *170*:43–58.

P077
PRIOR EXPOSURE TO NICOTINE INCREASES THE REWARDING EFFECTS TO PSYCHOSTIMULANTS AND Δ9-TETRAHYDROCANNABINOL (THC) IN MICE AND ZEBRAFISH

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Marijuana and tobacco are substances frequently used by adolescents. Epidemiological studies have shown that the use of tobacco usually precedes the use of marijuana.[1] Nicotine can therefore act, on the brain, as a "gateway drug", an effect that seems to be a response to the drug *per se*,[2] but there are no data regarding its possible gateway effect in relation to amphetamine derivatives. Zebrafish is a valuable model for high throughput drug discovery and screening and can be used to investigate some aspects of neuropsychiatric disorders, including addiction.[3] On these bases our aim was to investigate the gateway effect of nicotine on the rewarding properties of Δ^9-THC and MDMA, in mice and zebrafish, using the classical conditioned place preference (CPP) task. Mice were exposed for seven weeks (3 times/day) to standard (cig) and electronic (e-cig) cigarettes while zebrafish to nicotine (1 mg/L) dissolved in the water tank for two weeks. At different intervals of nicotine/cig/e-cig withdrawal (wdw) animals were tested in CPP paradigm after treatment with Δ^9-THC (mice: 0.01 mg/kg/i.p.; zebrafish: 0.001–0.1 mg/kg/i.m.) and MDMA (zebrafish: 0.1 mg/kg/i.m.). During wdw, mice exposed to e-cig/cig showed a greater Δ^9-THC induced-CPP than control group, showing less CP-55.940 stimulated GTPγS binding of CB_1 receptors an increase in GluR1 receptor density in the nucleus accumbens. In zebrafish preliminary studies indicated an increased response to the rewarding effects of MDMA during wdw. Our results show that chronic nicotine exposure induces in mice altered responses to Δ^9-THC- and in zebrafish to MDMA-induced CPP.

Keywords: tobacco cigarette, conditioned place preference, 3,4-methylenedioxymethamphetamine, 2,5-dimethoxy-4-bromo-amphetamine hydrobromide (DOB), *para*-methoxyamphetamine (PMA)

References:

[1] JA Pipkin et al, Drug Alcohol Depend, **2014**, 142:341–4.
[2] DB Kandel et al, N Engl J Med, **2014**, 371(21): 2038–9.
[3] A Stewart et al, Rev Neurosci, **2011**, 22(1):95–105.

P078
EFFECTS OF CHRONIC ANTIPSYCHOTICS ADMINISTRATION ON AKT-DEPENDENT SIGNALLING PATHWAYS IN RAT BRAIN CORTEX

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The Akt-dependent signaling pathway is an emerging candidate for playing a role in schizophrenia. This pathway acts as a molecular systems integrator and regulates protein synthesis, transcription, autophagy, metabolism, and organelle biogenesis and maintenance. The aim of the present study was to evaluate the status of key proteins involved in Akt-dependent signalling pathways, Akt, phospho-Akt (Ser473), GSK3β, phospho-GSK3β (Ser9), rpS6 and phospho-rpS6 (Ser235/236), in the brain cortex of rats chronically treated with haloperidol, risperidone or clozapine. Animals (n = 10) were treated (every 12 h, 21 days) with saline (1 ml/kg, i.p.), clozapine (5 mg/kg, i.p.), risperidone (0.5 mg/kg, i.p.) or haloperidol (0.5 mg/kg, i.p.). Western-blot technique was used to quantify protein levels in total homogenates of rat brain cortex. Both haloperidol and clozapine induced a significant decrease (−37% and −32% respectively, $p < 0.05$) in the active phosphorylated form of Akt (phospho-Akt (Ser473)) while no changes were found in Akt total form. Conversely, haloperidol significantly increased (+26%, $P < 0.05$) GSK3β but no phospho-GSK3β (Ser9). Clozapine did not produced significant changes in both proteins. Risperidone was not able to modulate the levels of none of the proteins evaluated. No changes were observed in S6 and phospho-rpS6 (Ser235/236) protein levels in the brain cortex of rats treated with any antipsychotic. These findings suggest that antipsychotics may modulate both density and phosphorylation of some proteins of Akt-signalling pathways.

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Keywords: antipsychotics, haloperidol, risperidone, clozapine, Akt, GSK3β, S6, rat brain

P079
BEHAVIOURAL EVALUATION OF A TRANSLATIONAL ANIMAL MODEL OF SCHIZOPHRENIA

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Schizophrenia (SZ) is a chronic and disabling psychiatric disorder affecting about 1% of the population worldwide. Schizophrenia comprises positive and negative symptoms as well as cognitive deficits. Epidemiological and experimental studies indicate that infections during the gestational period represent a risk factor to develop SZ along lifetime, which in combination with stressful events in adolescence may lead to the SZ onset. The aim of the present study was to create a translational "double-hit" animal model of SZ in male and female mice, based in maternal immune activation (MIA, hit-1)—injection of poly(I:C) to pregnant dams, 7.5 mg/kg i.p.—and social isolation (SI, hit-2) in the peri-pubertal period (3–11 weeks). In the four experimental groups (hit-1, hit-2, double-hit and control) locomotion and anxiety were assessed using the Open Field Test (OFT), and the cognitive status (declarative/episodic memory) was evaluated by means of the Novel Object Recognition Test (NORT). No differences were observed in the spontaneous locomotor activity between any of the groups, neither in females nor in males. However, an increase in the percentage of time spent in the centre of the OFT was significantly associated to the hit-1 (MIA) only in female mice ($F[1,53] = 4.252$; $P = 0.044$, $n = 57$). Moreover, a significant decrease in the discrimination index in the NORT was also associated to the hit-1 (MIA) in the subgroup of female mice ($F[1,55] = 7.266$; $P = 0.0093$, $n = 59$). These preliminary results indicate that MIA produces a greater impact in female mice inducing an anxiolytic-like phenotype and cognitive impairments.
Supported by Basque-Government (IT616/13) and MSCA-2016-IF-C.Muguruza.
Keywords: schizophrenia, animal model, maternal immune activation, social isolation, sex differences

P080
EVALUATION OF LONG-TERM DESIPRAMINE ADMINISTRATION ON α_2- ADRENOCEPTORS REGULATING NORADRENALINE AND SEROTONIN RELEASE IN RAT FRONTAL CORTEX

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Antidepressant drugs take time to develop their effect, typically 2–3 weeks. It has been shown that desensitization of α_2-adrenoceptors is a common response to the chronic treatment with antidepressant drugs that increase synaptic noradrenaline (NA) concentration. The present study was undertaken to elucidate the effect of repeated treatment with the tricyclic antidepressant desipramine (DMI) (twice daily, 14 days. 72 hours of washout period) on the frontal cortex (FC) noradrenergic and serotonergic transmission by rat brain microdialysis, focusing on the effect of α_2-adrenoceptors sensitivity. Acute administration of the α_2-adrenoreceptor agonist clonidine (0.3 mg/kg i.p.) decreased NA in FC ($E_{max} = -45 \pm 7\%$; $P < 0.0001$). Administration of the α_2-adrenoreceptor antagonist RX821002 (1 mg/kg i.p.) enhanced NA concentration ($E_{max} = 268 \pm 48\%$; $P < 0.0001$). For α_2-adrenoreceptor-mediated serotonin (5-HT) release inhibition ($E_{max} = -30 \pm 9\%$; $P < 0.0001$), a higher dose of clonidine was needed (0.9 mg/kg i.p.). RX821002 administration (1 mg/kg i.p.) did not modulate 5-HT concentration in the FC. After long-term desipramine treatment, a high dose clonidine injection (0.9 mg/kg i.p.) decreased NA concentration in the FC in control ($E_{max} = -42 \pm 12\%$; $P < 0.0001$) and treated groups ($E_{max} = -40 \pm 12\%$; $P < 0.0001$). In the same way, when 5-HT concentration was measured after clonidine administration (0.9 mg/kg i.p.) a similar decrease of 5-HT was observed in control ($E_{max} = -60 \pm 10\%$; $P < 0.0001$) and treated groups ($E_{max} = -59 \pm 6\%$; $P < 0.0001$). These findings indicate that long-term administration of DMI is not able to desensitize the α_2-adrenoreceptor subpopulation that exert an inhibitory control on 5-HT release by serotonergic terminals in the FC.
Supported by the Basque Government (IT616/13). I.M-A. is recipient of a Predoctoral Fellowship from the Basque Government.
Keywords: α_2-adrenoreceptor, prefrontal cortex, desipramine, noradrenaline, serotonin, clonidine

P081
SPINOPHILIN EXPRESSION IN POSTMORTEM PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA: EFFECT OF ANTIPSYCHOTIC TREATMENT

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Spinophilin is a scaffold protein that modulates excitatory synaptic transmission and dendritic spine morphology. Spinophilin also regulates the intracellular signaling of some receptors that play a role in the pathophysiology of schizophrenia and are targets of antipsychotics. For those reasons, it is of interest to study spinophilin expression in the brain of subjects with schizophrenia. Spinophilin protein expression was determined by Western Blot in the postmortem prefrontal cortex of forty-eight subjects: twenty four subjects with an antemortem diagnosis of schizophrenia, and twenty four controls with no history of psychiatric disease; matched by age, gender, and postmortem delay. Twelve of the schizophrenia subjects were under antipsychotic treatment at death (positive blood toxicology) and twelve were antipsychotic-free (negative toxicology). Spinophilin was measured in synaptosomes and in postsynaptic membrane fractions (PSD), using actin immunoreactivity as loading control. Two immunoreactive bands were detected for spinophilin at ~120 and ~95 kDa. The ~120 kDa band showed no significant differences between schizophrenia and control subjects neither in synaptosomes nor in PSD. The ~95 kDa band was significantly reduced in synaptosomes (-15%, $P = 0.0067$) and in PSD (-15%, $P = 0.0383$) in schizophrenia subjects compared with controls. This reduction was also observed in antipsychotic-treated subjects, both in synaptosomes (-24%, $P = 0.0028$) and in PSD (-26%, $P = 0.0135$), but no differences were observed in antipsychotic-free subjects. In conclusion, the present results suggest that antipsychotic treatment reduces the ~95 kDa band spinophilin expression in prefrontal cortex of schizophrenia subjects.
Supported by grants from the Spanish MINECO (SAF2013-48586-R) and the Basque Government (IT616/13).
Keywords: spinophilin, schizophrenia, postmortem human brain, antipsychotics, prefrontal cortex

P082

VESICULAR GLUTAMATE TRANSPORTER MODULATES BEHAVIOURAL RESILIENCE IN RODENTS: ADVANCE IN THE SEARCH FOR INNOVATIVE TARGETS FOR DEPRESSION

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Increasing evidence indicates that aberrant plasticity at glutamate synapses is a key feature of susceptibility to stress, and represents a promising target for innovative depression therapies.[1] Here we focused on glutamate neuroplasticity in the nucleus accumbens (NAc), the prime hub of reward circuit, in susceptibility to stress, and assessed whether the pharmacological inhibition of glutamate release may promote resilience in rodents. Synaptic plasticity in the NAc of stress-susceptible mice was evaluated by immunofluorescence and confocal microscopy of input-specific synaptic markers (vesicular glutamate transporters (VGLUT)-1 and 2, PSD95) and morphology.[2,3] The contribution of subcortical excitatory input to the NAc in mediating a susceptible phenotype was assess by circuit-specific optogenetics.[3,4] Lastly, behavioural and neuro-functional effects of glutamate release inhibition were evaluated by systemic administration of BYA-2, novel VGLUT inhibitor.[5] Stress-susceptible mice showed decreased VGLUT-1- and increased VGLUT-2 axon terminals, in the absence of post-synaptic alterations of PSD95, spine density or type in the NAc. Optogenetic stimulation of the thalamic excitatory input to the NAc increased latency to eat in the novelty suppressed feeding test, showed a trend in decreasing sucrose preference, while it did not altered behavioural reactivity in open field and elevated plus maze. On the other hand, BYA-2 was effective in promoting swimming in the forced swim test and region-specific c-fos expression in the reward circuit. Taken together, these data suggest a role for subcortical excitatory projections to the NAc in inducing stress-susceptibility and point at VGLUT as potential target for promoting resilience and develop next-generation glutamate-based antidepressant therapies.

Keywords: synaptic plasticity, nucleus accumbens, vesicular glutamate transporter, resilience, glutamate-based antidepressant

References:

[1] J.W. Murrough, C.G. Abdallah CG, S.J. Mathew SJ. *Nat Rev Drug Discov*., **2017** *16(7)*, 472–486.

[2] G.E. Hodes, M.L. Pfau, I. Purushothaman, H.F. Ahn, S.A. Golden, D.J. Christoffel, J. Magida, A. Brancato, A. Takahashi, M.E. Flanigan, C. Ménard, H. Aleyasin, J.W. Koo, Z.S. Lorsch, J. Feng, M. Heshmati, M. Wang, G. Turecki, R. Neve, B. Zhang, L. Shen, E.J. Nestler, S.J. Russo. *J Neurosci.* **2015** *35(50),* 16362–76.

[3] R.T. Jr Fremeau, K. Kam, T. Qureshi, J. Johnson, D.R. Copenhagen, J. Storm-Mathisen, F.A. Chaudhry, R.A. Nicoll, R.H. Edwards. *Science*. **2004** *304(5678*), 1815–9.

[4] D.J. Christoffel, S.A. Golden, J.J. Walsh, K.G. Guise, M. Heshmati, A.K. Friedman, A. Dey, M. Smith, N. Rebusi, M. Pfau, J.L. Ables, H. Aleyasin, L.A. Khibnik, G.E. Hodes, G.A. Ben-Dor, K. Deisseroth, M.L. Shapiro, R.C. Malenka, I. Ibanez-Tallon, M.H. Han, S.J. Russo. *Nat Neurosci.* **2015** *18(7),* 962–4.

[5] J. Kehrl, J.C. Althaus, H.D. Showalter, D.M. Rudzinski, M.A. Sutton, T. Ueda. *Neurochem Res*. **2017** *42(6)*, 1823–1832.

P083

IMPLICATION OF BDNF PATHWAYS IN THE BASOLATERAL AMYGDALA FOLLOWING NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL IN A CONDITIONED PLACE AVERSION PARADIGM

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Brain-derived neurotrophic factor (BDNF) regulates neuron synaptic plasticity and plays a vital role in learning and memory in multiple brain areas. The amygdala is a brain structure especially important for the storage of fear memories, being necessary for both fear acquisition and extinction. Therefore, we used male mice and performed conditioned place aversion (CPA) paradigm to study the role of the precursor of BDNF (proBDNF) and BDNF mature form on plasticity-related processes that occur within the basolateral amygdala (BSA) during opioid withdrawal conditioning. The animals were rendered dependent on morphine by intraperitoneal injection of increasing doses of morphine (10–60 mg/kg). Negative state associated with naloxone (1 mg/kg s.c.)-precipitated morphine withdrawal was examined by using CPA. The extracellular-regulated kinase (ERK), CREB, proBDNF and BDNF expression were detected in BSA by immunoblotting. Mice subjected to conditioned morphine withdrawal robustly expressed CPA, which was accompanied by significantly increased levels of BDNF mature form without significant changes in proBDNF or pCREB. However, after CPA expression we observed a significant decrease in pERK expression. Altogether, these results indicate that other pathways, such as PKA or CaMK-IV, but not ERK, or CREB could be implicated in the activation of BDNF in the BSA. Formation and expression of aversive memories associated with conditioned withdrawal in morphine-dependent mice requires BDNF expression in the BSA.

Keywords: BDNF, morphine, aversión

P084

THE ROLE OF GUO AND UA SIGNALING IN THE EFFECTS ASSOCIATED TO THE FUNCTIONAL INTERPLAY BETWEEN CAMP AND CGMP SYSTEM IN THE CNS

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Growing evidence suggests that the guanine-based purines stand out as key player in cell metabolism and in signaling pathways involved in neurodegenerative disorders.[1] The nucleobase Guanine (GUA) is generated from the nucleoside Guanosine (GUO) in a reaction catalyzed by the purine nucleoside phosphorylase (PNP),[2] whereas its degradation to xanthine is mediated by Guanine deaminase (GDA), also called cypin.[3] GUO and GUA are both able to activate cGMP through different signaling pathways leading to neurite outgrowth[4] or cognitive improvement,[5] respectively. Conversely, neither GUO nor GUA have been implicated in the activation of the cAMP system, which is widely involved in the modulation of cognitive functions.[6,7] Therefore, we investigated, in SH-SY5Y cells, whether the activation of cAMP could affect extracellular levels of GUO and GUA, the expression and activity of PNP and GDA, as well as the expression of several key molecules involved in the signaling cascades converging on the cGMP formation. Interestingly, the EPAC-specific cAMP analogue 8-pCPT-2′-OMe-cAMP (8-Me) and, to a less extent, the PKA-specific 6-Bnz-cAMP analogue, enhanced cGMP formation induced by GUA, whereas they did not affect cGMP-mediated neuritogenesis induced by GUO. 8-Me was also able to increase cypin expression, a cytosolic

PSD-95 interactor regulating neuritogenesis, and reduced that of PNP, thus eliciting an accumulation of extracellular GUO parallel to a reduction of GUA. Collectively, our data suggest that the concomitant activation of both cAMP-cGMP systems in SH-SY5Y cells resulted to be synergic for dendrite elongation and differentiation and represents a viable path forward for treating age-related disorders.

Keywords: guanosine, guanine, cGMP, cAMP, nitric oxide, CNS

References:

[1] Di Liberto V., Mudò G., Garozzo R., Frinchi M., Fernandez-Dueñas V., Di Iorio P., et al., *Front. Pharmacol.*, **2016**, 7:158, https://doi.org/10.3389/fphar.2016. 00158.
[2] Rathbone M., Pilutti L., Caciagli F., Jiang S., *Nucleos. Nucleot. Nucl.,* **2008**, 27, 666–672.
[3] Miyamoto S., Ogawa H., Shiraki H., Nakagawa H., *J. Biochem*, **1982**, 91, 167–176.
[4] Bau C., Middlemiss P. J., Hindley S., Jiang S., Ciccarelli R., Caciagli F., et al., *Purinerg. Signal.* **2005**, 1, 161–172.
[5] Zuccarini M., Giuliani P., Frinchi M., Mudò G., Serio R.M., Belluardo N., et al., *Front. Pharmacol.,* **2018**, 9:110. https://doi.org/10.3389/fphar.2018.00110.
[6] Kelly M.P., *Cell Signal.*, **2018**, 42, 281–291.
[7] Cabrera-Pastor A., Malaguarnera M., Taoro-Gonzalez L., Llansola M., and Felipo V., *Sci. Rep.*, **2016**, 6:33124. https://doi.org/10.1038/srep33124.

P085
REDOX IMBALANCE AND DRUG-INDUCED PSYCHIATRIC DISORDERS: FROM RODENT MODELS TO HUMAN PATHOLOGY

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Drug abuse increases the risk of developing psychiatric disorders.[1] Redox imbalance has recently gained great importance as crucial contributor for the development of drug abuse-induced neurotoxicity. One of the major source of free radicals in the brain is represented by NOX enzymes which have been shown to play a key role in the development of neuropathological alterations observed in animal models of psychosis,[2] as well as in post mortem brain samples of subjects with a psychiatric anamnesis.[3] In this study, we aimed to investigate the link between drug-induced neurotoxicity and NOX modifications in the brain by using a rodent model, obtained by perinatal administration of sub-anaesthetic doses of ketamine, with an approach toward the human pathology by using post mortem brain samples of a cocaine abuser affected by excited delirium syndrome and blood samples of drug abusers with an associated psychiatric diagnosis. Results showed that, in mice, ketamine administration during the perinatal life resulted in an increased locomotor activity as well as decreased discrimination abilities, accompanied by enhanced NOX2 and 8OHdG (a marker of oxidative stress) levels in both prefrontal cortex and nucleus accumbens. An increase of NOX2 and oxidative stress-related markers was also detected in the cortex of the cocaine-abuser, as well as in blood samples of the alive drug abusers with an associated psychiatric diagnosis. These findings suggest that NOX2 is a reliable biomarker for drug-induced psychiatric disorders and open new insights for novel therapeutic approaches based on the selective inhibition of this enzyme.

Keywords: drug abuse, oxidative stress, NOX enzymes, psychiatric disorders

References:

[1] Kirk et al., *Oslo, Norway: Knowledge Centre for the Health Services at The Norwegian Institute of Public Health (NIPH)*, **2008**, No. 25-2008.
[2] Schiavone and Trabace, *Pharmacol Res.* **2016** *107*:195–204.
[3] Schiavone et al., *Transl Psychiatry*. **2016** *17*;6:e813.

P086
BRAIN CARBONIC ANHYDRASES INHIBITION IMPAIRS FEAR MEMORY EXTINCTION IN RATS

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Fear extinction is an active form of learning defined by the attenuation of a learned response following non-reinforced exposure to a previously fearful stimulus. Such phenomenon is mediated by similar neural circuits in rodents and humans and therefore the interest in fear extinction as a translational model is raising much interest. Recent evidence indicates a role for central carbonic anhydrases (CAs) in fear memory acquisition and consolidation,[1,2,3] but nothing is known about its role in extinction. Thus, here we explored the impact of CAs activity on memory extinction. The effects of CAs inhibitors acetazolamide and compound 18 or CA activator D-phenylalanine were evaluated using the extinction of contextual fear conditioning paradigm in rats.[4] Systemic administration of acetazolamide (30 mg/Kg, i.p.) impaired, while injection of D-phenylalanine (300 mg/kg, i.p.) facilitated extinction memory consolidation. Co-treatment with acetazolamide prevented D-phenylalanine-induced promnesic effect. No behavioural alterations were detected after systemic treatment with Compound 18 (30 mg/kg, i.p.), a CA inhibitor unable to cross the blood brain barrier. Extinction deficits were also observed following acetazolamide (10 nmols/side) infusion locally into the CA1 region of the hippocampus, the basolateral amygdala or the ventromedial prefrontal cortex. However, acetazolamide was ineffective when infused into the substantia nigra. Neurochemical experiments are being carried out to further support our results. These findings are the first demonstration of brain CAs role in the extinction of fear memory, suggesting its potential as an innovative target for the development of novel drugs for the treatment of disorders characterized by maladaptive fear responses.

Keywords: fear extinction, carbonic anhydrases, acetazolamide, D-phenylalanine

References:

[1] Canto de Souza L, Provensi G, Vullo D, Carta F, Scozzafava A, Costa A, Schmidt SD, Passani MB, Supuran CT, Blandina P. Neuropharmacology, **2017**, 118, 148–156.
[2] Yang MT, Chien WL, Lu DH, Liou HC, Fu WM. Neuropharmacology, **2013,** 67, 412–418.
[3] Sun MK, Alkon DL. J Pharmacol Exp Ther, **2001**, 297(3), 961–967.
[4] de Carvalho Myskiw J, Furini CR, Schmidt B, Ferreira F, Izquierdo I. Proc Natl Acad Sci USA, **2015** 112(2),E230-3.

P087
SEROTONIN-2A HOMODIMERS ARE NEEDED FOR SIGNALLING VIA BOTH PHOSPHOLIPASE A_2 AND PHOSPHOLIPASE C IN TRANSFECTED CHO CELLS

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Different ligands differentially activate phospholipase A_2 (PLA_2) and phospholipase C (PLC) signalling pathways that are coupled to the serotonin 2_A (5-HT_{2A}) receptor, a class-A G-protein coupled receptor (GPCR). The serotonin 5-HT_{2A} receptor has been shown to be expressed as a homodimer displaying some ligands negative

cooperativity between protomers in the PLA_2 signalling pathway.[1] We hypothesized that the homodimeric complex is the minimum functional unit required for activation of the PLA_2 and PLC pathways by the serotonin 5-HT_{2A} receptor. To investigate this hypothesis, we partially blocked the serotonin 5-HT_{2A} receptors with ritanserin and measured PLA_2 and PLC activity simultaneously. We subsequently added the competitive antagonist spiperone to release the inactivator through a crosstalk mechanism and thus allow the dimer to return to a reactive state. Partial inactivation of the homodimer by ritanserin binding decreased the activity of the receptor by 59 ± 13% and 70 ± 4% in the PLA_2 and PLC pathways respectively ($P < 0.001$), with no difference in the potency of the serotonin (5-HT) was observed. The subsequent binding of spiperone released ritanserin due to the crosstalk between protomers and recovery of the receptor activity to 74 ± 7% and 72 ± 4%. Negative cooperativity between protomers in the dimer was maintained during arachidonic acid (AA) release after blocking ritanserin, as indicated by the biphasic inhibition curves for clozapine over 1 μM serotonin (5-HT). These findings provide evidence that serotonin 5-HT_{2A} receptors must be expressed as homodimers in order to activate both the PLA_2 and PLC signalling pathways.[2,3]

Keywords: serotonin 2_A receptor, phospholipase A_2, phospholipase C, homodimer, crosstalk, minimum functional unit

References:

[1] Brea J, Castro M, Giraldo J, López-Giménez JF, Padín JF, Quintián F, Cadavid MI, Vilaró MT, Mengod G, Berg KA, Clarke WP, Vilardaga JP, Milligan G, Loza MI, *Mol. Pharmacol*., **2009**, *75*, 1380–1391.
[2] Teitler M, Klein MT, *Pharmacol. Ther*., **2012**, *133*, 205–217.
[3] Iglesias A, Cimadevila M, Cadavid MI, Loza MI, Brea J, *Eur. J. Pharmacol*., **2017**, *800*, 63–69.

P088
ACETYLCHOLINESTERASE IMMOBILIZATION ON MICROPLATES FOR HIGH-THROUGHPUT SCREENING OF INHIBITORS

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Acetylcholinesterase (AChE) is involved in the termination of nerve impulse transmission at cholinergic synapses by catalyzing the rapid hydrolysis of the neurotransmitter acetylcholine in the central and peripheral nervous systems. AChE inhibition leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission. Hence, this enzyme is the primary target of acetylcholinesterase inhibitors applied as relevant drugs and toxins. Reversible inhibitors include drugs applied in neurodegenerative (Alzheimer's and Parkinson's diseases) or neurological (autism) disorders treatment, and toxic carbamates used as pesticides, whereas irreversible acetylcholinesterase inhibitors include organophosphorus compounds (insecticides and nerve agents).[1] Robust and reliable assays amenable for high-throughput screening are necessary in the discovery and development of AChE inhibitors. Enzyme immobilization on microplates facilitates carrying out reversion assays, re-use of microplates and reduction of costs in high-throughput drug screening campaigns. In this work, an enzyme assay for the screening of AChE inhibitors was developed with AChE immobilized on 96-well microplates via entrapment in a polymeric matrix of a mixture of poly(vinyl alcohol) (PVA) and PVA-SbQ. Polymerization was initiated by irradiation with UV light (366 nm) in presence of AChE. AChE activity was determined by evaluating with Ellman's reagent the production of thiocholine from acetylthiocholine. Entrapment conditions were optimized to maximize the Z-prime of the assay (Z' = 0.7). Finally, dose-response curves of model AChE reversible (donepezil) and irreversible (Chlorpyrifos) inhibitors were performed to assess the assay suitability for the screening of AChE inhibitors in high-throughput screening campaigns.

Keywords: Acetylcholinesterase inhibitors, high-throughput drug screening, acetylcholinesterase entrapment, microplate assay development

References:

[1] M. B. Čolović, D. Z. Krstić, T. D. Lazarević-Pašti, A. M. Bondžić, V. M. Vasić, *Curr. Neuropharmacol.*, **2013**, *11(3)*, 315–335.

O010
ULTRAMICRONIZED PALMITOYLETHANOLAMIDE IMPROVES LEARNING AND MEMORY IN A TRIPLE TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE THROUGH A COMBINATION OF ANTI-INFLAMMATORY AND NEUROPROTECTIVE EFFECTS

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Alzheimer's disease (AD) is a neurodegenerative disorder responsible for the majority of dementia cases in elderly people. Current treatments provide inadequate symptomatic relief as several distinct pathological processes are thought to underlie the decline of cognitive and neural function seen in AD. This suggests that the efficacy of treatment requires a multitargeted approach. In this context, palmitoylethanolamide (PEA) provides a novel potential adjunct therapy that can be incorporated into a multitargeted treatment strategy. We used young and adult 3×Tg-AD mice that received ultramicronized PEA (um-PEA, a formulation endowed with best bioavailability) for 3 months via a subcutaneous delivery system. Mice were tested with a range of cognitive and non-cognitive tasks, and potential neuropathological mechanisms were assessed post-mortem by western blot, RT-PCR, and immunofluorescence. Our data demonstrate that um-PEA rescues behavioral impairments and reduces Aβ formation, the phosphorylation of tau proteins, and promotes neuronal survival in the CA1 subregion of the hippocampus. Finally, um-PEA normalizes astrocytic function, rebalances glutamatergic transmission, and restrains neuroinflammation. The efficacy of um-PEA is particularly potent in younger mice, suggesting its potential as an early treatment. These data demonstrate that um-PEA is a novel and effective promising treatment for AD with the potential to be integrated into a multitargeted treatment strategy in combination with other drugs.

Keywords: Alzheimer's disease, 3×Tg-AD, um-PEA, pharmacology, neuroscience

O011
ADRA2A GENE EXPRESSION AND EPIGENETIC REGULATION IN POSTMORTEM PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA

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Cognitive deficits in subjects with schizophrenia (SZ) remain unsolved or even aggravated by antipsychotic treatment. Cognitive impairment in SZ might relate to prefrontal cortex (PFC) dysfunction. Activation of α_{2A}-adrenoceptors by selective agonist guanfacine improves disorders with PFC dysfunctionality. Thus, this study aimed to evaluate α_{2A}-adrenoceptor-encoding ADRA2A gene expression and epigenetic

regulation in post-mortem PFC of SZ subjects. Subjects with antemortem diagnosis of SZ (n = 24) were matched to control subjects (n = 24) for age, gender, and post-mortem delay. SZ subjects were classified as antipsychotic–treated (n = 12) or antipsychotic-free (n = 12) based on the presence or absence of antipsychotics in blood at autopsy time. ADRA2A gene expression was determined by RT-qPCR. Epigenetic regulation of ADRA2A gene was studied in part of the SZ subjects (n = 11) by chromatin immunoprecipitation (ChIP) of acetylated and methylated histones. ADRA2A promoter region was amplified by qPCR on immunoprecipitated DNA. Results were analysed by Student's two-tailed t-test. ADRA2A gene expression was unaltered in the whole SZ group and in antipsychotic-free SZ subjects. However, ADRA2A gene expression was selectively upregulated in antipsychotic-treated SZ subjects (+93% over controls, P = 0.042). Permissive H3K4me3, AcH3, AcH3K9, AcH3K27, AcH4K5, AcH4K16 and repressive H3K27me3 histone modifications at ADRA2A promoter were unaltered in SZ subjects. To our knowledge, this is the first time ADRA2A gene expression and epigenetic regulation in SZ are studied. Enhanced ADRA2A gene expression in antipsychotic-treated SZ subjects might relate to antipsychotic drug mechanism. Preliminary results in this study show that antipsychotic-induced histone acetylation or methylation at ADRA2A may not be involved in this effect.

Keywords: schizophrenia, post-mortem brain, antipsychotic drug, gene expression, epigenetic, histone acetylation, histone methylation

O014
REINTERPRETING ANOMALOUS COMPETITIVE BINDING EXPERIMENTS USING G PROTEIN-COUPLED RECEPTOR HOMODIMERS: CONSEQUENCES OF RADIOLIGAND-COMPETITOR ALLOSTERIC INTERACTIONS

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An increasing number of G protein-coupled receptors (GPCRs) have been reported to be expressed in the plasma membrane as dimers. Since most ligand binding data are currently fitted by classical equations developed only for monomeric receptors, the interpretation of data could be misleading in the presence of GPCR dimers. On the other hand, the equations developed from dimer receptor models assuming the existence of two orthosteric binding sites within the dimeric molecule offer the possibility to directly calculate macroscopic equilibrium dissociation constants for the two sites, an index of cooperativity (D_C) that reflects the molecular communication within the dimer and, importantly, a constant of radioligand-competitor allosteric interaction (K_{AB}). Here, we provide a practical new way to fit competitive binding data that allows to interpret apparently anomalous results, such as competition curves that could be either biphasic, monophasic or bell-shaped depending on the assay conditions. Considering a radioligand-competitor allosteric interaction allows fitting these data both under simulation conditions and in real radioligand binding experiments. Our approach is novel because it is the first that, assuming the formation of receptor homodimers, is able to explain several experimental results previously considered erroneous due to their impossibility to be fitted. We also deduce the radioligand concentration responsible for the conversion of biphasic to monophasic or to bell-shaped curves in competitive radioligand binding assays. In addition, we demonstrate that bell-shaped curves in competitive binding experiments constitute evidence for GPCR homodimerization.

Keywords: G-protein coupled receptor, pharmacological parameter, allosteric modulation, protein-protein interaction, receptor homodimer, dimer receptor model, molecular cross-talk

O015
MUTATED HUNTINGTIN OVEREXPRESSION IS ASSOCIATED TO ALTERED EXCITABILITY AND EXOCYTOSIS IN CHROMAFFIN CELLS FROM THE R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Adrenal medullary chromaffin cells (CCs) from transgenic mouse models of some neurodegenerative diseases have been reported to undergo functional changes of cell excitability, ion currents, cytosolic calcium transients ($[Ca^{2+}]_c$), the quantal release of catecholamines and the kinetics of the exocytotic fusion pore, with respect their wild type (WT) counterparts. We here present a thorough investigation on the functional changes undergone by CCs from 2-months (2 m) and 7-months (7 m) aged WT and R6/1 mouse model of Huntington's disease (HD), stimulated with acetylcholine (ACh) or K^+. With respect WT cells, some of the changes next summarized were already observed in HD mice at a pre-disease stage (2 m); however, they were more pronounced at 7 months, when motor deficits were clearly established. They were as follows: (i) huntingtin overexpression as nuclear aggregates in CCs; (ii) smaller CC size with decreased dopamine β-hydroxylase expression, indicating lesser number of chromaffin secretory vesicles; (iii) reduced adrenal tissue catecholamine content; (iv) membrane hyperpolarisation with reduced ACh-evoked action potentials; (v) reduced Na^+ currents; (vi) reduced $[Ca^{2+}]_c$ transients with faster clearance; (vii) diminished quantal secretion with smaller vesicle quantal size; (viii) faster kinetics of the exocytotic fusion pore, pore expansion, and closure. These profound changes demonstrate that the altered neurotransmitter release occurring in the brain of HD patients also occur in peripheral sympathetic-like adrenal CCs. As several of those changes occur at pre-disease stages, their monitoring could potentially be used as markers for early disease diagnosis.

Keywords: Huntington's disease, R6/1 mice, chromaffin cells, exocytosis

O016
METABOTROPIC GLUTAMATE RECEPTOR 5 AS A POTENTIAL TARGET IN ALS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron (MN) death, whose aetiology is not clear, although glutamate (Glu)-mediated

excitotoxicity represents one major factor.[1,2] Group I metabotropic glutamate receptors (mGluR1 and mGluR5) may be implicated in ALS, since they are largely over-expressed during disease progression and involved in altered cellular processes.[3,4,5] In this scenario, we recently demonstrated that mGluR1 and mGluR5 at Glu synapses produces abnormal Glu release[5] and that knocking-down mGluR1 in $SOD1^{G93A}$ mice significantly prolongs survival and ameliorates disease progression.[6]

Aim: To study the function of mGluR5 in ALS, we investigated the effects of the genetic down-regulation of mGluR5in $SOD1^{G93A}$ mice ($SOD1^{G93A}mGluR5^{+/-}$) or its ablation ($SOD1^{G93A}mGluR5^{-/-}$) and the pharmacological treatment of $SOD1^{G93A}$ mice with the mGluR5 NAM, CTEP.[7]

Results: $SOD1^{G93A}mGluR5^{+/-}$ mice showed delayed disease onset and prolonged survival probability, accompanied by spinal MN preservation, decreased astrocyte and microglia activation and normalization of the excessive cytosolic $[Ca^{2+}]_i$ and Glu release. Unexpectedly, motor skills were improved in male $SOD1^{G93A}mGluR5^{+/-}$ mice only. $SOD1^{G93A}mGluR5^{-/-}$ presented a more evident amelioration of all disease features, including motor skills, both in males and females. Furthermore, we treated 90 days-old $SOD1^{G93A}$ mice with CTEP (2 mg/kg/48hs;4 mg/kg/24hs) until death. The lower dose CTEP-treated-$SOD1^{G93A}$ mice showed a significant prolonged survival probability only in female mice, paralleled by improved clinical parameters. The higher dose CTEP produced a marked clinical amelioration, both in female and male $SOD1^{G93A}$ mice.

Conclusion: These results support the idea that mGluR5 represent a useful target to counteract ALS.

Keywords: amyotrophic lateral sclerosis, excitotoxicity, type I metabotropic glutamate receptors

References:

[1]. E. Bogaert, C. d'Ydewalle, L. Van Den Bosch, *Neurol Disord Drug Targets*., 2010, 9, 297–304.

[2]. AE. King, A. Woodhouse, MT. Kirkcaldie, JC. Vickers, *Exp Neurol*., 2016, 1, 162–171.

[3]. E. Aronica, MV. Catania, J. Geurts, B. Yankaya, D. Troost, *Neurosc*., 2001, 105, 509–20.

[4]. D. Rossi, L. Brambilla, CF. Valori, C. Roncoroni, A. Crugnola, T. Yokota, DE. Bredesen, A. Volterra, *Cell Death Diff*., 2008, 15, 1691–700.

[5]. F. Giribaldi, M. Milanese, T. Bonifacino, PIA. Rossi, S. Di Prisco, A. Pittaluga, C. Tacchetti, A. Puliti, C. Usai, G. Bonanno, *Neuropharmacol*., 2013, 66, 253–63.

[6]. M. Milanese, F. Giribaldi, M. Melone, T. Bonifacino, I. Musante, E. Carminati, PIA. Rossi, L. Vergani, A. Voci, F. Conti, A. Puliti, G. Bonanno, *Neurobiol. Dis.,* 2014, 64, 48–9.

[7]. L. Lindemann, G. Jaeschke, A. Michalon, E. Vieira, M. Honer, W. Spooren, R. Porter, T. Hartung, S. Kolczewski, B. Büttelmann, C. Flament, C. Diener, C. Fischer, S. Gatti, EP. Prinssen, N. Parrott, G. Hoffmann, JG. Wettstein, *J. of Pharmacol. and Exp. Therap*. 2011, 339, 474–86.

OPEN INNOVATION

P089
MAGISTRAL FORMULA INTENDED FOR ANIMALS IN A PHARMACY FROM LEÓN (SPAIN) IN THE PERIOD 2012–16

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Magistral formulation is one of the ancestral activities that persists over time, and whose current task is the elaboration of medicinal products by a pharmacist that are not manufactured by the pharmaceutical industry. The increasing coexistence of pets with humans has not only made it essential for the veterinary profession to protect the health of the population by preventing zoonoses, but also to ensure the health and welfare of pets. The veterinarian must address those therapeutic needs that are not completely covered by the industry. To carry out this study, we used the registry of all prescriptions made by veterinarians for the preparation of oral magistral formula in a pharmacy from León (Spain) in the period 2012–2016. After reviewing the prescriptions of magistral formula elaborated in this pharmacy in the period considered, it was seen that veterinarians prescribed only a 0.8% of them. No significant differences were found in the number of magistral formulas made each year, although in 2013 and 2014 the number of prescriptions was lower than in the other years. Taking into account the pharmacological activity, the most frequent prescribed formula contained active substances that act in the central nervous system, being the most used potassium bromide (41%) and gabapentin (14%). Considering their pharmaceutical form, the capsules were formulated in the largest number (53%), followed by the solutions (40%) and suspensions (7%). Most of the formulas were used in diseases that were not treated or diagnosed until a few years ago in domestic animals.

Keywords: magistral formula, veterinary prescription, pharmacy

P090
CHARACTERIZATION OF THE CELLULAR CONTENT OF FAT GRAFTS IN THE SURGICAL TREATMENT OF PATHOLOGIC DYSPHONIA

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Many laryngeal disorders can affect the vocal fold producing dysphonia and pneumonia. Injection laryngoplasty is one of the main procedures to treat these disorders. However, there is no optimal injectable substance that is stably integrated. Currently, autologous fat grafts are beginning to be used with very positive results. The objective of this work is the characterization of this fat graft in order to improve the surgical treatment. Liposuction samples (5 men and 1 woman, aged 22–69 years) are obtained at the time of surgery. After the surgery, the fat excess is processed by 7 different methods: 3 min centrifugation with 5 or 15 passes between syringes, centrifugation without passes, 6 min centrifugation; and also by decanting or by decanting and washing with saline solution; and combining the sample with lidocaine. The cells are isolated with collagenase and the viability evaluated by trypan blue. The cells are cultured in DMEM/F12 with 10% FBS and 5% CO_2. The pre-adipocyte phenotype is evaluated by Oil red staining, and proliferation index by culture of cells at 24 h, 48 h and 96 h followed by hematoxylin staining. The obtained results indicate that the cellular viability is >95% in all cases. A greater number of total cells and preadipocytes is obtained by decanting compared to centrifugation. However, the proliferation index is significantly higher in centrifugation samples with respect to decanting. More studies are in progress for determining the contribution of different cellular subpopulations to the stability and regenerative properties of fat graft in these patients.

Keywords: fat grafting, pre-adipocyte, dysphonia

P091
A COMPARISON OF CLINICAL RISK INDEX FOR BABIES (CRIB-II), SCORE FOR NEONATAL ACUTE PHYSIOLOGY (SNAP-II) AND SNAPPE-II IN PREDICTING PARENTERAL NUTRITION NECESSITY IN LOW BIRTH WEIGHT PRETERM NEONATES

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Advances in perinatal care have made it possible to improve survival of low birth weight neonates. Clinical risk index for babies (CRIB-II), score for neonatal acute physiology (SNAP-II), and SNAP-perinatal extension-II (SNAPPE-II) have been used as mortality predictors for preterm infants. Feeding intolerance is very frequent in preterm neonates, and the development of an early effective biomarker for its prediction could be useful for carrying out a proper feeding strategy. Our aim was to compare the ability of CRIB-II, SNAP-II and SNAPPE-II in predict the feeding intolerance and parenteral nutrition necessity in preterm neonates.

Methods: A retrospective cohort study on preterm neonates' born at Jaen Hospital Complex with low birth weight and ≤36 weeks of gestation was done. Epidemiological, clinical and clinical scores CRIB II, SNAP-II and SNAPPE-II were recorded.

Results: 255 low birth weight preterm neonates, 131 males (51.4%), aged ≤32 weeks of gestation (71%), were enrolled at our hospital. Parenteral nutrition needed were significantly higher in preterm neonates weighed 2500–1500 g (73.3%) and ≤1000 g (87%). CRIB-II, SNAP-II and SNAPPE-II mean values were higher in neonates group subjected to parenteral nutrition compared with oral nutrition ($P < 0.05$). CRIB-II and SNAPPE-II scores significantly correlated with parenteral nutrition days ($P < 0.05$). Overall mortality rate was 11%. The 78.6% of all deceased infants needed parenteral nutrition.

Conclusion: Clinical Risk Index for babies (CRIB-II) better than SNAPPE-II correlated with the feeding intolerance and thus the parenteral nutrition days in preterm neonates with low birth weight.

Keywords: parenteral nutrition, preterm neonates, CRIB-II, SNAP-II, SNAPPE-II

P092
CLINICAL IMPACT OF MODERATE AND SEVERAL POTENTIAL DRUG- DRUG INTERACTIONS IN MEDICAL INPATIENTS. COHORT STUDY

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Background: The drug-drug interactions (DDIs) are a problem that has been associated with high morbidity, mortality, and healthcare cost, especially in vulnerable population, such as inpatients. However, the studies that support these conclusions are scarce and show methodological problems, which makes it difficult to extrapolate the results obtained. For this reason, we believe that is necessary to determine the association between potential DDIs on severe clinical outcomes in inpatients.
Methods: We conducted a prospective and concurrent cohort study. The target populations were medical non-critical and critical inpatients ≥18 years. We evaluated the presence of moderate and severe potential DDIs and its association with mortality during hospitalization or until 30 days after hospital discharge; length of hospital stay and hospital readmissions within two weeks after hospital discharge. Control of confounding factors was performed.
Results: We identified 1170 potential DDI. We found that potential DDI was related to a longer hospital stay, where patients with at least one potential DDI had a higher average hospital stay than those without potential DDI (8.7 days vs 5.4 days, $P = 0.003$). We did not find impact on other clinical outcomes. The results did not change when adjusted for age, polypharmacy and comorbidity.
Conclusions: We found a high prevalence of DDI, which may increase the hospital length of stay. This could be increase the morbidity and healthcare cost.
Keywords: drug interactions, morbidity, inpatients

P093
CANNABIDIOL, VIA SIGMA 1 RECEPTORS, ALLEVIATES NMDA-INDUCED SEIZURES AND STROKE DAMAGE

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The phytocannabinoid cannabidiol (CBD), unlike delta-9-tetrahydrocannabinol the main constituent of the marijuana plant, is devoid of psychoactive properties and exhibits no direct binding to endocannabinoid receptors 1 and 2.[1] During recent years, evidence has accumulated regarding the therapeutic potential of CBD in the prevention or attenuation of various human CNS diseases, including neuropsychiatric disorders, epilepsy, chronic neurodegenerative diseases, ischemic stroke, neuropathic allodynia and certain types of cancer. CBD pharmacology is far from being completely understood, as multiple mechanisms of action have been proposed. Discarding CBD as an antagonist of NMDARs, we evaluate the possibility that this phytocannabinoid might modulate NMDA receptor transmission through sigma type 1 receptors (σ1Rs).[2] With this aim, we investigated the effects of CBD on three animal models that involve the overactivity of the NMDAR. Our data reveals that CBD administration to mice, potentiates morphine-evoked antinociception and reduces by approximately half, the duration of the NMDA-induced convulsive seizure episodes seizures, with the total absence of death. Also, the administration of CBD improved stroke outcomes (approximate 75% reduction in the infarct size) after permanent cerebral ischemia. The positive effects of CBD in these paradigms were reduced by σ1R agonists such as PRE084 and PPCC. Thus, our study indicates that CBD displays antagonist-like activity at σ1Rs to counteract the negative effects of NMDAR activation. This new idea may help with understanding the pharmacology of CBD, and it provides new approaches in the treatment of several brain-related disorders. Supported MINECO SAF2015-65420R and MSSSI, PND 2014-012.
Keywords: cannabidiol, NMDA, sigma1 receptors, ischemic stroke, epilepsy
References:
[1] R. G. Pertwee, The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br. J Pharmacol., **2008**, 153, 199–215.
[2] M. Rodríguez-Muñoz, P. Sánchez-Blázquez, M. Merlos, J. Garzón-Niño, Endocannabinoid control of glutamate NMDA receptors: the therapeutic potential and consequences of dysfunction. Oncotarget, **2016**, 7, 55840–55862.

P094
FENFLURAMINE DIMINISHES NMDA RECEPTOR-MEDIATED SEIZURES VIA ITS MIXED ACTIVITY AT SEROTONIN 5HT2A AND TYPE 1 SIGMA RECEPTORS

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An excess of glutamatergic neurotransmission, particularly at NMDARs, is essential to the clinical manifestations of epilepsy,[1] and drugs such as fenfluramine reduce NMDAR overactivity without triggering the undesirable side effects caused by direct antagonists.[2,3] Fenfluramine is metabolized into norfenfluramine in vivo, which shows greater affinity at serotonin 5HT2Rs than fenfluramine. In this study, the anticonvulsant activity of fenfluramine was compared to that of the selective sigma 1 receptor (σ1R) antagonist S1RA in an animal model of seizures induced by icv administration of NMDA. Either compound reduced the appearance of most NMDA-induced signs. Fenfluramine was more efficacious at reducing rearing, while norfenfluramine outperformed its parent compound in abolishing clonic convulsions and tonic seizures. Both compounds protected the mice from NMDA-provoked death. In the presence of the σ1R agonist PPCC, the capacity of fenfluramine and norfenfluramine to diminish certain signs of the NMDA syndrome was impaired. In *in vitro* experiments, we found that fenfluramine and norfenfluramine disrupted the regulatory association of the σ1R with NR1 subunits of NMDAR, an effect that was also produced by σ1R antagonists and prevented by PPCC. Our data indicate that the agonist activity of fenfluramine and norfenfluramine on 5HT2ARs is essential to their anticonvulsive effects and that antagonism at σ1Rs increases their efficacy to inhibit NMDAR overactivity. Thus, fenfluramine circumvents the negative side effects of direct NMDAR antagonists and may improve the quality of life of subjects affected by such proconvulsant dysfunctions. Supported MINECO SAF2015-65420R
Keywords: fenfluramine, norfenfluramine, NMDA, sigma1 receptors, epilepsy, anticonvulsants
References:
[1] Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. Pharmacol Rev. 1999; 51: 7–61.
[2] Schoonjans AS, Lagae L, Ceulemans B. Low-dose fenfluramine in the treatment of neurologic disorders: experience in Dravet syndrome. Ther Adv Neurol Disord. 2015; 8: 328–338.
[3] Ceulemans B, Boel M, Leyssens K, Van RC, Neels P, Jorens PG, Lagae L. Successful use of fenfluramine as an add-on treatment for Dravet syndrome. Epilepsia. 2012; 53: 1131–1139.

P095
MEDICINES UNDER ADDITIONAL MONITORING: CRITERIA FOR INCLUSION, DISPENSATION CONDITIONS AND SAFETY

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In 2013 the European Union introduced a new legislation to medicines that require additional monitoring by regulatory authorities.[1] These medicines are labelled with a black triangle in the information for healthcare professionals and patients. The objective of our study was to analyze these medicines and to review their safety according to the reports of the Spanish Agency of Medicines and Health Products (AEMPS). Based on the list published by the European Medicines Agency in January 2017 (EMA/245297/2013 Rev.41),[2] criteria to be assigned as medicines under additional monitoring, types of medicines included and dispensation conditions were studied. Furthermore, the safety reports published by the AEMPS, related to these medicines, were reviewed. A total of 316 medicines under additional monitoring were analyzed. The most common criterion to be assigned was: new active substance [n = 197 (62.3%)], that were mainly included in the pharmacological groups: L (antineoplastic and immunomodulating agents) [n = 59 (29.9%)] and J (anti-infectives for systemic use) [n = 32 (16.2%)]. Other common criteria for inclusion were: to require a post-marketing safety study [n = 52 (16.5%)] and to be a biologic medicine, although not a new active substance [n = 49 (15.5%)]. In relation to the dispensation conditions, nearly two thirds of them were authorized with restricted conditions. Until January 2017, the AEMPS published 14 safety reports related to medicines under additional monitoring. Identification and knowledge of medicines under additional monitoring should to make easier their benefit/risk assessment and to improve their safety use in clinical practice.

Keywords: medicines, additional monitoring, black triangle, pharmacovigilance

References:

[1] Regulation (EU) No 1235/2010 of the European Parliament and of the Council of 15 December 2010 amending, as regards pharmacovigilance of medicinal products for human use, Regulation (EC) No 726/2004 laying down Community procedures for the authorization and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency, and Regulation (EC) No 1394/2007 on advanced therapy medicinal products Text with EEA relevance - reg_2010_1235_en.pdf Available from: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2010_1235/reg_2010_1235_en.pdf [accessed 27.03.18].

[2] European Medicines Agency. Medicines under additional monitoring. Available from: http://www.ema.europa.eu/docs/es_ES/document_library/Other/2013/04/WC500142430.pdf [accessed 27.03.18].

P096
BIC CASSETTE AS A NEW TOOL TO ENABLE NOVEL BIOLOGICAL TARGETS

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Finding new active compounds against a biological target is an equation with many variables. Screening can provide accurate data and is generally the method of choice. In academia, many biological groups focus their research on target identification, with the final objective of enabling the pharmacological validation and exploring the therapeutic relevance. In the last decade, many institutions have built drug discovery facilities where HTS centers have emerged to facilitate the discovery of chemical probes. These screening facilities integrate automation and the most advanced biological technologies but sometimes have difficulties to acquire high quality chemical libraries. In 2017, Lilly expanded the Open Innovation Drug Discovery program to include the Emerging Biology offer. We designed the Biology Interrogation Compound (BIC) Cassette, a diversity library of privileged compounds to be distributed to scientific institutions interested in testing novel biological hypothesis in therapeutic areas of common interest. The BIC cassette is a powerful tool for screening campaigns based on structural diversity with the appropriate drug-like properties. It does not overlap with commercially available libraries and comes in two different sizes (21K or 4K), pre-plated in DMSO solution at volumes tailored to suit assay requirements. All compounds in the BIC cassette have a set of analogs for immediate follow-up to establish a small-scale structure–activity relationship. The academic institutions share with Lilly the biological results generated with the BIC cassette and Lilly discloses the structures of active compounds. Based on the results, findings may form the basis for a drug discovery collaboration around a novel biological hypothesis. This poster will describe the scientific rationale behind BIC, the business model, operational details and performance metrics.

Keywords: open innovation, collaboration, drug discovery, screening, novel biological targets

PAIN

P097
GENETIC EXPRESSION STUDY ON GLIAL CELLS ISOLATED FROM SPINAL CORD OF OXALIPLATIN-TREATED NEUROPATHIC MICE

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Neuropathy is a chronic pain arising from central or peripheral nervous system damage. One of its causes is the prolonged treatment with chemotherapeutics agents, like oxaliplatin. Sustained oxaliplatin administrations in rodents was described to cause pain and to evoke strong oxidative stress in spinal cord, by inducing glial cells activation, which are increasingly considered as modulators of pain. Therefore, we decided to study the changes in expression of eleven pain-related genes in glia isolated from the spinal lumbar tract of oxaliplatin-treated mice with evident neuropathy, with respect to non-treated healthy animals. After 14 days of oxaliplatin administrations, the explanted nervous tissues were dissociated and magnetically sorted for glia via antibody-conjugated magnetic microbeads. Isolation of glia from adult cerebral tissue has been widely tested and fine-tuned, but less has been experimented on adult spinal cord. FACS analysis characterization revealed that, differently from our expectations, positive-sorted fractions contained a miscellaneous of glia and oligodendrocytes, 37% and 47% out of the sorted population, respectively. A real time PCR was performed on the mRNA extracted from that population, analysing the pain- or excitotoxicity-related genes ET-1, FGF-2, GFAP, c-JUN, serpin-3N, LCN2, VEGF-A, EAAT-2 and the protective genes IL-1α, PTX3, SEMA3A. This pilot study highlighted a strong upregulation of pro-pain genes and a less prominent, but however relevant, increase of protective gene expression in oxaliplatin-treated mice. Future efforts will focus on improving purity of isolations and enlarging gene expression analysis.

Keywords: Neuropathy, oxaliplatin, pain, glial cells, real time PCR, FACS

P098
THE SPINAL ADMINISTRATION OF THE CHEMOKINE CCL4 CAN ACTIVATE ANALGESIC MECHANISMS IN MICE

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The role played by the chemokine CCL4 (MIP-1β) in nociceptive processing at the spinal cord has not been profusely explored. Data reported so far suggest that this chemokine could be involved in the amplification of nociceptive processing and participate in neuropathic pain.[1] Contrasting with this view, the experiments here presented show that the intrathecal (i.t.) administration of CCL4 can inhibit thermal nociception in mice. Thus, the i.t. administration of 0.3–3 ng of CCL4 produced analgesia measured by the unilateral hot plate that lasted for 8 h. In accordance, the i.t. administration of 3 ng of CCL4 reduced the immunohistochemical expression of Fos protein in the I-II layers of the spinal cord following thermal nociceptive stimulation of a hind paw. This analgesic effect remained unmodified by the administration of the opioid receptor antagonist naloxone, the cannabinoid receptor antagonists AM251 (CB1) or SR144528 (CB2), the NO synthase inhibitor L-NMMA or the $GABA_A$ receptor antagonist bicuculline. However, it was prevented by the spinal coadministration of selective antagonists of CCR5 (DAPTA), $GABA_B$ (phaclophen) or muscarinic receptors (atropine). Furthermore, CCL4-evoked analgesia was also blocked by administering the microglial inhibitor minocycline but not the inhibitor of astroglial cells, aminoadipate. Based on these data, we propose a hypothetic model in which CCL4 could act on CCR5 receptors expressed in spinal microglial cells, thus promoting the release of acetylcholine. Next, according to previous reports,[2] acetylcholine could induce analgesia through the release of endogenous GABA and the subsequent activation of $GABA_B$ receptors.

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Keywords: CCL4, CCR5, analgesia, intrathecal, GABA-B receptors, muscarinic receptors, microglia, mouse

References:

[1] Rojewska E, Zychowska M, Piotrowska A, Kreiner G, Nalepa I, Mika J., *Front Immunol l.*, 2018, *9*, 1–21.

[2] Li DP, Chen SR, Pan YZ, Levey AI, Pan HL. *J Physiol.*, 2002, *543*, 807–818.

O007
PAIN THRESHOLD EVALUATION AFTER PF-3845, A FAAH INHIBITOR, ADMINISTRATION IN CAFETERIA-INDUCED ABSTINENCE IN RAT: ROLE OF CANNABINOID AND OPIOID RECEPTORS

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Obesity by cafeteria diet (highly palatable foods), is linked to behavioral disorders, such as, addiction-like deficits in the brain reward system and pain sensitivity.[1–6] The wearing off of the hedonical rewarding properties of these foods may gradually lead negative reinforcement so that consumption becomes necessary to prevent or relieve negative states that would result from abstinence.[7] Endocannabinoids play an important role in this scenario, also modulating opioid system.[8] In this study, we investigated whether the long-term exposure to a cafeteria diet could modify pain sensitivity, whether the pharmacological manipulation, by PF-3845, a FAAH inhibitor, can ameliorate this alteration and the mechanisms involved. Animals were subject to chow only (CO) or extended access (EA) with ad libitum access to cafeteria diet for 40 days and then EA rats were undergo an "abstinence" period of 28 days. Pain threshold was evaluated by hot plate and tail flick tests, at day 40, and at the end of the abstinence period (day 68). On day 40, EA showed a significant increase of threshold pain respect CO rats along with an upregulation of CB1 and MU receptors in brain. On day 68, no difference in pain sensitivity was found between groups. Finally, PF-3845 (10 mg/kg) induced a

significant analgesic effect and up regulation of MU, but no CB1. In conclusion, long exposure to high-palatable food produces an increase of threshold of pain, due to up regulation of CB1 and MU receptor, and endocannabinoid manipulation during abstinence period through opioidergic system.

Keywords: opioid, endocannabinoid, cafeteria diet; abstinence

References:

[1] Dong C. *Int. J Obes Relat Metab Disord* **2004,** *28*; 790–5.
[2] Roberts RE *Int. J Obes Relat Metab Disord* **2003,** *27*; 514–21.
[3] Simon GE *Arch Gen Psychiatric* **2006,** *63*; 824–30.
[4] Iannitti T. *Exp Physiol* **2001,** *97*; 1236–45.
[5] Davis JF *Behav Neuroisci* **2008,** *122*; 1257–63.
[6] Geigev BM *Neuroscience* **2009,** *159*; 1193–9.
[7] Parylak SL *Physiol Behan*. **2011,** 25; 149–56.
[8] Welch SP. Int Rev Psychiatry. **2009,** *21*;143–51.

RECEPTORS ION CHANNELS

P099
CYSTEINE 148 IS A KEY RESIDUE IN HUMAN 5-HT2A RECEPTOR EXPRESSION

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Serotonin 2A (5-HT_{2A}) receptor is a G-protein coupled receptor (GPCR) with a conserved disulfide bridge between Cys148 (transmembrane helix 3) and Cys227 (extracellular loop 2), that has been reported to be reduced by dithiothreitol, altering receptor binding and function, but not its homodimerization.[1] However, its importance in the correct protein folding has been assessed in other GPCRs, where membrane expression is impaired in the absence of both cysteines.[2] Our hypothesis was that C148-C227 disulfide bridge may condition receptor expression and cellular location. Thus, our aim was to evaluate how this receptor trafficking can be modified by disrupting the disulfide bridge is broken, by means of mutagenesis approach. For this purpose, QuikChange II XL site-directed mutagenesis kit was used to generate three constructions from pcDNA3-myc-5-HT_{2A}-eYFP (WT): pcDNA3-myc-5-HT_{2A}-C148A-eYFP, pcDNA3-myc-5-HT_{2A}-C227A-eYFP and pcDNA3-myc-5-HT_{2A}-C148A/C227A-eYFP. Subsequently, 0.2 μg/well of each plasmid were transfected into HEK293 cells by using FuGENE® 6. 24 hours later, cellular nuclei and reticulum were stained by Hoechst 33342 dye and ER- tracker, respectively, and fluorescence microscopy images were obtained in Operetta (High Content Imaging System). It was observed that mutation of C148A prevents receptor synthesis as no expression was detected by fluorescence microscopy. This suggests that this cysteine is crucial for the correct expression of the receptor. Neither the expression levels for C227A nor C148A/C227A were decreased when compared to WT. Together with previous data, these results allow the identification of C148 as a key residue for both correct receptor expression and trafficking, as well as for its activation by ligands.

Keywords: Serotonin 2A receptor, disulfide bridges, receptor expression

References:

[1] Iglesias A, Cimadevila M, la Fuente RA, Martí-Solano M, Cadavid MI, Castro M, Selent J, Loza MI, Brea J. *Eur. J. Pharmacol.*, **2017**, *815*, 138–146.

[2] Rummel PC, Thiele S, Hansen LS, Petersen TP, Sparre-Ulrich AH, Ulven T, Rosenkilde MM. *Mol. Pharmacol.*, **2013**, *84*, 335–345.

P100
MUSCARINIC RECEPTOR ACTIVATION IN RESTING MOUSE CHROMAFFIN CELLS INDUCE OPPOSING ELECTROPHYSIOLOGICAL RESPONSES: FROM BLOCKADE OF SPONTANEOUS FIRING ACTIVITY TO RHYTHMIC BURSTING

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Excitation–secretion coupling in adrenal chromaffin cells is largely controlled by cholinergic receptors. Nicotinic acetylcholine receptors (nAChR) ensure a rapid catecholamine release while muscarinic acetylcholine receptors (mAChRs) play a regulatory role. There is clear evidence about the mAChR control of secretion[1] but also uncertainties about the molecular mechanisms behind it.[2] Mouse chromaffin cells (MCCs) are shown to possess spontaneous slow firing activity, which is converted into sustained firing of higher frequency during cell depolarization by ACh. We thus felt of interest to study whether muscarinic stimulation acts on cell firing patterns. Here we report that perfusion of the muscarinic agonists methacholine (100 μM), oxotremorine M (10 μM) or muscarine (30 μM) have variable effects on MCCs membrane potential (V_m) and spontaneous firing. We observed for all agonists that 27% cells underwent hyperpolarization plus depolarization responses (-13 mV followed by +8 mV), 27% of cells responded with only depolarization responses (+4 mV) and 34% of cells exhibited only hyperpolarization responses (-5 mV). Depolarization always induced an increase in firing frequency (from 0.23 to 1.01 Hz) reaching a burst pattern in a 31% of the cells. Hyperpolarization decreased the frequency and even silenced the spontaneous firing. In these cells, depolarization correlated with inward currents of 9 pA and hyperpolarization with an outward current of 5 pA. We further investigated three described pathways involved in the activation of mAChR: the activation of a non specific cationic current and the block of TASK or Kv7 channels. Using histamine, XE991 (both K_V7 channel blockers) or A1899 (TASK channel blocker),[7,6] we could clarify that the depolarizing effect is mainly due to the activation of a non specific cationic current. The mechanism behind hyperpolarization still remains unclear as well as the reason of the opposite effect on MCCs. On the basis of these results we postulate that these variable muscarinic responses bay obey different behaviours between adrenaline- and noradrenaline-containing MCCs during the activation of mAChRs. Funding: SAF 2013-44108-P grant to AGG; FPI BES-2014-069005 and EEBB-I-17-12697 grant to I. M-L., Ministerio de Economía y Competitividad, España. The continued support of Fundación Teófilo Hernando is also acknowledged.

Keywords: Chromaffin cell, muscarinic acetylcholine receptor, Kv7 channel, cell excitability

References:

[1] Inoue, M., et al., Am J Physiol Cell Physiol, **2012**. 303: p. C635–44.

[2] Olivos, L. and A.R. Artalejo, Acta Physiol (Oxf), **2008**. 192: p. 213–20.

[6] Inoue, M., et al., J Neurochem, **2008**. 106: p. 1804–14.

[7] Wallace, D.J., C. Chen, and P.D. Marley, J Physiol, **2002**. 540: p. 921–39.

P101
HALLUCINOGENIC LIGAND (±)DOI MODULATE DOPAMINE D_2 SIGNALING BY ACTIVATING 5-HT_{2A} PROTOMERS OF A $5\text{-HT}_{2A}/D_2$ HETEROOLIGOMER

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G-protein coupled receptors (GPCRs) oligomerization phenomena may to lead to alterations in the intracellular signaling, modulating the effect of the physiological ligands of these receptors. The 5-HT_{2A} receptors have been observed to be expressed as heterodimers with the D_2 receptor, causing alterations in intracellular signaling.[1] Both receptors are targets for antipsychotic drugs used to treat schizophrenia.[2] Our hypothesis was that 5-HT2A/D2 heterooligomer may condition the activation of these receptors by hallucinogenic ligands. The aim of

the present work was to study the effect exerted by the hallucinogenic, 5-HT_{2A} agonist, (±)DOI [1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine] over dopamine-induced D_2 receptor signaling. For this purpose, we have used a cell line of human embryogenic kidney (HEK293) constitutively overexpressing D_2 receptors and expressing 5-HT_{2A} receptor in an inducible way doxycycline-dependent. In order to analyze the effect of dopamine at D_2 receptor in presence and absence of (±)DOI agonist 5-HT_{2A} we have used a cAMP–Gs Dynamic Kit (*Cisbio*). We have demonstrated that the activation of 5-HT_{2A} receptor by (±)DOI decreased the efficacy of dopamine to inhibit the production of cAMP depending of $G_{i/o}$ with E_{max} values of 58.9 ± 8.5% and 29.9 ± 7.0%* in absence and presence of (±)DOI, respectively (*$P < 0.05$ compared with dopamine control curve in absence of (±) DOI). This effect was reverted in the presence of the 5-HT_{2A} antagonist Ketanserin. In view of the results, we conclude that the activation of $5HT_{2A}$ receptor by the hallucinogenic agonist (±)DOI modulated the signal pathway dependent on $G_{i/o}$ from the D_2 receptor in HEK293 cells co-expressed both receptors. This modulation may condition the response of both receptors to typical and atypical antipsychotic drugs.

Keywords: Heterodimerization, crosstalk, D_2 receptor, 5-HT_{2A} receptor

References:

[1] S. Lukasiewicz, A. Polit, S. Kedracka-Krok, K. Wedzony, M. Mackowiak, M. Dziedzicka-Wasylewska, *Biochim. Biophys. Acta,* 2010, 1347–1358.

[2] L. Albizu, T. Holloway, J. Gonzalez-Maeso, S. C.Sealfon, *Neuropharm*., 2011, 770–777.

P102
SPATIOTEMPORAL LOCATION OF THE FRACTALKINE RECEPTOR CX_3CR1 AND ITS NATURAL GENETIC DOUBLE VARIANT V249I/T280M

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The chemokine receptor CX_3CR1 is a GPCR that plays an important role in inflammation and immunity as well as in neuron-microglia communication in the CNS, and whose only ligand known is fractalquine.[1] Genomic studies have identified two nsSNPs in the CX_3CR1 gene that lead to four genetic variants of the receptor; the double variant CX_3CR1-V249I/T280M has been associated with different diseases.[2–6] We aimed to investigate the possible impact of this variant on the spatiotemporal location of the receptor. The distinct internalization and trafficking profiles of the CX_3CR1 receptor were quantified by kinetic BRET assays. The CX_3CR1-WT-RLuc8 and CX_3CR1-V249I/T280M-RLuc8 receptors were coexpressed in HEK293 cells with BRET acceptors that are localized to different compartments: KRas-Venus at the plasma membrane, Rab5a-Venus in early endosomes, Rab7a-Venus in late endosomes/lysosomes and Rab11-Venus in recycling endosomes.[7–8] Our results indicated that the CX_3CR1-V249I/T280M variant internalizes more efficiently than the WT receptor in response to 10 nM fractalkine, as shown by the highest decrease with KRas (**$P < 0.01$-30 min;***$P < 0.001$-15 min; ****$P < 0.0001$-45 and 60 min, two-way ANOVA with Sidak´s multiple comparison test†) and the highest increase with Rab5a (**$P < 0.01$-15 and 45 min;***$P < 0.001$-60 min; ****$P < 0.0001$-30 min†) in the BRET signal. The interaction of both receptors with Rab7 was similar meaning a similar degradation, however the signal with Rab11 (*$P < 0.05$-30 and 45 min;**$P < 0.01$-60 min†) was higher for the double variant involving a more efficient recycling for this receptor. These results expand our current knowledge on the dynamic and compartmentalization of the CX_3CR1 receptor and its double polymorphism and their possible implications in physiological and pathological conditions.

Abbreviations: GPCR, G protein-coupled receptor; CNS, Central Nervous System; nsSNPs, non-synonymous single nucleotide polymorphisms; WT, wild-type; BRET, bioluminescence resonance energy transfer.

Keywords: GPCR, CX_3CR1, spatiotemporal location, BRET

References:

[1] Réaux-Le Goazigo A, Van Steenwinckel J, Rostène W, Mélik Parsadaniantz S. Current status of chemokines in the adult CNS. Prog Neurobiol. 2013; 104: 67–92.

[2] Faure S. Rapid Progression to AIDS in HIV+ Individuals with a Structural Variant of the Chemokine Receptor CX3CR1. Science. 2000; 287 (5461): 2274–7.

[3] Feldman GJ, Parvizi J, Levenstien M, Scott K, Erickson JA, Fortina P, et al. Developmental dysplasia of the hip: Linkage mapping and whole exome sequencing identify a shared variant in CX3CR1 in all affected members of a large multigeneration family. J Bone Miner Res. 2013; 28 (12): 2540–9.

[4] Combadière C, Feumi C, Raoul W, Keller N, Rodéro M, Pézard A, et al. CX3CR1-dependent subretinal microglia cell accumulation is associated with cardinal features of age-related macular degeneration. J Clin Invest. 2007; 117 (10): 2920–8.

[5] Sirois-Gagnon D, Chamberland A, Perron S, Brisson D, Gaudet D, Laprise C. Association of common polymorphisms in the fractalkine receptor (CX3CR1) with obesity. Obesity. 2011; 19 (1): 222–7.

[6] Wu J, Yin RX, Lin QZ, Guo T, Shi GY, Sun JQ, et al. Two polymorphisms in the Fractalkine receptor cx3cr1 gene influence the development of atherosclerosis: A meta-analysis. Dis Markers. 2014; 2014.

[7] Lan TH, Liu Q, Li C, Wu G LN. Sensitive and High Resolution Localization and Tracking of Membrane Proteins in Live Cells with BRET. 2012; 13 (11): 1450–6.

[8] Jensen DD, Godfrey CB, Niklas C, Canals M, Kocan M, Poole DP, et al. The bile acid receptor TGR5 does not interact with β-arrestins or traffic to endosomes but transmits sustained signals from plasma membrane rafts. J Biol Chem. 2013; 288 (32): 22942–60.

P103
VMAT2 LIGAND TETRABENAZINE FACILITATES EXOCYTOSIS THROUGH ER CA2+ RELEASE IN BOVINE CHROMAFFIN CELLS

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The vesicular monoamine transporter 2 (VMAT2) is expressed in the membrane of presynaptic secretory vesicles in the CNS. Its blockade by tetrabenazine (TBZ) causes the depletion of dopamine at striatal basal ganglia; this is the mechanism underlying its long-standing use in the treatment of Huntington´s chorea and other hyperkinetic disorders. In the frame of a project aimed at studying the process of exocytosis from vesicles with partial emptying of their neurotransmitter, we unexpectedly found that TBZ facilitated exocytosis. This study was therefore planned to characterize such effect. We used bovine chromaffin cells (BCCs) that were challenged with repeated 5-s pulses given at 1-min intervals. With repeated pulsing the exocytotic catecholamine release responses were gradually decaying. However, when cells were exposed to 1–3 μM TBZ, the responses were mildly augmented and the rate of decay was delayed. Facilitation of exocytosis was not due to blockade of Ca^{2+} entry through plasmalemmal voltage-activated Ca^{2+} channels (VACCs) because TBZ blocked the whole-cell Ca^{2+} current. However, TBZ mimicked the facilitatory effects of exocytosis elicited by 1 μM of the L-subtype of VACCs BayK8644, an

effect that was blocked by nifedipine, an inhibitor of VACCs. On the basis that TBZ augmented the secretory responses to caffeine (but not those of histamine), we here rise the hypothesis that TBZ is facilitating exocytosis by mobilising Ca^{2+} through an action on the ryanodine receptor channel the endoplasmic reticulum. This is consonant with the existence of a healthy Ca^{2+}-induce-Ca^{2+} release mechanism that is activated by K^+ depolarisation of BCCs. Supported by SAF 2016-78892-R

Keywords: catecholamine release, tetrabenazine, ryanodine receptor, chromaffin cell

P104
NOVEL RGD-LIGANDS DIFFERENTLY MODULATE α5β1 INTEGRIN-MEDIATED ADHESION, SIGNALING AND TRAFFICKING

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Integrins are α/β heterodimeric membrane receptors that mediate cell-cell and cell-extracellular matrix (ECM) interactions. As important sensors of the cell microenvironment, integrins regulate crucial aspects of cellular functions and have been implicated in various diseases including tumor cell proliferation and migration, inflammation, thrombosis and autoimmune diseases.[1,2] Among integrins, α5β1 integrin is over-expressed in novel tumor vessel and has been implicated in tumor development and cancer cell invasion. Membrane trafficking pathways influence α5β1's capacity to promote invasion and metastasis.[3] To date, several monoclonal antibodies and small molecules targeting integrins have been approved for the treatment of multiple sclerosis, Crohn's disease and thrombosis.[4] Therefore, considering integrins as valuable drug target, the current study was performed to elucidate the effects of small molecules on integrin-mediated cell adhesion and signaling in K562 cells (endogenously expressing α5β1 integrin) and on trafficking in HEK293 cells transfected with a plasmid coding for α5-EGFP by confocal microscopy to assess α5β1 integrin internalization. We have identified selective and potent agonists and antagonists of α5β1 integrin, able to modulate cell adhesion, signaling and trafficking. Interestingly, we observed that α5β1 integrin agonists are able to activate integrin-mediated signaling and to induce α5β1 integrin internalization while antagonists prevent fibronectin-induced ERK activation and integrin internalization. Most effective compounds may represent lead compounds for the development of new therapeutic agents: integrin antagonists may be employed as anti-cancer or anti-angiogenic drugs, whereas integrin agonists may be used as shuttles for selective delivery of therapeutic payloads and diagnostics.

Keywords: integrins, cell adhesion, signaling, trafficking, cancer

References:

[1] D. V. Iwamoto, D.A. Calderwood, *Curr Opin Cell Biol.,* **2015,** 36:41–7.
[2] A. Tolomelli, P. Galletti, M. Baiula, D. Giacomini, *Cancers*. **2017**, 9(7): pii: E78.
[3] C. Rüegg, A. Mariotti, *Cell Mol Life Sci*., **2003** 60(6):1135–57.
[4] K. Ley, J. Rivera-Nieves, W.J. Sandborn, S. Shattil, *Nat Rev Drug Discov*., **2016**, 15(3):173–83.

TEACHING PHARMACOLOGY

P105
CONTINUOUS ASSESSMENT AS PART OF THE EVALUATION SYSTEM: RELATIONSHIP WITH EXAM RESULTS

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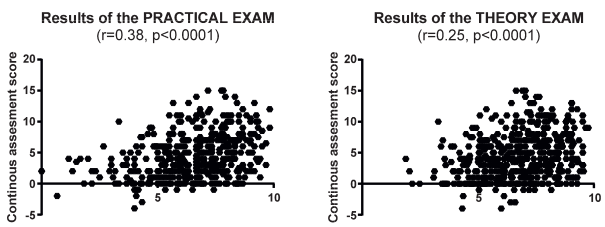
The Bologna Process advocates the student being at the centre of the learning process. As part of the adaptation of the Medicine undergraduate degree to this new system, we have included an estimation of the contribution of each student to the practical classes of the subject of Pharmacology (3rd year) as part of the evaluation process. The present study analyses this evaluation process and its relationship with more established methods of assessment (i.e., examinations). The subject is comprised of 15 practical classes (2 h), conducted by four teachers, in groups of 18–24 students. After each session, the teacher assigns a mark (between −2 and 2) to each student to reflect the student's attitude towards the learning process (continuous assessment). The accumulated points are used to slightly (≤6%) modulate the exams' results and determine the final grade. The students are informed of this evaluation to stimulate their interest in the subject and their motivation. The analysis of the data recorded during the last three years (2015/18) shows a positive and significant correlation between the final scores of the abovementioned continuous assessment and: a) the punctuations of each teacher (r: 0.80, 0.78, 0.62, 0.59, all $P < 0.001$); b) the results of the examinations, especially those of the practical exam.



This method of continuous assessment seems to provide a reliable estimation of students' attitude towards learning, in that a consistency is observed between the evaluations of different teachers and higher scores are associated with better results, especially in the exam aimed to evaluate transversal skills.

Keywords: continuous assessment, examinations.

P106
SPANISH NETWORK FOR INNOVATIVE EDUCATION IN PHARMACOLOGY: TWO YEARS SHARING EXPERIENCES

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"The teaching and learning international survey (TALIS), European Commission 2013" concluded that the more teachers participate in educational networks, the more likely they are to apply innovative pedagogies. With this perspective, the Spanish inter-university network for innovative education in Pharmacology has been created in 2015 as a common space for sharing experiences of learning innovation. 68 participants belonging to fifteen Spanish universities are associated to this project that utilizes a Moodle platform to share teaching materials. Actually, the shared material includes cases and problems for seminars or practical sessions, videos, activities based on films, TV series, literary texts or press news, social network strategies, gamification, computer-based simulation software or practice notebooks as well as evaluation and self-assessment systems, which came from 29 innovation projects. To analyze the efficacy of the network, a questionnaire was submitted to all participants which includes questions about the utility of the shared projects and the preferences for future network activities. A total of 42 answers (62%) have been received. 64% of the survey participants have implemented and 84% have consulted the network material. Only 6 participants have not used or consulted any of the shared projects, but all of them plan to consult them in the near future. The survey about preferences indicates that problem-based learning activities, preparation of flipped class material and practical sessions were the more interesting activities to share in a future network meeting. These results confirm the contribution of the network to the improvement of pharmacological innovation. Funded by Vice-Rectorate for Training Policies and Educational Quality (UVEG) (UV-SFPIE-GER 15-314985, UV-SFPIE-GER16-418743, and UV-SFPIE-GER17-588356).

Keywords: interuniversity network, pharmacology teaching, innovation.

P107
POSITIVE PROGRESSION IN STUDENTS' TRANSVERSAL SKILLS AS A RESULT OF THE METHODOLOGICAL CHANGES INTRODUCED BY ADAPTATION OF A UNDERGRADUATE MEDICINE DEGREE PROGRAMME TO THE BOLOGNA PROCESS

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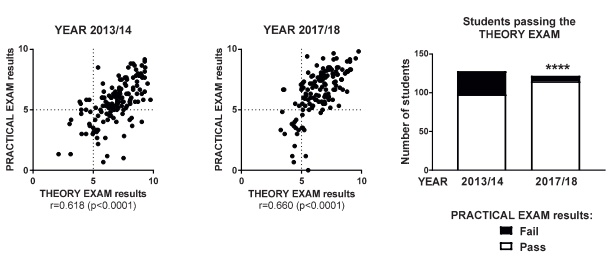
The implantation of the Bologna Process (2011/12) has represented significant changes for the teaching of Pharmacology within the Medicine undergraduate degree (3rd year) at the University of Valencia. The subject, previously organized in 50 lectures plus 40 hours of practical sessions, was reduced to 18 lectures and 38 hours of seminars and practical classes. Therefore, the subject programme was re-structured to increase the relevance of practical teaching and, following the Bologna spirit, to provide students with knowledge and skills for a lifelong learning in pharmacology. The present study analyses the relationship between the results obtained in theory and practical exams in our university during the period 2013–2018. The teaching staff in charge of the subject and the teaching and evaluating methods employed have remained constant throughout this period. The analysis revealed stable median results in theory exams and a progressive improvement in the evaluations of student's practical performance. When the results of both exams were analysed individually, a significant and positive correlation was observed between the two, but a



significant number of students with good results in the theory exam failed the practical exam (23%; 2012/13). Five years later, the initial discordance between these evaluations observed for some students has been clearly reduced (6%, 2017/18; $P < 0.0001$; Chi-square).

We believe that the implantation of the Bologna Process has highlighted weaknesses in the students' transversal skills (e.g. searching for information, analysis of results, deductive capacity, etc.) necessary for an up-to-date, subject-specific knowledge. Fortunately, these shortcomings have improved over the five-year study period.

Keywords: evaluation, transversal skills, practical exam, theory exam

P108
ACTIVE ROLE OF STUDENTS IN THEIR EVALUATION PROCESS: RELATIONSHIP BETWEEN THEORY, PRACTICE AND SEMINARS

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Traditionally, the teacher has had the main role in students' learning. Bologna process advocates for an active role of students, acting the teacher as a guide. We have implemented this theory in the subject of Pharmacology in the Medicine undergraduate degree of the Jaume I University, including seminars in which students (25% of the total number) participate in the generation of knowledge and in the evaluation of their learning process. Thus, the aim of this study was to compare this type of evaluation with traditional theory and practical exams. Seminars (25 students approximately) consist of 6 different units which are not included in the theory program. Students are divided into 6 teams (4 students each); each team develops one of the units and presents it to their colleagues. All the teams formulate questions about each topic for an exam composed with some of them. Data was recorded during the last two years (2015/17) showing a significant increase in the results of seminar versus those of both theory and practice (Figure 1). There is a positive correlation between the results of theory and practice ($r = 0.4668$; $P < 0.0001$) but the mark of seminar did not correlate with that of theory or practice. These results indicate that active role of the students´ in their learning process improves their attention and motivation to acquire pharmacological skills.

Keywords: evaluation, seminars, active role of students

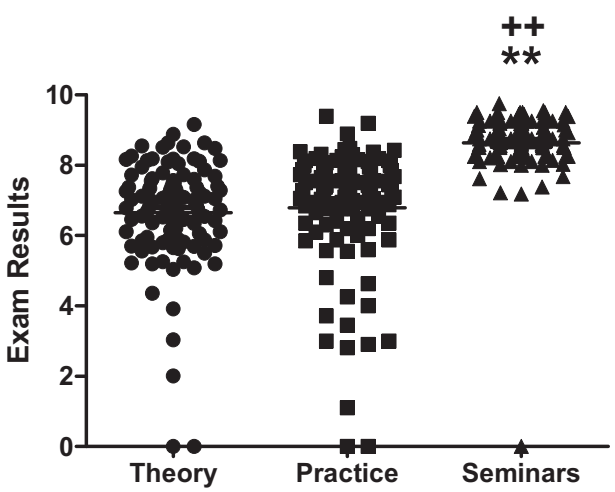

Figure 1. Results of theory, practice and seminars. Results are shown as mean ± SEM of two academic years (2015/16, $n = 85$ and 2016/17, $n = 103$); $^{**}P \leq 0.01$ vs. theory and $^{++}P \leq 0.01$ vs. practice.

P109
A PHARMACOLOGY PRACTICAL CLASS ABOUT THE USE OF EXPERIMENTAL ANIMALS AND ALTERNATIVE METHODS

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Introduction: Although the use of experimental animals in Pharmacology courses could be avoided, it is important that the students know the problems and characteristics of their utilization, since it could be worthy for their professional future especially if they engage in research, ensuring a high level of protection for animals that still need to be used in procedures.

Materials and Methods: 90 students of the Pharmacology course (second year of the degree in Nursing) were enrolled in the study. Students followed a practice divided in two sessions in which the neuroleptic effect of promazine was evaluated in mice, an analysis of the legislation on the protection of animals (Directive 2010/63/EU[1] and Spanish Royal Decree 53/2013[2]) carried out and an alternative method to the use of experimental animals seen. At the end of the practice, a survey was given to the students to assess their opinion about the practice developed.

Results: The answers given to the different items were very positive (mean score over 4) except for 2 items (mean value over 3.5). The highest score was obtained for the affirmation *I consider important the use of alternative techniques for learning Pharmacology* with a mean value of 4.51. Students also considered very difficult the design of alternative methods for the replacement of experimental animals.

Conclusions: The development of this practice was useful for the students to understand the employment of experimental animals in research and teaching, as well as the legislation applied.

Keywords: experimental animals, alternative method, pharmacology, practical classes

References:

[1] European Commission. *OJEU L* 2010, 276, 33–79.
[2] Ministerio de la Presidencia. *BOE* 2013, 34, 11370–421.

P110
POTENTIAL VALUE OF THE PRESS DURING DEGREE TRAINING

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Press coverage of health, may be of educational interest in acquiring skills during university degrees, e.g. medicine, as well as public acquisition of scientific culture.[1] In order to quantify and evaluate the potential of the press on these aspects we have created a media reports registry (with science news about health in general and drugs in particular) for 16 years (2001–2016) using the largest national daily newspaper in circulation in Spain as the main source. The registry integrates 7581 news articles, 1957 (25.81%) correspond to drugs, which means 122.3 articles / year, figures equivalent to those of other authors.[2] Of the 506 (25.8%) news articles that refer to medications, 353 can be classified into groups J, G and L of the ATC classification, 456 (23.3%) within Substances and Methods prohibited in sport, 248 (12.7 %) as illegal drugs and the rest classified into different topics related to toxicity, costs of medications, pharmaceutical industry activities, drug development, distribution and dispensing, advertising, fraud or medicalization. The distribution of the identified aspects suggests that the newspaper is a documentary source for the initiation of students in various topics of medicines.

Keywords: active learning, medical terminology, press news, self-learning

References:

[1] Cantabrana B, Diez B, Bordallo J, Sánchez M, Hidalgo A. FEM **2013,** 16, 145–151.
[2] Revuelta G. *Gac. Sanit*. **2006,** 20 (Supl 1), 203–208.

P111
OBSERVATORY OF PHARMACOLOGICAL NEWS IN SOCIAL NETWORKS

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Social networks provide an efficient means of disseminating news related to health. However, they also allow the spread of false news. In order to encourage the participation of students in their learning and stimulate their critical spirit, we proposed the analysis of pharmacological news accessible through social networks. For this purpose, we designed a strategy based on *Problem Based Learning* techniques successfully used in our Department.[1] As a distinctive feature, students were prompted to actively contribute to news collection and volunteer as Coordinator Students. This activity was offered in three subjects of Pharmacy Degree: Pharmacology I (Year 3), Pharmacology II (Year 4), and Clinical Pharmacy and Pharmaceutical Care (Year 5), with specific organizational requirements. During the preparation phase, Coordinators were designated, working groups of 4–5 students were formed and news items were uploaded to an open forum in the virtual classroom. Coordinators collaborated with supervising teachers, selecting the news items to be analysed in terms of: veracity, reliability of the source, and potential impact. Finally, students had to produce a multimedia document to be presented during seminar lessons. Supervising teachers reported an enthusiastic response from most students who produced creditable analyses, although some were reluctant to participate or present their work in public. Surprisingly, many students lacked multimedia skills and preferred PowerPoint presentations, lengthening presentation time. Improvable aspects included poor time-management by students and supervision required more hours than anticipated. In conclusion, this activity helps students build critical attitudes and work transversal competences in order to give a sound opinion. Funded by the Vice-Principal for Training and Educational Quality Policies (UV-SFPIE_RMD17-588677).

Keywords: problem based learning, critical analysis, collaborative learning, coordinator student

References:

[1] M.C. Recio, M. Noguera, M. Montesinos, M. Úbeda, M. Ivorra, M. Terencio, M. D'Ocon, M. Ferrándiz. Encouraging transverse competences in the new undergraduate degree in pharmacy: critical news analysis as a tool in teaching pharmacology. *EDULEARN11 Proceedings*, **2011**, pp. 2488–2495.

P112
IMPROVING THE TEACHING-LEARNING PROCESS IN A PHARMACOLOGY MODULE AFTER THE IMPLEMENTATION OF A PROJECT-BASED LEARNING (PBL) AND THE CREATION OF ARTIFACTS

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Introduction: Some shortfalls (absenteeism, reduced involvement in the subject) in the teaching-learning process were found for the module "Pharmacology applied to over-the-counter drugs". This is an optional module for the fourth year of the Pharmacy Degree at the University of Granada. These shortfalls are defined based on the requirements of the European Higher Education Area.

Aim: To evaluate the impact of the implementation of an innovative teaching programme. This programme would allow the extraction of information about the teaching practice to get a higher student motivation regarding knowledge acquisition, attitudes and skills needed for their future professional performance.

Methods: Methodologies of project-based learning[1] and creation of artifacts[2] have been used to achieve the objectives proposed for each topic area. In addition, students have completed questionnaires before and after the implementation of these methodologies. The questionnaires were based on the key issues addressed during the classes.

Results: The results of the initial and the final questionnaires showed a positive development of the students' learning compared to previous academic years. Moreover, a reduced absenteeism (29%) and a higher number of created artifacts (90%) demonstrated a higher students' motivation after the implementation of these methodologies. However, a 19.5% increase in the number of withdrawals from this module was found, which was associated with this new learning method.

Conclusions: Teachers and students agree that the teaching-learning process is much more complete and attractive when project-based learning is implemented since it encourages students' participation and critical thinking.

Keywords: project-based learning, teaching, artifacts, pharmacology

References:

[1] Trujillo, F. Aprendizaje basado en proyectos. Educación infantil, educación primaria y educación secundaria. Ministerios de educación, cultura y deporte. Madrid. 2017.
[2] Trujillo, F (coord.), Conecta13. Artefactos digitales. Una escuela digital para la educación de hoy. Grao. Barcelona. 2014.

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UCMSPACE-COMUNICA))), UN ESPACIO WEB COMO HERRAMIENTA DE APRENDIZAJE DE CARÁCTER MULTIDISCIPLINAR PARA LA MEJORA DE LA COMUNICACIÓN CIENTÍFICA

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En los últimos años se observa un déficit creciente en el alumnado para la presentación pública de los conocimientos adquiridos. Como solución se propone el diseño, ejecución y evaluación de un espacio web -**UCMspace-comunica**)))-, utilizable como herramienta de aprendizaje eficaz, multidisciplinar, que capacite para la presentación de contenidos en forma de presentaciones orales y escritas y facilite la labor tutorial del profesor. El espacio web, incluido en el entorno oficial de la UCM, se estructura en páginas. La primera se emplea como canal para dirigir a los estudiantes, a través del método científico mediante tres llamadas: Céntrate, enlazada a la presentación de las fuentes de información científica; Actúa, dirigida hacia la profundización en el tema propuesto; y Comunica, en la que se incluyeron las metodologías más adecuadas para presentar comunicaciones orales y pósteres. Asimismo se incorpora a la web un sistema de tutoría virtual para responder a las demandas planteadas por los estudiantes. La evaluación de la eficacia de la herramienta de aprendizaje frente a sistemas clásicos de acción tutorial, se planteó aleatoriamente a dos grupos de alumnos ("herramienta" y "control"), de asignaturas troncales (Biología Vegetal, Farmacología General, Farmacología Especial y Farmacoterapia, Farmacognosia y Fitoterapia) y optativa (Hematología Farmacéutica) de diferentes cursos del Grado en Farmacia. Los resultados fueron óptimos, siendo la principal fortaleza el aumento en la capacidad para atender a mayor número de alumnos. Sin embargo, se reconoce como debilidad la resistencia del alumno al aprendizaje autónomo.

Keywords: aprendizaje virtual, farmacología.

P114
A STUDY ON THE KNOWLEDGE, ATTITUDE AND PRACTICE OF GENERIC MEDICINES AMONG THE DOCTORS IN A TERTIARY CARE TEACHING HOSPITAL IN NORTH EAST INDIA

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Healthcare expenditure is increasing steadily worldwide. In developing countries out-of-pocket payment is as high as 80% of healthcare spending.[1] Generics are less expensive than brand-name drugs with same therapeutic effect but many doctors hold negative views of generics and resist prescribing.[2,3] To evaluate the knowledge, attitude and practice of doctors regarding generic medicines and to explore the factors hindering and favoring generic drug prescribing if any. It is a cross-sectional questionnaire-based study in a tertiary care teaching hospital. All doctors working in the hospital during the study period were participated and filled up the structured and pre-validated questionnaires and analyzed. Close to three quarters of the participants had good attitude about the efficacy and safety of generic and majority of doctors actively prescribe it but a high number of doctors (72%) were of the view that generic were manufactured to the poorer quality but cheaper than brand name drugs. The Majority of respondents believed that their prescribing decision is influenced by lots of factors. These results suggest that there are a significant number doctors concerns about the quality of generics and this negative perceptions are likely to be barriers to a wider acceptance of generics.[4] In order to have a better understanding of generic, the doctor must be well informed about the generic during their academic career resulting in savings to healthcare budgets.

Keywords: generic medicines, knowledge, attitude, practice, doctors, India

References:

[1] The World Medicines Situation WHO/EDM/PAR/2004.5. Geneva. www.searo.who.int/. . ./Reports_World_Medicines_Situation.pdf (2004).
[2] Haas JS, Phillips KA, Gerstenberger EP, et al. *Ann InternMed* 2005; 142: 891–897.
[3] Shrank WH, Liberman JN, Fischer MA, et al. Ann Pharmacother 2011;45:31–8.
[4] Kumar R, Hassali MA, Saleem F, et al. J Pharm Policy Pract 2015;8:11.

P115
INTERPROFESSIONAL TEACHING FROM THE PHARMACY AND CONSULTING LAB TO THE REAL MARKET

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This project is framed in the context of new teaching methodologies aimed facing a new economic, social and cultural pharmacy globalization reality.[1,5] This is an interprofessional education study in evolution. With the aim at working with the competences promoting students integral formation, in values, creativity, and professional performance.[3,4] This project has followed the Challenge Based Learning methodology via the Consulting Lab, and designed to encourage collaboration among different subjects, i.e. to approach pharmacists to the real sales market environment. Pharmacy students, as specialists in the pharmaceutical products, have been capable of designing and elaborating them. Within the Consulting Lab, Business Management students study and provide their analysis and advice to pharmacists students on the current and real market demands, comparing similar product sales from different competitors. Students are grouped with team works specialized in innovation management and carried out the study and design of the best strategic plans, based on the needs and specific demands of the client and the market. The preliminary results suggest that interprofessional education and collaborative practices are highly valued by students (9.2/10) and had positive attitudes towards it. In addition, both enhance the closeness of students to pharmaceutical industry. This student high satisfaction, implies a stimulus to academics to improve and innovate new methodologies integrated in models of transversal and interprofessional competences that fulfill to European framework of higher education.[2]

Keywords: interprofessional education, pharmacy, business management, innovation management, undergraduate learners

References:

[1] Curr Pharm Teach Learn. 2017 Jul;9(4):729–736. Interprofessional education and distance education: A review and appraisal of the current literature. McCutcheon LRM1, Alzghari SK2, Lee YR3, Long WG4, Marquez R5.
[2] Med Educ. 2012 Jan;46(1):58–70. Interprofessional education: a review of context, learning and the research agenda. Thistlethwaite J1.
[3] Med Teach. 2007 Oct; 29(8):735–51. A best evidence systematic review of interprofessional education: BEME Guide no. 9. Hammick M1, Freeth D, Koppel I, Reeves S, Barr H.
[4] Res Social Adm Pharm. 2017 Nov 2. S1551-7411(17)30388-1. A comprehensive systematic review of pharmacy perspectives on interprofessional education and collaborative practice. El-Awaisi A1, Joseph S2, El Hajj MS3, Diack L4.
[5] Pharmacy (Basel). 2016 Sep 26;4(4). Interprofessional Education (IPE) and Pharmacy in the UK. A Study on IPE Activities across Different Schools of Pharmacy. Patel N1, Begum S2, Kayyali R3.

O005
DEVELOPMENT OF A BLOG ABOUT PHARMACOLOGY OF DRUGS OF ABUSE IN SPANISH ROCK AND POP SONGS

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Introduction: Pharmacology is often seen as a tedious subject by students. Thus, motivating students is one of the most important and difficult challenges teachers face. Several innovative approaches have been performed to improve engagement, motivation and performance among pharmacology students.[1–2] Listening to music under different contexts is usually an important part of student's free time. Showing how pharmacology surrounds us in our day to day, as part of rock and pop Spanish songs and, in consequence, using these songs in the lessons, could be of benefit to improve the motivation and performance of the students.

Objectives: To approach pharmacology to everyday life of pharmacology students and the general population, as well as increase motivation, liking for the subject and performance among students.

Methodology: Following a well graded final grade work (Trabajo de Fin de Grado, TFG) on the subject of drugs of abuse in Spanish rock and pop songs, a strategy to spread pharmacology awareness and also increase motivation among subject's students was analysed and then developed.

Results: A blog with the results of the previous research as different entries, links to the mentioned songs, the referred drugs and a comments section will be freely accessible at https://lamusicadeldesasosiego.wordpress.com/.

Conclusions: The multimedia data from the created blog will be utilised as a supporting and motivating aid in the class of pharmacology and they will also remain available for the general population.

Keywords: pharmacology, blog, drugs of abuse, music, rock and pop, songs, teaching, motivation

References:

[1] White PJ, Naidu S, Yuriev E, Short JL, McLaughlin JE, Larson IC, *Am J Pharm Educ.,* **2017**, 81(9), 10–23.

[2] Karaksha A, Grant G, Anoopkumar-Dukie S, Nirthanan SN, Davey AK, *Am J Pharm Educ*., **2013**, 77(6), 125.

TOXICOLOGY

P116
FINGER PRICK CAPILLARY MIR-122 IS A BIOMARKER OF PARACETAMOL HEPATOTOXICITY

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Objectives: Paracetamol (acetaminophen) overdose is the most common cause of acute liver failure in western countries. Several strategies have been used to define the risk of acute liver injury (ALI) and to decide whether to treat patients with N-Acetylcysteine (NAC). Despite such strategies, a small proportion of patients will still develop liver damage because their hepatotoxic risk is underestimated.[1] To improve risk stratification, new markers are currently under investigation. microRNA-122 (miR-122) has recently been demonstrated to be a marker of liver injury following paracetamol overdose. We have previously demonstrated that capillary miR-122 faithfully reflects the venous concentration.[2] This study was undertaken to confirm the reliability of capillary miR-122, measured in a finger prick blood drop, as biomarker of paracetamol induced ALI. Capillary miR-122 promises to facilitate rapid point of care diagnosis with minimal sample preparation.

Methods: Thirty-eight patients with paracetamol overdose were enrolled in this study. All patients were treated with the SNAP 12-hour NAC regimen (300 mg/kg body weight). Ten hours after beginning NAC, a capillary blood drop was obtained through a finger prick. miR-122 was measured by quantitative polymerase chain reaction in collected samples. Results were compared with alanine aminotransaminase activity (ALT). miR-122 was also compared between patients without liver injury (ALT < 50 IU/L), with borderline liver injury (ALT 50–100 IU/L) and with ALI (ALT > 100 IU/L). Non-normal data comparisons were made using the Mann-Whitney U test. Pearson's correlation test was used for correlative analysis. Results were considered significant when $P < 0.05$.

Results: Capillary miR-122 was positively correlated with ALT measured at 10 hours and at 20 hours after commencing NAC ($r = 0.83$, $P < 0.0001$; $r = 0.96$, $P < 0.0001$, respectively). MiR-122, measured after 10 hours of NAC treatment, was significantly higher in patients who developed ALI (ALT > 100) at 10 ($N = 2$, $P = 0.0036$) and 20 hours ($N = 2$, $P = 0.0062$) after the beginning of NAC treatment.

Conclusion: Our data support the reliability of capillary miR-122 as predictive marker of liver damage caused by paracetamol overdose and confirm its utility as a simple and minimally invasive diagnostic test when collected by finger prick.

References:

Nicholas D Bateman Paracetamol poisoning: beyond the nomogram Br J Clin Pharmacol. 2015; 80: 45–50.

Bastiaan AD Vliegenthart, Cécile Berends, Carmelita MJ Potter, et al. MicroRNA-122 can be measured in capillary blood which facilitates point-of-care testing for drug-induced liver injury. Br J Clin Pharmacol 2017; 83: 2027–2033.

P117
POTENTIALLY USE OF GALLIUM NITRATE FOR "LOCAL" ADMINISTRATION IN PULMONARY INFECTION INDUCED BY *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa is the most common infecting organism in chronic lung disease as Cystic Fibrosis (CF). *P. aeruginosa* adapts to avoid immune clearance and resists antibiotics via efflux pumps. Thus, a no antibiotic strategy is being explored for the eradication of *P. aeruginosa* from the airways of CF patients.[1] Fe(III) is essential for *P. aeruginosa* surviving and Ga(III) disrupt microbial iron metabolism.[2] Our aim was to evaluate the advantages of using a solution of gallium nitrate/sodium citrate (ratio 1:2), as innovative "no-antibiotic" molecules, administered intratracheally, in terms of toxicity and biodistribution. Firstly, at scheduled time intervals, treated and untreated animals were used to obtain bronchoalveolar lavage (BAL), lung and blood. Data shows that after 48 h, 72 h and 14 days from intratracheal administration of Ga(III), no significant increase in neutrophils and macrophages in BAL of treated groups was observed compared with control. To investigate systemic toxicity, different blood and serum parameters were analysed, and no hematic, hepatic and renal toxicity were observed in treated animals. We confirmed the safe use of aerosolized gallium nitrate (single administration), by evaluating the expression of different proteins (COX2, MMP9, iNOS) as index of local inflammation in lungs homogenates. Furthermore, the in vivo studies bio-distribution, showed a higher significant amount of Ga(III), analysed by inductively coupled plasma-optical emission spectrometry (ICP-OES), in lungs after intratracheal administration compared to the intravenous. In conclusion, the local administration of gallium nitrate could represent a safe and promising inhalable drug candidate in the treatment of pulmonary infection induced by *P. Aeruginosa.*

Keywords: toxicology, cystic fibrosis, infection, inflammation, open innovation

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References:

[1] Rogers G.B., Hoffman L.R., Döring G. *J. Cyst. Fibros*, **2011**, 10; 387–400.

[2] Kaneko Y., Thoendel M., Olakanmi O. *J. Clin. Invest,* **2000***,* 68; 5619–5627.

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SPASMOLYTIC ANTICHOLINERGICS OTILONIUM AND PINAVERIUM TRIGGER MITOCHONDRIAL-MEDIATED APOPTOSIS IN RAT CORTICAL NEURONS

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In the frame of a repositioning programme with cholinergic medicines in clinical use searching for neuroprotective properties, we surprisingly found that spasmolytic antimuscarinics otilonium and pinaverium exhibited neurotoxic effects in neuronal cultures. This property was not shared by other cholinergic medicines and thus, we decided to characterize such (in principle) paradoxical action in primary cultures of rat embryo brain cortical neurons. Neurotoxicity was time- and concentration-dependent, exhibiting approximate $EC_{50's}$ of 5 μM. Seven antimuscarinic drugs endowed with a quaternary ammonium, and another 10 drugs with cholinergic activities carrying in their molecule a ternary ammonium did not exhibit neurotoxicity. Both drugs caused a concentration-dependent blockade of K^+-elicited $[Ca^{2+}]_c$ transients. Cyclosporine A, a blocker of the mitochondrial permeability transition pore, prevented the neurotoxic effects of otilonium and pinaverium monitored as the fraction of cells undergoing apoptosis. Furthermore, the caspase-9 and caspase-3 inhibitor Ac-LEHD-CHO mitigated the apoptotic neuronal death of both drugs by around 50%. Data are compatible with the hypothesis that otilonium and pinaverium elicit neuronal death by activating the intrinsic mitochondrial -mediated signaling pathway of apoptosis. Of interest was the fact they did not exhibit cytotoxicity in sympathetic-like chromaffin cells neither in SH-SY5Y human neuroblastoma cells. Clinically, the neurotoxic effect of otilonium and pinaverium may not have safety consequences because both drugs possess a quaternary ammonium and as they are orally administered to trent irritable bowel syndrome and other enteric processes, they may not cross the blood-brain barrier. Additionally, otilonium and pinaverium may be used as chemical tools to study neuronal apoptosis through the intrinsic mitochondrially mediated pathway.

Keywords: otilonium, pinaverium, apoptosis, mitochondria, cortical neurons.

P119
COMPARATIVE STUDY OF IN VIVO AND IN VITRO TOXIC POTENCY OF DIARRHEIC SHELLFISH TOXINS

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Diarrheic shellfish poisoning (DSP) is an alimentary human intoxication of worldwide incidence caused by the consumption of seafood that bioaccumulated marine phycotoxins. Okadaic acid (OA), as the reference compound, and their analogues dinophysistoxin-1 (DTX1) and dinophysistoxin-2 (DTX2) are the main toxins responsible for DSP. However, there is limited data on the toxicity of OA analogues. This is the first comparative study of *in vivo* and *in vitro* toxic potency of the DSP toxins using accurate well-characterized standards. With this purpose, we studied *in vivo* effects of the oral administration of OA and analogues to Swiss female mice. All toxins induced similar symptoms, but the time of appearance of diarrhea was delayed in mice treated with DTX1, however this was the compound with the higher oral toxic potency. The protein phosphatase (PP) inhibition assay allowed the determination of the *in vitro* potency of DSP. The order of potency was as follows: DTX1 > OA > DTX2 in good agreement with the *in vivo* results. However the ratio between the oral toxicity of each analogue and that of OA is higher for DTX1 and lower for DTX2 suggesting a reevaluation of the mechanism of toxicity of DSP toxins.

Keywords: okadaic acid, dinophysistoxin-1, dinophysistoxin-2, protein phosphatase 2A

P120
SODIUM BUTYRATE LIMITS VALPROIC ACID-INDUCED HEPATOTOXICITY: *IN VITRO* AND *IN VIVO* EVIDENCE

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Valproic acid (VPA), widely used as antiepileptic drug, is potentially hepatotoxic causing reversible or fatal liver injury.[1] Recent evidence highlighted the involvement of oxidative and mitochondrial damage and impairment of fatty acid β-oxidation (FAO) in VPA detrimental effects,[2,3] even if the molecular mechanisms are overlooked. Recently, we demonstrated that butyrate restores FAO and reduces hepatic inflammation and mitochondrial dysfunction in steatotic and obese mice.[4,5] Here, we have evaluated the effect of sodium butyrate (BuNa) in limiting VPA-induced hepatotoxicity and FAO impairment and associated oxidative and mitochondrial damage using *in vitro* and *in vivo* models. HepG2 cells were stimulated, in presence or not of BuNa (0.5–1 mM), with a high concentration of VPA (2 mM) as previously described,[2] choosing 48 hour of incubation to avoid alterations of cell viability. BuNa counteracted VPA toxic effect, dampening hepatocyte oxidative damage and mitochondrial dysfunction, as demonstrated using Seahorse analyzer or polarographical and spectrophotometrical measurements. ROS, MDA and SOD-2 expression were also determined in cell lysates. Finally, we showed butyrate activity in restoring FAO (PPAR-α expression and CPT1 activity), compromised by VPA. Protective effects of butyrate were confirmed in epileptic WAG/Rij rats treated or not with VPA (300 mg/kg/day) contextually or not to BuNa (30 mg/kg/day), for 6 months. In these animals, BuNa reduced VPA-induced hepatic biomarkers in serum, and improved liver FA metabolism and inflammation. In conclusion, our data indicate the potential therapeutic effect of butyrate in counteracting VPA-induced hepatotoxicity and in limiting this common adverse reaction related to VPA chronic administration.

Keywords: valproic acid (VPA), sodium butyrate, hepatotoxicity, oxidative stress, mitochondrial dysfunction, fatty acid oxidation

References:

[1] M.M. Schmid, R.W. Freudenmann, F. Keller, et al. *Pharmacopsychiatry*, **2013**, 46, 63–68.
[2] T. Komulainen, T. Lodge, R. Hinttala, et al., *Toxicology*, **2015**, 4, 331, 47–56.
[3] K. Begriche, J. Massart, M.A. Robin, et al., *J Hepatol.*, **2011**, 54 (4):773–94.
[4] M.P. Mollica, G. Mattace Raso, G. Cavaliere, et al. *Diabetes*, **2017**, 66(5):1405–1418.
[5] G. Mattace Raso, R. Simeoli, R. Russo, et al., *PlosOne,* **2013**, 8 (7):e68626.

P121
IDENTIFICATION OF THE NA+/K+-ATPASE ISOFORMS CORRELATED WITH THE IN VITRO SENSITIVITY TO PALYTOXIN

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Palytoxins (PLTXs) are highly toxic compounds identified in marine *Palythoa* zoanthids, *Ostreopsis* dinoflagellates and *Thricodesmium* cyanobacteria. Human poisonings are associated with ingestion of contaminated seafood or with inhalation and/or cutaneous exposure to these compounds during *Ostreopsis* blooms or during cleaning operations of *Palythoa*-containing aquaria. Epidemiological and molecular evidences suggest a different inter-individual sensitivity to PLTXs, possibly related to genetic-dependent differences in Na^+/K^+-ATPase expression, the molecular target for these toxins. Thus, to identify the specific Na^+/K^+-ATPase isoforms correlated with the *in vitro* sensitivity to PLTX, 46 healthy donors were enrolled and their sensitivity to PLTX was evaluated by means of PBMC cytotoxicity (MTT assay) and erythrocytes hemolysis. The results were correlated with the gene expression for the mostly expressed isoforms of the α (α1, α3) and β (β1, β2, β3) Na^+/K^+-ATPase subunits (real time PCR). Considering the concentration giving the 50% of the effect (EC_{50}), a significant variability was recorded (median EC_{50} hemolysis = 2.9×10^{-10} M; interquartile range = $1.4x10^{-10}$-$1.1x10^{-9}$ M; median EC_{50} MTT = $1.4x10^{-10}$ M; interquartile range = $3.1x10^{-11}$ to $6.5x10^{-10}$ M). Cells sensitivity to PLTX appears to be related with gene expression of specific α and β isoforms of the Na^+/K^+-ATPase. In particular, regarding the hemolysis assay, a significant positive correlation was found between EC_{50} values and β2 gene expression (r = 0.3048, *P* value = 0.0394; Spearman correlation). Regarding the MTT assay, a significant positive correlation was found between EC_{50} values and α3 gene expression (r = 0.3739, *P* value = 0.0105; Spearman correlation). On the whole, these data indicate a significant correlation between specific Na^+/K^+-ATPase isoforms and the *in vitro* cells sensitivity to PLTX.

Keywords: palytoxin, ostreopsis, Na^+/K^+-ATPase, genetic variants, in vitro toxicity

P122
IN VITRO PRO-INFLAMMATORY EFFECTS OF GRAPHENE AND GRAPHENE OXIDE ON HUMAN SKIN KERATINOCYTES AND MONOCYTES

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Graphene is a novel nanomaterial consisting of a single atom thick two-dimensional (2D) sheet of sp2-hybridized carbon atoms.[1] Graphene-based nanomaterials (GBNs), including the highly oxidized form of chemically modified graphene (graphene oxide, GO), are becoming promising tools for several applications in the fields of nanotechnology and biomedicine. Despite the huge GBNs development, little is currently known about their impact on human health. In particular, skin toxicity of GBNs remains largely unexplored, despite cutaneous contact can be one of the major exposure routes to these nanomaterials during their production, use and discharge. Recently, we demonstrated that GBNs induce a significant reduction of HaCaT skin keratinocytes viability.[2] Therefore, this study was aimed to investigate whether subcytotoxic concentrations of two GBNs (a few layer graphene, FLG, and a commercial graphene oxide, GO) could exert *in vitro* pro-inflammatory effects in HaCaT skin keratinocytes. Cells exposure to FLG or GO (0.1 and 1.0 μg/mL) for 4 h, followed by 20 or 68 h recovery in fresh medium, induced a significant release of inflammatory mediators (granulocyte macrophage colony stimulating factor, GM-CSF; tumor necrosis factor-α, TNF-α; interleukin (IL)-1α, IL-6 and IL-8). However, HaCaT cells exposed to FLG or GO did not significantly influence THP-1 monocytes migration or differentiation towards macrophages or dendritic cells, suggesting that the inflammatory stimuli by HaCaT keratinocytes exposed to GBNs does not promote a significant activation of THP-1 monocytes. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 696656.

Keywords: graphene, skin, toxicity, inflammation

References:

[1] K. S. Novoselov, A. K. Geim, S. V. Morozov et al., *Science,* **2004**, *306*, 666–669.

[2] M. Pelin, L. Fusco, V. León et al., *Sci. Rep*., **2017**, *7*, 40572.

PHARMACOGENETICS & PHARMACOGENOMICS

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EFFECT OF CLARITHROMYCIN RESISTANCE AND CYP2C19 POLYMORPHISM ON THE EFFICACY OF THERAPIES FOR *HELICOBACTER PYLORI* INFECTION IN CHILEAN PEOPLE: COHORT STUDY

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Introduction: The eradication of Helicobacter pylori (HP) is closely related to the prevention and/or treatment of gastritis, peptic ulcer, gastric cancer and various extragastric diseases. The eradication rate resulting from standard triple therapy has declined due to resistance to clarithromycin (CR), but could also be affected by availability related to the proton pump inhibitor, which is due to part to the CYP2C19 polymorphisms. There are inconsistent studies on the roles of this polymorphism according to resistance to clarithromycin.
Objective: We evaluate the effects of CYP2C19 polymorphisms in HP infection in Chilean adults with clarithromycin resistance.
Methods: In this prospective study, 90 consecutive adult patients with *H. pylori* infection were recluted and assigned to 14 day regimens of concomitant therapy with omeprazole (20 mg), amoxicillin (1000 mg) and clarithromycin (500 mg), twice daily for 14 days. All diagnoses of *H. pylori* infection were based on a rapid urease test. Gene polymorphisms and antimicrobial susceptibility were determined using Real time PCR system and molecular assessment by PCR- RFLP , respectively. *H. pylori* eradication was defined as a negative 13C urea breath test at least 4–6 weeks after the completion of treatment.
Results : The overall eradication rate resulting was 69.4%. The rate of clarithromycin resistance was 22.2%. The eradication rate was higher in patients with CYP2C19 extensive metabolizer/clarithromycin resistance (27.8%) and lowest in Intermediate metabolizer (IM)/ clarithromycin susceptibility (83.3%) ($P < 0.001$).
Conclusion: Clarithromycin resistance had a synergistic effect with CYP2C19 EM on regimen efficacy. In a population with a high rate of clarithromycin resistance it could be useful to know the CYP2C19 polymorphism to choose the best therapy. Studies with high simple size are needed to verify this conclusion.
References:
[1] N. Author1, N. Author2, N. Author3, *J. Our. Nal.*, Year, *Vol.*, page–page. (Helvetica 9 pt).
[2] N. Author1, N. Author2. Book title. ed. n°, Publisher, Publisher City. Year.

P124
IMPROVING PHARMACOGENETIC PREDICTION OF EXTRAPYRAMIDAL SYMPTOMS INDUCED BY ANTIPSYCHOTICS

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Acute extrapyramidal symptoms (EPS) are serious adverse reactions to antipsychotic (AP) drugs. In previous work we developed a pharmacogenetic predictor of AP induced EPS based on four genes (*AKT1, FCHSD1, RPTOR* and *DDIT4*) involved in mTOR regulation.[1] The main objective is to improve this predictor by increasing its biological plausibility and replication in independent populations. Firstly, we re-sequence the four genes using targeted next-generation sequencing ($N = 88$). Then, of all identified SNPs, we predict functionality "in silico" using a web based tool (SiNoPsis).[2] Functionality of selected SNPs are test using *in vitro* luciferase reporter assays. Further, using functional SNPs, we develop a new predictor utilizing several machine learning algorithms (Discovery Cohort, $N = 131$) and replicate it in two independent cohorts (Replication Cohort 1, $N = 113$; Replication Cohort 2, $N = 113$). According to: the SiNoPsis classification; the linkage disequilibrium, and; the result of the preliminary association analysis, 12 of all identified SNPs, were selected for further analysis. 90% of these SNPs proved to be functional, since the transcription of the reporter gene was modified by the different alleles of each SNP. After prioritization, four SNPs in two genes were used to develop the pharmacogenetic predictor of AP-induced EPS. The model constructed using the Naïve Bayes algorithm achieved a 66% of accuracy in the Discovery Cohort, and similar performances in replication cohorts (Replication Cohort 1, 63%; Replication Cohort 2, 64%). The result is an improved pharmacogenetic predictor of AP induced EPS, which is more robust and generalizable than the original.
Keywords: pharmacogenetics, personalized medicine, antipsychotic, extrapyramidal symptoms, machine learning, next generation sequencing
References:
[1] Mas S, Gassó P, Ritter MA et al, *Eur. Neurospychopharmcol.*, 2015, 25, 51–59.
[2] Boloc D, Rodríguez N, Gassó P, et al, *Bioinformatics*, 2017, https://doi.org/10.1093/bioinformatics/btx570.

P125
EFFICIENCY OF THE GENOTYPING OF DPYD AND UGT1A1 IN THE PREVENTION OF SERIOUS SIDE EFFECTS TO TREATMENT IN COLON CANCER

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Patients carriers of *28 allele of the UGT1A1 gene and variants in the DPYD gene, have a high risk of serious adverse effects after the administration of irinotecan and fluoropyrimidines, respectively. The alleles *2A, *13 and rs67376798 of the DPYD gene are considered the most susceptible. We consider our series of 556 patients of the Oncology Department susceptible to treatment with the drugs involved. The methodology used was: 1. Directed genotyping of the variant *2A, *13 and rs67376798 of DPYD. 2. Sequencing of the A(TA) nTAA region of the UGT1A1 gene. We identified 38 patients with the variant UGT1A1*28 in homozygous. In addition, a patient with UGT1A1*1*36. We identified 1 heterozygous patient for the variant DPYD *2A and 5 patients heterozygous for the variant rs67376798. We have not detected the allele *13. Two of the carriers met clinical criteria for analysis of high risk genes of breast cancer and/or familial ovarian cancer, and Lynch Syndrome, respectively; in both cases the pharmacogenetic analysis was carried out on the relatives affected. The integration of the molecular analyzes performed on tumor DNA in the set of carriers, contributed to the dose adjustment in the 5 patients carrying high risk variants, and the appearance of serious side effects was avoided. The remaining 551 patients treated did not present any serious adverse reaction. The intergenomic nature of the data obtained and the pharmacogenetic study of the carriers helps the precision medicine necessary for clinical management.

Keywords: DPYD, UGT1A1, fluoropyrimidine, irinotecan

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CLINICAL GENETICS AND PHARMACOGENETICS SKILLS AND COMPETENCIES FOR THE EUROPEAN GENERAL PRACTITIONER

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Introduction: The training programs of the specialty of General Practitioner (GP) or Family Medicine remain heterogeneous at a EU level. Henceforth, in some European countries lack the skills and competencies in clinical genetics and pharmacogenetics for the GP. This poster is a proposal of these skills and competencies.

Material and Methods: This study has been carried out through a qualitative group of twelve experts selected by these three criteria: GP specialists, interested in clinical genetics and from different Spanish regions. During a four-hour session with moderator was discussed what kind of skills and competencies should be included in the curriculum of the trainee for GP for clinical genetics and pharmacogenetics.

Results: These are the skills and competencies that we have identified: *Skills*: (1) To know to build a detailed and a three generations pedigree according to the standardized nomenclature. (2) To know the different patterns of inheritance and to know to identify them in the pedigrees of the attended families. (3) To know the derivation criteria from the primary care to the centers of reference. (4) To know the most common genetic diseases in primary care. (5) To know pharmacogenetics and pharmacogenomics of frequents drugs in clinic and most common and practical tests. (6) To know to communicate to the patient the possible genetic problems that he/she faces with an accessible language. (7) To know the ethical and legal limits that imply to know the genetic status of a person/family. *Competencies*: (1) Identification of individuals at risk for a genetic condition, (2) Preconceptional counseling from primary care, (3) Knowledge of the prenatal diagnosis techniques, (4) Teratology: to know all the resources, (5) Follow-up of the patients with a genetic disease (i.e.: Down syndrome), (6) Identification of psychosocial problems in the rare diseases context. (7) To know the different kind of pharmacogenetics and genetic testing, (8) To know the resources of pharmacogenetics and clinical genetics in the Internet, (9) To know the clinical reference centers and how to derive the patients, and (10) To know the own limitations because we don't deal with being the "geneticist of primary care".

Conclusions: It is very important to adapt the current regulation across Europe in order to include specific skills and competencies in pharmacogenomics, pharmacogenetics and clinical genetics for the curriculum of the GP trainee. A rotation of these professionals during a month in a service of clinical genetics would complement the current competence-knowledge matrix and would grant better tools to improve the quality of the service provided to the patients.

P127
MIR-193A MIMICS AS NEW POTENTIAL THERAPEUTIC AGENTS IN CUTANEOUS MELANOMA

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MicroRNAs (miRNAs) are a class of small, non-coding RNAs that negatively regulate gene and other non-coding transcripts expression. MiRNAs play relevant functions in cancer biology and "miRNA mimics" may represent new potential innovative therapeutic agents restoring normal function of endogenous tumor suppressive miRNAs[1] MiR-193a acts as tumour suppressor in different types of cancer.[2–4] In melanoma, it is down-expressed in tissues and in plasma of patients,[5,6] but its role as oncosuppressor has not been yet clearly established. Aim of this study was to analyse the effects of miR-193a mimics transfection in melanoma cells to evaluate their potential role as therapeutic agents against cutaneous melanoma. miR-193a-3p and miR-193a-5p mimics were transfected with lipofectamine in three different melanoma cell lines with different B-RAF mutation status. Their effects on cell viability and migration, p-Akt, p-Erk, B-Raf protein levels and epithelial-mesenchymal transition (EMT) were evaluated. *A* significant decrease of cell viability and migration ability was induced by both miR-193a mimics in transfected cells. In addition, they decreased B-Raf protein levels and phosphorylation of Akt and Erk proteins. Finally, also Vimentin and E-Cadherin protein levels were significantly changed in transfected-cells: Vimentin expression was significantly reduced, while E-cadherin expression was significantly increased. Overall our results indicate the potential of both miRNA mimics to interfere with melanoma cell proliferation, survival and metastatization, independently from B-RAF mutation status of melanoma cells. So, our data suggest that miR193a mimics may represent potential therapeutic agents reducing melanoma progression. Future experiments will be aimed at investigating this therapeutic strategy in an *in vivo* cutaneous melanoma model.

Keywords: miR-193a-3p, miR-193a-5p, cutaneous melanoma, tumor suppressor

References:

[1] Maitri et al., (2016) EBioMedicine; 12: 34–42.
[2] Williams et al., (2015) Oncotarget; 6(27):23480–95.
[3] Jian et al., (2016) Tumour Biol.; Jul;37(7):8941–9.
[4] Yu et al., (2015) Oncogene; 34(4):413–23.
[5] Caramuta et al. (2010) J Invest Dermatol.;130(8):2062–70.
[6] Fogli et al., (2017) Tumour Biol.;39(5):1010428317701646.

P128
SCAFFOLDING PHARMACOGENETICS

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Pharmacogenetics (PGt) is a relatively new science and it is expected that not many people know about it. Moreover, it has not been studied widely in Health Sciences degree programs in Spain. A survey was designed to test the interest of general population (n = 190) about this science in a Spanish region (Aragón). Although the arithmetic mean of age was 37 years, there were two big groups of different populations including Secondary School students and retired persons. In general, 48% of people who participated in the survey have heard about PGt term although only 4 people related to understand "enough" about it and none of them were satisfied with their level of comprehension. Furthermore, not many people (37%) would rely on pharmacists as a credible source of such knowledge, mainly younger ones. Some strategies have been designed previously to educate patients about PGt testing[1] but community pharmacists would be easier to train. It would be desirable that they should understand PGt in order to counsel appropriately and provide optimal pharmaceutical care to their patients. But, in general, healthcare professionals have not shown to have enough knowledge about PGt.[2] A booklet with the most relevant applications of this area of knowledge, main concepts and basic vocabulary has been developed by Pharmacy students on the subject Pharmacogenetics and Pharmacogenomics (fifth year) at San Jorge University. After the revision period, it will be spread among community pharmacists at Aragón region. Its usefulness will be tested afterwards.

Keywords: pharmacy students, community pharmacist, patients

References:

[1] R. Mills, M. Ensinger, N. Callanan, S.B. Haga, *J. Pers. Med.* 2017, 7(2), 4.

[2] K.S. Just, M. Steffens, J.J. Swen, G.P. Patrinos, H-J. Guchelaar, J.C. Stingl, *Eur. J. Clin. Pharmacol.* 2017, 73(10), 1247–1252.

P129
GENETIC INFLUENCE ON THE WEIGHT GAIN DURING THE PHARMACOLOGICAL TREATMENT IN PROVANDS WITH ASD

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Introduction: Autism Spectrum Disorders (ASD) are severe neurodevelopmental disorders diagnosed in early childhood. Between 50–60% of children with ASD receive at least one psychotropic drug (e.g. antipsychotics, antidepressants, and stimulants). However, not all ASD patients respond to antipsychotic treatment. Many of them have severe side effects, among which weight increase is observed in 67–83% of treated cases. The antipsychotic-induced weight gain is higher than the observed in adult patients and carries a high risk of severe metabolic disorders. In addition, weight gain is the main reason for abandoning treatment, which negatively affects the patient's chances of improvement. Previous studies have shown an association between genetic polymorphisms in serotonin receptors (5-HT2C) and metabolic enzymes (CYP2D6) and drug-induced weight gain.

Objectives: The aim of this study is to investigate genetic markers that allow the identification of ASD patients susceptible to weight gain and metabolic disorders during pharmacological treatment, in which preventive or alternative treatments can be applied.

Methodology: A candidate gene association study was carried out in a cohort of N = 143 ASD probands (88% boys, 12% girls) treated with psychotropic drugs. 29 polymorphisms in 18 genes related to drug pharmacodynamics and pharmacokinetics and/or previously associated with weight gain were investigated.

Results: Statistical analyses, considering age and gender as covariates, revealed marginal associations between BMI and genetic polymorphisms in BDNF (P = 0.05), CNR1 (P = 0.03), DRD3 (P = 0.03), HTR2A (P = 0.02) and LEPR (P = 0.02). However, no clear associations were found with weight gain during treatment, except for a significant association with a DRD2 polymorphism (rs1801028, P = 0.002).

Conclusions: Our results suggest that genetic variants in DRD2 could contribute to weight gain during drug treatment in ASD probands.

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THE ONCOLOGICAL GENOMICS TOWARDS PRECISION MEDICINE: MAKING THE MOST OF GENETIC PROFILING THROUGH NEXT GENERATION SEQUENCING AND COGNITIVE COMPUTING SOFTWARE

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Background: Complete tumour molecular profiling provides valuable information, not only in the diagnostic context, but also on personalized optimal therapeutic alternatives. Furthermore, enables tumour's progression monitoring. These challenging expectations can be met through emerging technologies such as NGS, which allows simultaneous characterization of all candidate genes that may be involved in cancerous processes.

Methods: 17 samples from different oncological diagnostic stages were analysed using different NGS panel-strategies (TruSight-170, and Acute Myeloid Leukemia and Chronic Lymphocytic Leukemia custom services form Sistemas Genomicos) and HiSeq/MiSeq platforms. The resulting data were interpreted by Watson for Genomics cognitive computing software.

Results: In patients with solid tumours, whose first-line treatment failed or with limited treatment options (angiosarcoma, glioblastoma), tumour genetic profiling detected alternative drugs under investigation and classified patients as candidates for clinical trials recruitment. A complete treatment response was observed in renal tumour group of patients. Regarding identified variants, some were already associated with a specific therapy. In fact, for one variant in the *TSC2* gene, the recommended therapy matched with the one given. Finally, in the case of oncohaematological tumours, it was possible to evaluate the genomic profile throughout the disease (diagnosis, relapse and recurrence) as well as its clonal evolution. This approach allowed the selection of drugs that led to the maximum beneficial effect.

Conclusions: Genetic characterization of tumours through NGS studies, complemented by evidence-based therapeutic intervention options, provides accurate molecular diagnosis, tumour sub-classification and treatment options. In addition, this strategy offers clinicians new information to evaluate their patients.

Keywords: personalized medicine, next-generation sequencing, somatic tumours

P131
THE EMERGENCE OF MDMA: SUPER STRENGTH ECSTASY PILLS

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Background: In recent years, the market has seen the emergence of "ecstasy" tablets with a much higher content of MDMA, with very distinct shapes, colours and logos. This phenomenon has been linked to the apparent recovery of the "ecstasy" market, particularly in Europe.[1]

Methods: The concentration of MDMA has been analysed in 171 specimens, seized from 2016 to 2018. The UNODC recommendations, a GC-FID, with Helium as a carrier gas, have been used for the determination.

Results: The results obtained, shows that 11.69 % of the samples, were pills of high concentration of MDMA (180–275 mg per pill), and a 23.39 % of the tablets contained more than the usual amount per pill (75–150 mg).[2] The most common brands of strength pills are included in the next table.

Brand	Amount MDMA mg./pill	% SSEP*
Silver	209.16–166.09	40.9%
Audi	264.92–246.59	9.1%
Monkey	201.11–155.88	18.2%
db	317.20–157.33	22.7%
Moncler	233.81–206.91	9.1%

*Super strength ecstasy pills.

Conclusions: Both, the brands and the striking colours, make these tablets, appetizing for consumers; in addition, these drugs can be purchased in the dark net. The same shape and brand didn't have the same amount of MDMA. The tolerance usually developed to MDMA, could be the reason why these tablets have appeared, however, the consumption of higher doses increases the side effects (mandibular tension, hangovers, etc.), the super strength pills contains so much MDMA, that side effects can wear to death the new consumers.[3]

Keywords: amphetamines, ecstasy, MDMA, dopamine, serotonin, neuronal damage

References:

[1] EMCDDA Rapid Communication Series. *EMCDDA, Recent Changes in Europe's MDMA/Ecstasy Market: Results from an EMCDDA Trendspotter Study*, (Luxembourg, Publications Office of the European Union, 2016).

[2] Vidal Giné C, Ventura Vilamala M, Fornís Espinosa I, Gil Lladanosa C, Calzada Álvarez N, Fitó Fruitós A, Rodríguez Rodríguez J, Domíngo Salvany A, de la Torre Fornell R. *Crystals and tablets in the Spanish ecstasy market 2000–2014: Are they the same or different in terms of purity and adulteration?*. Forensic Sci Int. **2016** Jun; 263:164–8.

[3] Edland-Gryt M, Sandberg S, Pedersen W. *From ecstasy to MDMA: Recreational drug use, symbolic boundaries, and drug trends*.(Int J Drug Policy**. 2017**). Dec; 50:1–8.

P132
THE BIOAVAILABILITY OF INHALED TETRAHYDROCANNABINOL (THC) IN JOINTS

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Introduction: The aim of this work is study the bioavailability of inhaled tetrahydrocannabinol (THC) in joints. Only 10% to 25% of THC passes into the bloodstream. The dose absorbed is from 0.2 mg to 4.4 mg. The minimum dose to produce pharmacological effects is from 2 mg to 22 mg.[1]

Materials and Methods: Twenty-six samples of the whole joint seized, were analyzed by GC-FID. Whole joint weight, net joint weight (without filter), percentage of THC and mg. of THC in each joint were measured. It is supposed a bioavailability from 10% to 25%.

Results:

- The net weight average oscillates from 1.54 g to 0.50 g.
- The THC percentage: from 10% to 2%.
- The total THC in a cigarette is from 85 mg to 12 mg.
- The THC bioavailability observed is from 1.20 mg. to 18.25 mg.

Conclusions: The amount of THC in the Cannabis Sativa cigarette is variable because the results are affected by different factors: the size of the cigarette and the different herb content into the cigarette. Relating to bioavailability, the THC dose absorbed is higher than the one described by other authors.

References:

[1] Ballcells Olivero, M. Toxicologia del cannabis. Adicciones Revista on line . Vol.12 (2000), City. Year.

	Total W.(g.)	Net W.(g.)	THC percentage	THC (mg)	Availability. 10%	Availability 25%
Media	0.9399	0.7848	4.99	39.65	3.96	9.89
Mediana	0.9350	0.7650	4.60	38.50	3.81	9.55
Moda	0.63[a]	0.77	4[a]	27.00[a]	1.20[a]	2.90[a]
Desv. típ.	0.25364	0.25322	1.954	19.38	1.933	4.839
Varianza	0.064	0.064	0	375.5	3.73	23.42
Mínimo	0.63	0.50	2	12	1.20	2.90
Máximo	1.57	1.54	10	85	8.46	21.15

[a]The minor value.

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CONTRIBUTION OF THE CYP2C9 PHARMACOGENETIC ANALYSIS TO THE IMPLEMENTATION OF PRECISION MEDICINE IN THE CLINICAL PRACTICE

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Introduction: *CYP2C9* is a highly polymorphic and one of the most important contributors to drug metabolism.[1] *CYP2C9**2 and *3 alleles reduce the enzymatic activity of oral anticoagulants, non-steroidal anti-inflammatories and many neurological drugs among others.
Methods: The 5SPM model of Precision Medicine[2] was clinically applied to 204 patients with problems related to therapeutic response. After collecting clinical and therapeutic data, DNA samples were obtained using MagNAPure Compact® (Roche). According to the prescribed drugs, *CYP2C9* genotype was determined in 114 patients by Real Time PCR in LightCycler 2.0 and 480 (Roche) using FRET probes.
Results: Up to 10 medical specialties requested this analysis, 52% from the Psychiatric Unit. 46% of the patients were polymedicated and statistically significant differences were observed in the number of drugs among specialties ($P = 0.004$). According to the Anatomical Therapeutic Chemical Classification System, the N group (Nervous System) was the most frequent (45.7%). At least one CYP2C9 substrate was prescribed in 55.8% of patients and 43% were carriers of a *CYP2C9* genotype related to low metabolization, being *1/*2 the most frequent. Significant association between the indication of the study and the patient's phenotype was observed, 42.6% of non-efficient phenotypes presented adverse effects vs 21.9% of efficient ($P = 0.023$).
Conclusions: A clear increase of adverse events is observed in carriers of *CYP2C9* genotypes associated to low metabolization, this together with the high number of patients treated with *CYP2C9* substrates highlights the relevance of implementing personalized pharmacogenetic studies in clinical practice, in order to increase the safety of patients.
Keywords: *CYP2C9*, phenotype, genotype, pharmacogenetic
References:
[1] Ann K. Daly, et al, J Pers Med 2018; 8: 1–31.
[2] Isidoro-García et al, CPPM 2016; 14: 1–11.

P134
TPMT: GENETIC POLYMORPHISMS, CLINICAL AND THERAPEUTIC IMPLICATIONS

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Introduction: Thiopurine S-methyltransferase (TPMT) metabolizes Azathioprine (AZA) and other thiopurine drugs. TPMT activity is inversely correlated with active metabolite accumulation and therefore it is also associated with drug efficacy and toxicity. Several *TPMT* genetic polymorphisms affecting enzymatic activity have been described, being the most relevant non-functional alleles in our population **3B* (c.460G>A), **3C* (c.719A>G) and **3A* (c.460G>A and c.719A>G). The most important toxic effect of AZA is dose-related hematologic toxicity and the risk is higher in those patients with intermediate (carrying one nonfunctional *TPMT* allele) or absent TPMT activity (two nonfunctional *TPMT* alleles). Patients who carry two nonfunctional *TPMT* alleles (homozygous) generally experience life-threatening myelosuppression when treated with conventional doses of AZA. The aim of the present study is to investigate allelic frequencies of *TPMT* polymorphisms in our population and elucidate how healthcare professionals in our hospital are considering these results as a guide for AZA personalized therapy.
Methods: Results of allelic variants *(*3A, *3B, *3C)* of the *TPMT* gene routinely analyzed in our laboratory from 2013 to 2017 were retrospectively studied. Results: from 249 patients 94.4% were homozygous for wild-type allele (*TPMT*1/*1)*. We identified 14 carriers of on mutated *TPMT* allele (heterozygous): *TPMT*1/*3A* ($n = 11$, 4.4%), *TPMT*1/*3B* ($n = 1$, 0.4%), *TPMT*1/*3C* ($n = 1$, 0.4%) and one homozygous carrier *TPMT *3A/*3A* ($n = 1$, 0.4%) with a severe AZA-related toxicity. A 64-year-old woman with autoimmune hepatitis was referred to our centre with pancytopenia and bacteraemia after starting treatment. AZA was immediately discontinued and results of the *TPMT* genotype test showed that the patient was homozygous for *TPMT*3A* allele. This case highlights the importance of TPMT genotyping prior to starting therapy.
Conclusions: *TPMT* genotyping can help identifying patients with deficiency of TPMT activity who have a high risk of AZA related toxicity. Although testing prior to AZA initiation may prevent life-threatening adverse drug reactions, it has not yet been completely implemented in clinical routine. More educational efforts have to be made to facilitate the clinical implementation of pharmacogenetics into patient management.
Keywords: *TPMT,* pharmacogenetics, azathioprine

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DETERMINATION OF THE METABOLOMIC FOOTPRINT IN WORKERS WITH SHIFT-WORK. PILOT STUDY

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Objective: To determine the metabolic footprint of serum and body fat in workers with one-week shift rotation.
Materials and Methods: prospective cohort (morning and night-14 days of follow-up, internal pilot study ($n = 30$), both sexes (20–60 years), who agreed to participate by signing the informed consent. We collect anthropometric, dietary, physical activity and metabolomics data; Student's t test and principal component analysis (PCA) were used in R statistics software 1.0.153.
Results: We have observed 23 subjects, 15 women and 8 men, age 30.7 ± 6.9. When the subjects were in the nocturnal shift they had an increased intake of carbs and lipids and we found statistically significant differences in the gain of body fat and amount of sedentary and sleep hours (Table 1); Octadecane and Cyclopentanol 3-methyl- are elevated when the subjects are in the night shift. The first is related to a high intake of saturated fats and the second is possibly involved in mechanisms related to the thyroid stimulating hormone, however the clinical trials are still inconclusive and Oxirane 2- methyl-3-propyl-cis is related to cholesterol metabolism.

Table 1 General Data

	MEAN		P VALUE
SEX (MALE)	8 (33%) φ		<.001§ *
AGE (years)	30.7 ± 6.9		
ROTATING TIME (years)	3 ± 4.9Ų		
	ANTHROPOMETRIC DATA		
	MORNING SHIFT	NOCTURNAL SHIFT	
	MEAN ± SD	MEAN ± SD	
BMI DAY 1 (kg/m²)	27 ± 4.2	27.2 ± 4.2	.04 *
BMI DAY 7 (kg/m²)	26.9 ± 4.2	27 ± 4.3	.36
FAT MASS DAY 1 (%)	30.9 ± 9.9	29.8 ± 9.5	.00032 *
FAT MASS DAY 7 (%)	30.1 ± 9.5	30 ± 9.5	.78
DIFFERENCE BMI (kg/m²)	.1 ± .2	.2 ± .3	.41
DIFFERENCE FAT MASS (%)	.9 ± 1.2	-.2 ± .9	.0045 *
	DIETARY INTAKE		
	MORNING SHIFT	NOCTURNAL SHIFT	
	MEAN ± SD	MEAN ± SD	
KCAL	3069.8 ± 1045.8	2992.5 ± 999.9	.73
CARBS (gr)	144.2gr ± 116.9 Ų	294.2 ± 201.2	.16 ¥
LIPIDS (gr)	104.9gr ± 67 Ų	136.8 ± 53.3	.15 ¥
PROTEINS (gr)	98.6 ± 35.1	96.7 ± 34.8	.78
FIBER (gr)	49.1 ± 15.9	44.7 ± 13.5	.19
SATURATED FAT (mg)	40.8gr ± 23.1 Ų	43gr ± 17.1 Ų	.25 ¥
MONOINSATURATED FAT (mg)	38.9 ± 19.6	41 ± 15.4	.60
POLINSATURATED FAT (mg)	20.2 ± 12.5Ų	24.2 ± 15.3	.82 ¥
CHOLESTEROL (mg)	289.2 263 Ų	302.7 ± 335.3 Ų	.52 ¥
	PHYSICAL ACTIVITY DATA		
	MORNING SHIFT	NOCTURNAL SHIFT	
	MEAN ± SD	MEAN ± SD	
SLEEP HOURS	6 ± 1.1	4.9 ± 1.1	.018 *
SEDENTARY HOURS	3 ± 1.2 Ų	5.8 ± 2.7	.003 ¥ *

*STATISTICAL DIFFERENCE; Φ frequency and percentage ; Ų Median and IQR ; ¥ Wilcoxon test; § Fisher test

Conclusions: These alterations of metabolites, along with a high intake of carbohydrates, lipids and more hours of sedentary life during the night shift could generate a higher gain of fat mass reflected in the morning shift.
Keywords: metabolomics, body fat, shift work.

References:
[1] O'Gorman A, Brennan L. Metabolomic applications in nutritional research: A perspective. J Sci Food Agric. 2015; 95(13):2567–70.
[2] Crispim CA, Padilha HG, Zimberg IZ, Waterhouse J, Dattilo M, Tufik S, et al. Adipokine Levels Are Altered by Shiftwork: A Preliminary Study. Chronobiol Int. 2012; 29(5):587–94.
[3] Proper KI, Van De Langenberg D, Rodenburg W, Vermeulen RCH, Van Der Beek AJ, Van Steeg H, et al. The relationship between shift work and metabolic risk factors: A systematic review of longitudinal studies. Am J Prev Med. 2016; 50(5):e147–57.

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NOVEL ANTAGONIST TARGETING EPHB1 RECEPTOR

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EphB receptors and their membrane-bound ephrin B ligands are expressed in the nervous system, where Ephrin B/EphB signaling is an important regulator of physiologic and pathologic processes. Specifically, Ephrin Bs/EphB1 pathway is implicated in the development and maintenance of different types of pain as well as in the tolerance and dependence to opiates. Furthermore, several studies have demonstrated that blocking or introducing mutations in EphB1 receptor rescues morphine-mediated analgesia in different pain models which suggest that EphB1 could represent a potential target in the treatment of pathologic pain and CNS diseases.[1] So far, has been poor studied novel molecules able to block EphB1 receptor, probably because its crystal structure has not been elucidated. However, Koolper and colleagues have reported the identification, by phage display, of peptides that bind selectively the ephrin binding pocket of the Eph receptors.[2] The peptide EWLSPNLAPSVR bind selectively EphB1 receptor, and from this sequence we developed and characterized different linear peptides aiming at identifying novel potential EphB1 receptor antagonists. Considering that the activation of ERK1/2 in response to EphB1 stimulation is involved in several pathological processes. We retained important firstly, to assay the ability of these peptides to modulate ERK1/2 phosphorylation by itself and to counteract ephrin B1-Fc-mediated activation. We observed, that when these peptides were administered alone did not activate ERK1/2 phosphorylation but when the peptides were administered 15 min before ephrin B1-Fc, two of these peptides counteracted ephrin B1-Fc-mediated activation of ERK1/2 phosphorylation, with an IC50 in the range of 0.8–1.21 μM.
Keywords: EphB1, Ephrin B1 Fc, pain, SNC, peptides, ERK1/2.

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PHARMACOGENETIC VARIANTS ASSOCIATED WITH TREATMENT RESPONSE IN NEUROBLASTOMA

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Background: Treatment for neuroblastoma (NB) is still suboptimal. The objective of this study was to identify single nucleotide polymorphisms (SNPs) playing an important role in chemotherapeutic induction treatment in terms of efficacy.
Patients and Methods: A pharmacogenetic study of 96 SNPs was performed in 60 genes. Samples from 104 NB patients were analyzed by MassArray (Agena Bioscience). The association of genotypes with event-free (EFS) and overall survival (OS) was evaluated using a Cox Elastic-Net model. The influence of genotypes on response to induction therapy (RIT) was assessed in 41 stage M high-risk (HR) NB patients treated with COJEC chemotherapeutic regimen.
Results: The strongest association with poorer OS was observed with rs1544410 GA variant (HR = 1.39) while rs7186128 GG (HR = 0.83) and rs1801133 TC (HR = 0.65) were associated with better outcome. Associations with lower EFS for rs45511401 GT (HR = 1.79), rs1544410 GA (HR = 1.75) and rs6539570 GG (HR = 1.61) were identified. On the other side rs4880 TC (HR = 0.72), rs3814058 TT (HR = 0.62) and rs2032582 GA (HR = 0.48) variants were associated with better EFS. Regarding RIT, rs726501 AG (OR = 2.87), rs3740066 GG (OR = 1.79) or rs2010963 (OR = 1.23) and rs1143684 TT (OR = 1.143) were associated with metastatic complete response. However, rs8133052 GG (OR = 0.53), rs4149056 TC (OR = 0.64), rs10276036 TT (OR = 0.67) and rs1544410 GA (OR = 0.68) were associated to incomplete metastatic response.
Conclusions: We identified polymorphisms in *VDR*, *MTHFR*, *ABCC1*, *NR1I2* and *ABCB1* genes associated with EFS and OS as well as in *XRCC1* and *ABCC2* genes influencing on HR patients' RIT. These associations should be replicated in a large independent cohort in multicenter studies.
Keywords: neuroblastoma, pharmacogenetics, response induction therapy.

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A 10 YEARS FOLLOW-UP STUDY ON EFFICACY AND TOXICITY OF ADJUVANT CHEMOTHERAPY ON COLORECTAL CANCER PATIENTS: HOW MUCH INFLUENCE FROM THE GENETICS?

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Background: We studied the predictive value of functional polymorphisms in genes involved in the oxaliplatin/fluorouracil pathway in colorectal cancer (CRC) patients.
Material & Methods: All patients were treated with curative intended surgery followed by adjuvant chemotherapy with FOLFOX (fluorouracil, leucovorin and oxaliplatin) regimen. Genomic DNA was extracted from formalin-fixed paraffin-embedded samples of 127 patients. The *TS* promoter region polymorphism (5'VNTR) was analyzed by sequencing and the *TS* 1494del6 polymorphism by fluorescent fragment analysis. Polymorphisms at *MTHFR, XRCC1, ERCC1, ERCC2, GSTP1* and *DPYD* were analyzed by SnapShot method. These genes and SNPs were selected on the basis of their reported associations with response or toxicity in CRC.
Results: The median age was 65.53 (27–80) years (66.9% male, 59.1% rectum). The median follow-up was 7.1 years (range, 0.5–11.3). At the end of follow-up, 50 patients (44.6%) had relapsed or died in the whole study population. None of the studied polymorphisms showed clinically relevant association with disease-free survival and overall survival. The TS3´UTR 6 bp/6 bp genotype was associated with a significantly increased risk of anemia G≥2 (OR: 6.0; 95% CI: 1.48–24.40; $P = 0.014$ in a recessive model). For rs25487, 34.7% of the patients harboring a G allele (G/G and G/A) developed diarrhea G≥2 compared with 15.1% of patients with an A/A genotype (OR: 3.53; 95% CI: 1.36–9.20, $P = 0.0064$ in a dominant model).
Conclusions: Polymorphisms in XRCC1 and TS are related to toxicities and none of the tested polymorphisms is likely to be a reliable marker of response to adjuvant FOLFOX therapy.
Keywords: colorectal cancer, pharmacogenetics, FOLFOX, toxicity, survival.

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PHARMACOGENETIC VARIANTS AND RESPONSE TO NEOADJUVANT SINGLE-AGENT DOXORUBICIN OR DOCETAXEL: A STUDY IN LOCALLY ADVANCED BREAST CANCER PATIENTS PARTICIPATING IN THE NCT00123929 PHASE 2 RANDOMIZED TRIAL

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Objectives: Taxanes and anthracyclines are widely used in the treatment of breast cancer, although the benefit is limited to a proportion of patients and predictive biomarkers for clinical outcome remain elusive.
Methods: We conducted a pharmacogenetic study in 181 patients with locally advanced breast cancer enrolled in a phase 2 randomized clinical trial (NCT00123929), where patients were randomly assigned to receive neoadjuvant single-agent docetaxel 100 mg/m2 ($n = 84$) or doxorubicin 75 mg/m2 ($n = 97$). We studied the association of 226 single nucleotide polymorphisms (SNPs) in 15 key drug biotransformation genes with neoadjuvant pathological tumor response [residual cancer burden (RCB) score] to docetaxel and to doxorubicin.
Results: We identified a significant association for rs162561, an intronic SNP located in the cytochrome P450 family 1 sub-family B member 1 (CYP1B1) gene, with tumor response in patients treated with single-agent docetaxel ($\beta = 1.02$; 95% confidence interval [CI] = 0.49–1.55; $P = 1.77 \times 10^{-4}$, PBonferroni = 0.079), and for rs717620, an SNP located in the promoter of the ATP-binding cassette sub-family C member 2 (ABCC2) gene, in patients treated with neoadjuvant doxorubicin ($\beta = 1.67$; 95% CI = 0.26–3.11; $P = 0$ .02, PBonferroni = 1).
Conclusions: We identified two polymorphisms in CYP1B1 and ABCC2 associated with tumor pathological response following docetaxel or doxorubicin neoadjuvant monotherapy, respectively. Although further validation is required, these variants could be potential predictive genetic markers for treatment outcome in breast cancer patients.
Keywords: pharmacogenetics, single nucleotide polymorphisms (SNPs), tumor response, neoadjuvant single-agent chemotherapy, docetaxel, doxorubicin, breast cancer.

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NOVEL CANDIDATE GENE ASSOCIATED WITH MTOR INHIBITOR RESPONSE IN RENAL CELL CARCINOMA

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Introduction: Inhibitors of the mammalian target of rapamycin (mTOR) are effective drugs for the treatment of renal cell carcinoma (RCC) and other tumor types. Mutations in key regulators of the mTOR pathway (e.g. *MTOR*, *TSC1* and *TSC2*) have been associated with improved response to these drugs.[1,2] However, a substantial part of responder patients do not carry mutations in these genes. We aimed to identify novel mTOR inhibitor predictive markers through next-generation sequencing in a large cohort of chromophobe RCC (chRCC) patients.

Materials and Methods: Whole exome sequencing was applied to three patients highly sensitive to mTOR inhibitors. Targeted sequencing and immunohistochemistry (IHC) of candidate genes was performed in an independent series of 96 chRCC. Rapamycin sensitivity and mTOR pathway activation was assessed using *in vitro* functional studies.
Results: WES revealed one single mutated gene shared by the three tumors analyzed. This gene, unrelated to mTOR pathway, had two somatic loss-of-function mutations and one missense variant, that were validated by IHC staining and Sanger sequencing. The inactivation frequency of the gene in an independent chRCC cohort was 3% (3/96), similar to TCGA-KICH. Depletion of the protein by shRNA and Crispr/cas9 in cell models increased the sensitivity to the mTOR inhibitor rapamycin. This occured without affecting the mTOR pathway effectors p-S6, p-S6K1 and p-AKT, suggesting that the sensitizing effects occurred through alternative effectors.
Conclusion: We propose a novel candidate gene associated with mTOR inhibitor response, which could contribute to select patients to successfully undergo therapy with these drugs.
Keywords: mTOR inhibitors, renal cell carcinoma, whole exome sequencing, predictive marker.
References:
[1] Kwiatkowski DJ, Choueiri TK, Fay AP, Rini BI, Thorner AR, De Velasco G, *et al.* Clin Cancer Res. 2016;22:2445–52.
[2] Rodríguez-Moreno JF, Apellaniz-Ruiz M, Roldan-Romero JM; Durán I; Beltrán L, Montero-Conde C, Cascón A, Robledo M, García-Donas J, Rodríguez-Antona C. J Natl Compr Canc Netw. 2017; 15:1310–15.

P141
VARIANTS IN INFLAMMATORY PATHWAY GENES ARE ASSOCIATED WITH DEPRESSION RESISTANT TO TREATMENT

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Introduction: Antidepressant drugs are the main treatment for major depression. However, about 30% of patients do not respond to these drugs and are considered treatment-resistant. Resistance to pharmacological treatment affects very negatively the patient, their caregivers and the health services. The causes of treatment failure are unclear. It has been suggested that inflammatory processes may be involved in treatment response. Several studies have observed an elevation of cytokine levels associated with inflammatory responses as a result of antidepressant treatments.
Hypothesis: Alterations in genes that encode proteins involved in inflammatory responses could be associated with treatment-resistant depression.
Methodology: One hundred and fifty-three patients with DSM-IV-TR diagnosis of major depression were included in the study (31% men, 69% women) of whom $N = 91$ were responders and $N = 62$ were resistant to antidepressant treatments. The depression severity was measured by the Hamilton scale (Hamilton Depression Rating Scale, HRSD-17, 1960). A total of 61 informative SNPs were investigated in 9 genes involved in inflammatory processes (*IL-1b, IL-2, IL-6, IL-6R, IL-8, IL-10, IL-18, TNF-alpha* and *IFN- γ*).
Results: Linear regression analyses including age and gender as covariates revealed several associations between the investigated genetic variants and resistance to antidepressant treatment: polymorphisms in the *IL6R* (rs57569414 and rs4075015), *IL18* (rs543810) and *IL1-beta* (rs1143643) genes were significantly associated with resistance to treatment ($P < 0.05$ in all comparisons).
Conclusions: Genetic variants of proteins involved in the inflammatory processes could explain, at least partially, the resistance to pharmacological treatment in patients with major depression.

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IDENTIFICACIÓN DE POLIMORFISMOS GENÉTICOS ASOCIADOS CON RESPUESTA SOSTENIDA A INFLIXIMAB EN ENFERMEDAD DE CROHN

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Introducción: Actualmente no se dispone de biomarcadores que ayuden a personalizar la decisión de sobre qué diana terapéutica actuar primero en enfermedad de Crohn (EC). Hasta la fecha no se conocen polimorfismos genéticos asociados a respuesta a medio o largo plazo a infliximab.
Objetivo: Identificar polimorfismos genéticos predictores de respuesta a medio y largo plazo a IFX en pacientes de EC.
Material y métodos: La población de estudio comprendió a 95 pacientes del Hospital General Universitario Gregorio Marañón diagnosticados de EC que fueron tratados o seguían en tratamiento con IFX o su biosimilar inflectra. Se seleccionaron 18 polimorfismos en 16 genes relacionados con respuesta a anti-TNFs en alguna enfermedad autoinmune.
Resultados: Los SNPs *TLR4* rs5030728 y *TNF-α* rs1800629 se asociaron a respuesta a IFX en EC a 12 meses. El 88.2% de los pacientes con el alelo G para el rs1800629 y el 93.9% con genotipo GG para el rs5030728 respondieron al tratamiento con IFX tras 12 meses ($P = 0.037$ y $P = 0.017$). Los SNPs *TLR4* rs5030728, TNF-α rs1800629 y *FCGR2A* rs396991 se asociaron con la respuesta a 24 meses ($P = 0.016$ $P < 0.001$ $P = 0.018$ respectivamente). Tres variantes genéticas se asociaron a respuesta sostenida a IFX a largo plazo: *TNF-α* rs1800629 ($P < 0.001$), *FCGR3A* rs396991 ($P = 0.018$) y *TNFRSF1B* rs1061624 ($P = 0.008$) (Figura 1). Este último es de especial relevancia ya que el 85% de los pacientes con genotipo GG siguen siendo respondedores tras más de 10 años de tratamiento.
Conclusiones: El genotipado de estas variantes genéticas en pacientes de EC puede ser utilizado para identificar pacientes cuya diana ideal sea un anti-TNF y a aquellos en los que lo ideal sea una diana terapéutica diferente.
Keywords: anti-TNFs, enfermedad inflamatoria intestinal, farmacogenética.

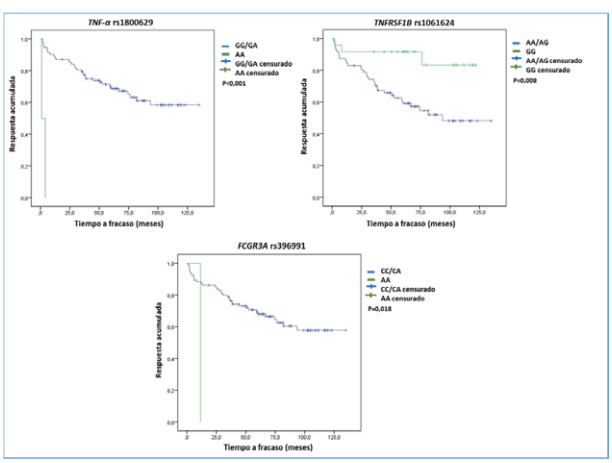

Figura 1 SNPs asociados a respuesta sostenida a infliximab a 10 años.

P144
POLYMORPHISMS ASSOCIATED WITH ANTI-TNF RESPONSE IN PSORIATIC ARTHRITIS

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Background: Psoriatic Arthritis is a chronic inflammatory autoimmune disease that results from the interaction between environmental and genetic factors. Tumor necrosis factor (TNFα) plays an important role in the development of this disease. Thus, anti-TNF drugs (adalimumab, etanercept and infliximab) are good therapeutic options for patients resistant to conventional disease-modifying antirheumatic drugs. Although anti-TNF agents are effective, not all the patients present an adequate response. Therefore, it is necessary to find biomarkers that could identify non responder patients to these therapies.
Purpose: To identify pharmacogenetic biomarkers that could predict anti-TNF drugs response in psoriatic arthritis patients.
Methods: DNA was isolated from peripheral blood cells of 20 psoriatic arthritis patients treated with anti-TNF drugs. Ten polymorphisms located in genes related with TNF were genotyped with the Illumina Veracode genotyping platform. Anti-TNFα drugs´ effectiveness was assessed by the improvement of arthritis and EuroQol Quality of Life Scales.
Results: rs1061624 *(TNFRSF1B)* and rs6920220 *(TNFAIP3)* SNPs were significantly associated with an improvement of 50% with respect the arthritis scale at 3 months in the univariate analysis. Nevertheless, this association was lost in the multivariate analysis. Furthermore, rs6920220 and rs610604 (*TNFAIP3*) SNPs showed a significant association with an improvement of EuroQol at 3 months of treatment both in the univariate and the multivariate analysis.
Conclusions: rs6920220 and rs610604 (*TNFAIP3*) have been associated with an improvement of EuroQol of PsA patients treated with anti-TNF drugs. Nevertheless, these biomarkers should be validated in large-scale studies before implementation in clinical practice.
Keywords: pharmacogenetics, arthritic psoriasis, anti-TNF drugs.

P145
PHARMACOGENETICS: A USEFUL TOOL IN THE COMPREHENSIVE MANAGEMENT OF THE PHARMACOTHERAPY OF PATIENTS IN TREATMENT WITH CLOZAPINE AND RISPERIDONE

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Introduction: Clozapine and risperidone are effective antipsychotic drugs for the treatment of schizophrenia. Despite this, there are a large number of factors that can influence on the lack of response to these drugs in patients. It is estimated that between 20–95% of an individual's response to medications is due to genetic variation.[1] The multidisciplinary approach of this study aims to identify and solve problems of indication, effectiveness, safety and adherence in patients diagnosed with schizophrenia and in treatment with clozapine and risperidone. Comprehensive medication management (CMM) and pharmacogenetic analysis have been used as complementary tools to get these results.
Materials and Methods: A total of 7 patients, diagnosed with schizophrenia, four in treatment with clozapine and four with risperidone (a patient with combination therapy) participated. The intervention consisted of CMM service[2] offered by the pharmacist. Moreover, a pharmacogenetic study of the genes involved in the metabolism and transport of these drugs (such as *CYP1A2, SLC6A4, HTR2A, CYP2D6*, and *ABCB1*) were carried out. Different polymorphisms of these genes were analysed.
Results: This study showed that patients with schizophrenia had many pharmacotherapeutic problems. Mainly, problems of drug safety, followed by problems of indication, and finally of effectiveness. The results of the genotype in addition to those of pharmacotherapeutic follow-up are useful to obtain data that allow us to generate personalized pharmacogenetic advice to each patient.
Conclusions: Pharmacogenetics can provide complementary information to solve the problems of pharmacotherapy.
Keywords: pharmacogenetics, clozapine, risperidone.

P146
NOVEL COPY NUMBER VARIANTS IN PHARMACOGENES CONTRIBUTE TO INTERINDIVIDUAL DIFFERENCES IN DRUG PHARMACOKINETICS

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Inter-individual variability in drug response constitutes a major challenge for clinical practice. These differences are at least partially shaped by single nucleotide variants (SNV) as well as copy number variants (CNVs) in genes that encode proteins involved in drug absorption, distribution, metabolism and excretion (ADME).[1,2] While single nucleotide variants have been extensively studied,[3] a systematic assessment of the CNV landscape in ADME genes is lacking. By leveraging novel NGS data sets, we here provide the first global panorama of the structural genetic diversity in pharmacogenes. We integrated data from 2504 whole genomes from the 1000 Genomes Project[4] and 59,898 exomes from The Exome Aggregation Consortium[5] from six major populations to identify CNVs in 208 relevant pharmacogenes. We next estimated the contribution of newly described structural variants to the variability in drug response. Novel exonic deletions and duplications were identified in 201 (97%) of the pharmacogenes analyzed. Novel deletions were population-specific and frequencies ranged from singletons up to 1%, and accounted for >5% of all loss-of-function alleles in up to 36% of the genes studied. We experimentally confirmed not previously described deletions in CYP2C19, CYP4F2 and SLCO1B3 by Sanger sequencing and assessed their allelic frequencies in selected populations. In conclusion, CNVs are an additional source of pharmacogenetic variability with important implications for drug response and personalized therapy. This, together with the important contribution of rare alleles to the variability of pharmacogenes, emphasizes the necessity of comprehensive next generation sequencing-based genotype identification for an accurate prediction of the genetic variability of drug pharmacokinetics.
Keywords: CNVs, drug pharmacokinetics, novel gene deletions, personalized medicine, pharmacogenes.

References:

[1] J. Li, L. Zhang, H. Zhou, M. Stoneking, K. Tang, *Hum. Mol. Genet*., 2011, *20*, 528–540.
[2] S. C. Sim, M. Kacevska, M. Ingelman-Sundberg, *Pharmacogenomics J.*, 2013, *13*, 1–11.
[3] M. Lek et al., *Nature*, 2016, *536*, 285–291.
[4] The 1000 Genomes Project Consortium, *Nature*, 2015, *526*, 68–74.
[5] D. M. Ruderfer et al., *Nat. Genet.*, 2016, *48*, 1107–1111.

P147
MICRORNAS ARE INVOLVED IN GLUCOCORTICOID RESISTANCE REVERSION BY RAPAMYCIN THROUGH SUPPRESSION OF THE JNK SIGNALING PATHWAY

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Glucocorticoids (GCs) are commonly used as therapeutic agents for inflammatory and autoimmune diseases, in the treatment of leukaemia and in the prevention of rejection in transplant patients. However, considerable inter-individual differences in their efficacy have been reported.[1] Several reports indicate that the immunosuppressive agent rapamycin, the inhibitor of mTOR, can reverse GC resistance in different cell lines,[2] but the mechanism involved in the synergistic effect of these agents has not yet been clarified. Recent studies showed that the abnormal expression of microRNAs (miRNAs) is associated with GC sensitivity.[3] On these bases, we explored the differential miRNA expression in a GC-resistant cell line (CCRF-CEM) after treatment with methylprednisolone (MP), rapamycin and in co-treatment using a TaqMan® Array and validated a candidate miRNA related to the JNK pathway. The expression analysis identified 70, 99 and 96 miRNAs that were differentially expressed after treatment with MP, rapamycin and MP + rapamycin, respectively. In order to detect the potentially altered pathways by differentially expressed miRNAs, the DIANA-miRPath software was used. Only two pathways were exclusively altered as a result of the co-treatment: the JNK and ErB. We validated one of the miRNAs potentially associated with the JNK pathway (Fig. 1), miR-331-3p, by qRT-PCR confirming its upregulation after co-treatment and the alteration of the JNK pathway by western blot. In conclusion, the combination of rapamycin with MP restores GC effectiveness through the regulation of different miRNAs, affecting JNK pathway, suggesting the important role of these pharmacoepigenetic factors in GC response.

Keywords: glucocorticoid, rapamycin, miRNA, JNK.

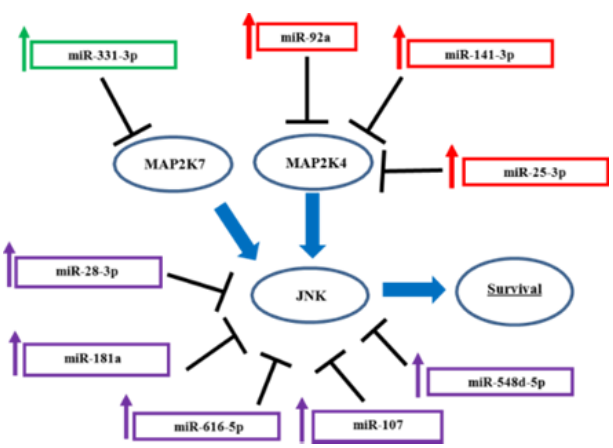

Figure 1 Deregulated miRNAs potentially involved in GC resistance reversion by rapamycin through suppression of the JNK pathway.

References:

[1] De Iudicibus S, Franca R, Martelossi S, et al., *World J. Gastroenterol.* 2011, *17*, 1095–108.
[2] Lucafo' M, Bravin V, Tommasini A, et al., *Clin Exp Pharmacol Physiol* 2016, *43*, 602–5.
[3] Rainer J, Ploner C, Jesacher S, et al., *Leukemia*, 2009, *23*, 746–752.

P148
P1-NTBD MIMETIC PEPTIDE DOWNREGULATES CX43 AND IMPROVES CHONDROCYTE PHENOTYPE IN OSTEOARTHRITIS

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Chondrocytes in articular cartilage undergo phenotypic changes and senescence, restricting cartilage regeneration and favoring osteoarthritis (OA) progression, a chronic disease characterized by degeneration of articular cartilage leading to physical disability and pain. Even OA is the most prevalent musculoskeletal disease worldwide, there is no cure or effective treatment. As in other wound healing disorders, chondrocytes from OA patients show a chronic increase in the protein connexin43 (Cx43),[1,2] which through the recruitment/release of signalling factors regulates signal transduction. Hence, Cx43 has been identified as a new therapeutic target for OA and other wound healing disorders.[3,4] In this study, we designed new mimetic peptides with binding motifs and phosphorylation sites of the C-terminal domain of Cx43 in order to downregulate Cx43 activity. Our results indicate that the peptide containing part of the tubulin-binding domain of Cx43 (P1-nTBD) decreases gap junction intercellular communication. P1-nTBD did not co-localized with Cx43, however significantly reduced Cx43 protein levels when chondrocytes from OA patients were treated in monolayer with different concentrations of the peptide. Further, downregulation of Cx43 led to redifferentiation of OA chondrocytes increasing Col2A1 and proteoglycans synthesis and reducing mesenchymal stem cells markers and cell proliferation, improving therefore the regenerative capacity of cartilage. Further studies will be performed to study the mechanisms of this peptide in order to assess its potential use as new pharmacological approach in OA. So far, our results indicate that that small peptide P1-nTBD has potential use for the treatment of OA in order to halt cartilage degeneration and foster regeneration.

Keywords: mimetic peptides, Cx43, chondrocytes, osteoarthritis.

References:

[1] Mayan, M.D., P. Carpintero-Fernandez, R. Gago-Fuentes, O. Martinez-de-Ilarduya, H.Z. Wang, V. Valiunas, P. Brink, and F.J. Blanco. 2013. *Am J Pathol*., 2013*, 182,*1337–1346.
[2] Mayan, M.D., R. Gago-Fuentes, P. Carpintero-Fernandez, P. Fernandez-Puente, P. Filgueira-Fernandez, N. Goyanes, V. Valiunas, P.R. Brink, G.S. Goldberg, and F.J. Blanco. *Ann Rheum Dis*., 2015, 74, 275–284.
[3] Grek, C.L., J. Montgomery, M. Sharma, A. Ravi, J.S. Rajkumar, K.E. Moyer, R.G. Gourdie, and G.S. Ghatnekar. *J Invest Dermatol*., 2017,*137*, 620–630.
[4] Varela-Eirin, M., J. Loureiro, E. Fonseca, S. Corrochano, J.R. Caeiro, M. Collado, and M.D. Mayan. *Ageing Res Rev*., 2018, *42*, 56–71.

P149
INFLUENCE OF GENETIC POLYMORPHISMS ON THE RESPONSE TO TRAMADOL AND IBUPROFEN IN PATIENTS WITH MODERATE-TO-SEVERE PAIN

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The aim of this study was to evaluate the impact of several polymorphisms on the analgesic effect of ibuprofen and tramadol. We analysed 81 patients with moderate-to-severe pain after dental surgery who randomly received ibuprofen arginine 400 mg or tramadol 50 mg for pain control. Patients recorded their pain intensity on a Visual Analogue Scale (VAS) to a maximum of 6 hours. We analysed 32 variants in metabolizing enzymes (cytochrome P450 (CYP), *COMT)*, transporters (*ABCB1*, *SLC22A1*) and receptors (*OPRM1*, *PTGS2*) using the custom SNP-array platform PharmArray.[1] Regarding ibuprofen, *CYP2C8* PM experienced significant lower VAS reduction at 6 h ($P = 0.02$). Conversely, although it was not significant ($P = 0.07$), *CYP2C9* PM presented a better response, possibly as a consequence of higher ibuprofen concentrations. There was no association between *CYP2B6*, *CYP2C19*, *CYP2D6*, *CYP3A5* and *PTGS2* and the response to ibuprofen. Regarding tramadol, the only subject who was *CYP2D6* UM experienced a higher VAS reduction at 1 h, which could be explained as *CYP2D6* mediates the formation of O-desmethyltramadol, which has a higher μ-opioid receptor affinity than tramadol. The only *CYP2D6* PM subject experienced a late response (no reduction at 1 h) due to the lower formation of O-desmethyltramadol. Patients carrying *ABCB1* minor alleles showed a greater response, along with patients carrying *OPRM1* 118G allele ($P = 0.049$). Patients with lack of *SLC22A1* active alleles experienced a worse response to tramadol. In summary, several polymorphisms have an impact in the efficacy of ibuprofen and tramadol in patients with acute pain. However, further research is needed with larger sample size to confirm our results.

Keywords: ibuprofen, tramadol, efficacy, pharmacogenetics.

References:

[1] Borobia AM, Dapia I, Tong HY, et al. Clin Transl Sci. 2018 Mar;11(2):189–199.

P150
STUDY OF GENETIC INFLUENCE ON TREATMENT RESPONSE IN AUTISM

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Introduction: Autism spectrum disorders (ASD) are severe and chronic neurodevelopmental disorders that affect about 0.5–1% of the population. 50–60% of ASD sufferers are treated with psychotropic drugs, including atypical antipsychotics, stimulants and antidepressants. However, about 30% of them do not respond adequately to treatment, and in some cases suffer a significant deterioration with subsequent treatment retrieval. Treatment failure and adverse reactions have a negative effect on patients' prognosis, often leading to noncompliance. The identification of biomarkers for the prediction of clinical outcome and side effects would help to improve treatment efficacy and safety in ASD patients. To date, only a few studies have focused on the genetic influence on treatment response in ASD. Previous studies have associated genetic polymorphism in serotonin receptors and metabolic enzymes with response to pharmacological treatment. However, those findings need confirmation. OBJECTIVES: the identification of pharmacogenetic markers for the prediction of response to pharmacological treatment in ASD.

Methodology: A candidate-gene association study was conducted in a cohort of 143 ASD probands (88% boys, 12% girls) treated with psychotropic drugs (113 methylphenidate, 30 antipsychotics, antidepressants, anxiolytics or mood stabilizers). Twenty-five polymorphisms within 15 genes previously associated with response to psychotropics and/or side-effects (ANKK1, BDNF, CNR1, COMT, DAT1, DRD2, DRD3, DRD4, HTR1A, HTR2A, ABCB1, CYP1A2, CYP2C19, CYP2D6 y CYP3A5) were investigated.

Results: Statistical analyses, considering age and sex as covariates, revealed marginal associations between polymorphisms in the genes ANKK1 (rs1800497) and DRD4 (exon 3VNTR) with treatment response ($P = 0.02$ and $P = 0.03$, respectively). Additionally, association was observed between polymorphisms in the genes ANKK1 (rs1800497), BDNF (rs6265), COMT (rs4633 & rs4680), and CYP3A5 (rs776746) and presence of adverse reactions ($P < 0.05$ for all comparisons).

Conclusions: our results suggest a genetic contribution to treatment variability and side-effects observed in ASD patients during pharmacological treatment. However, the clinical utility of these findings need further investigation.

P151
ROLE OF THE INFLAMMATORY ROUTE GENES IN SEXUAL DYSFUNCTION INDUCED BY ANTIDEPRESSANT DRUGS

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Introduction: Antidepressant drugs, and in particular selective serotonin reuptake inhibitors (SSRIs), are widely used as first-line treatment in depressive and anxiety disorders. However, they generate significant and continuous side effects in a large proportion of treated patients. Sexual dysfunction induced by antidepressants (SDA) is one of the most important adverse effects, with a prevalence of 20–70%, depending on the drug used. SDA negatively impacts the patient's quality of life and is a frequent reason for abandoning treatment. The identification of pharmacogenetic markers associated with SDA would help to predict and prevent its onset, thus improving the safety and effectiveness of antidepressant treatment. Although it is not the main target, antidepressants modulate the production of inflammatory cytokines and this may play a role in its therapeutic efficacy and its profile of adverse effects. Some studies have observed alterations in the levels of these cytokines in patients with erectile dysfunction but, to date, their possible role in SDA has not been investigated.

Hypothesis: Genetic variants in cytokines involved in inflammatory processes could contribute to SDA.

Methodology: Association study of candidate genes in a cohort of $N = 100$ patients treated with SSRI or dual antidepressants. The level of sexual dysfunction was assessed using the PRSexDQ-SALSEX questionnaire (Psychotropic-related-sexual-questionnaire, Montejo et al., 2000). 61 informational SNPs were genotyped in 9 genes involved in immune response (IL-1b, IL-2, IL-6, IL-6R, IL-8, IL-10, IL-18, TNF-alpha and IFN-γ) in the sample investigated. RESULTS: The statistical analyses (linear regressions considering the covariates age, sex, type of antidepressant and duration of the patient's treatment) revealed several associations. The most significant results showed clear associations between genetic variants in IL2 and sexual dysfunction ($P = 0.02$) and decreased libido ($P = 0.004$), and between genetic variants in TNF-α and delay and absence of orgasm (both $P = 0.003$).

Conclusions: Our results suggest that genetic variants of proteins involved in the inflammatory response could contribute to the sexual dysfunction observed in patients treated with SSRI antidepressants.

P152
DESIGNING AN AXIOM SPANISH BIOBANK ARRAY FOR ANALYSING RARE VARIATION IN SPANISH POPULATION

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The genetic architecture of the human complex diseases and traits has been largely explored over the years with hundreds of genetic variants identified with genome-wide association studies (GWAS). However, the genetic variants identified confer, in most cases, relatively small increments in disease risk[1] or explain only a fraction of the known heritability[2] for complex diseases, which poses the existence of variants related with genetic susceptibility to complex diseases that remains to be unravelled. It has been postulated that part of this "missing heritability" could be attributed to rare variation of moderate penetrance conferring important effects in the individual risk although global population risk remains lower.[3] Many studies have been starting to explore this possibility with interesting results for complex diseases such as autism[4] and also in pharmacogenomics studies.[5,6] In recent years, a large number of population genomic projects have explored the existence of an enormous amount of rare variation in human population genomes which has been observed to be population specific.[7,8,9] For this reason, we have designed in collaboration with Affymetrix (Thermo Fisher) an Axiom Spanish Biobank Array that encompass common variation of a variety of complex diseases enriched with rare variation from Spanish population and with pharmacogenetic variants with biologic relevance for pharmacogenetic response, which were selected from a recommended list of several consortiums and databases. Our main aim was to provide a tool for scientists to explore all this variation at an individual level in a feasible and easier manner.

Keywords: rare variation, complex diseases, pharmacogenetics, array.
Acknowledgments: We would like to specially thank to Ana Vega, Clara Ruiz, María Brión, Alejandro Blanco, Miguel González, Lara Besada, Carlos Flores and to the Affymetrix team for their contribution to the construction of the present array.

References:

[1] Manolio TA, Collins FS, Cox NJ, Nature, 2009, 461, 747–753.
[2] Lichtenstein P, Holm NV, Verkasalo PK, N Engl J Med. 2000, 343, 78–85.
[3] Robinson R, PLoS Biol, 2010, 8.
[4] Neale BM, Kou Y, Liu L, Ma'ayan A, Nature, 2012, 485, 242–245.
[5] Nelson MR, Wegmann D, Ehm MG, Science, 2012, 337, 100–104.
[6] Dopazo J, Amadoz A, Bleda M, Mol Biol Evol., 2016, 33, 1205–1218.
[7] Tennessen JA, Bigham AW, O'Connor TD, Science, 2012, 337, 64–69.
[8] Keinan A, Clark AG, 2012, 336, 740–743.
[9] Fu Y, Foden JA, Khayter C, Nat Biotechnol., 2013, 31, 822–826.

P153
IMPACT OF CYP AND ABCB1 POLYMORPHISMS ON THE PHARMACOKINETICS OF EFAVIRENZ IN HEALTHY VOLUNTEERS

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Background: Efavirenz (EFV) is a Human Immunodeficiency Virus non-nucleotidic reverse transcriptase inhibitor.[1] CYP2B6 has been described as its main metabolizing enzyme whereas CYP3A or CYP2A6 have been identified as secondary enzymes.[2] In this study, we evaluated the influence of polymorphisms in these enzymes and P-glycoprotein (ABCB1) on the pharmacokinetics of EFV.
Methods: Forty-seven healthy volunteers that received a 600 mg single oral dose of EFV were genotyped (with TaqMan™ probes in a StepOne Real-Time PCR System®) for eleven polymorphisms in the following genes: *CYP2B6*, *CYP2A6*, *CYP3A4*, *CYP3A5*, and *ABCB1*.
Results: *CYP2B6* G516T G/G genotype was associated to higher clearance EFV rates than C/T and T/T ($P = 0.025$); *CYP2A6* rs28399433 A/A genotype was associated to lower area under the curve (AUC) ($P = 0.037$) and higher volume of distribution (Vd) ($P = 0.038$) than A/T; *ABCB1* C3435T T/T genotype was associated to higher AUC ($P = 0.014$) and Cmax ($P = 0.011$) and to lower Vd ($P = 0.024$) in comparison to other genotypes. Multivariate analysis confirmed the influence of *CYP2B6* and *CYP2A6* polymorphisms on AUC, Cmax and Vd and discarded *ABCB1*. The influence of *CYP2B6* polymorphisms on EFV pharmacokinetics had already been reported.[3,4] Analogous to our results, "mutant" genotypes were associated to higher AUC or Cmax and lower clearance rates. Furthermore, we have demonstrated the involvement of CYP2A6 polymorphisms on EFV pharmacokinetics. Since this isoform metabolizes EFV into a different metabolite than CYP2B6, both isoforms may be significant regarding EFV pharmacokinetics, safety and efficacy.

Keywords: Efavirenz, pharmacokinetics, CYP2B6, CYP2A6, ABCB1.

References:

[1] H. Duarte *et al.*, 'Population Approach to Efavirenz Therapy', *J. Pharm. Sci.*, vol. 106, no. 10, pp. 3161–3166, Oct. 2017.
[2] A. Bienczak *et al.*, 'The impact of genetic polymorphisms on the pharmacokinetics of efavirenz in African children: Efavirenz pharmacogenomics in African children', *Br. J. Clin. Pharmacol.*, vol. 82, no. 1, pp. 185–198, Jul. 2016.
[3] T. N. Lam, K. H. Hui, D. P. C. Chan, and S. S. Lee, 'Genotype-guided dose adjustment for the use of efavirenz in HIV treatment', *J. Infect.*, vol. 71, no. 5, pp. 607–609, Nov. 2015.
[4] H. Gatanaga *et al.*, 'Successful Efavirenz Dose Reduction in HIV Type 1-Infected Individuals with Cytochrome P450 2B6 *6 and *26', *Clin. Infect. Dis.*, vol. 45, no. 9, pp. 1230–1237, Nov. 2007.

P154
GENETIC BIOMARKERS IN THE VEGF PATHWAY AS PROGNOSTIC FACTORS IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS TREATED WITH PLATINUM-BASED CHEMOTHERAPY

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Background: The Vascular Endothelial Growth Factor (VEGF) signaling pathway plays a critical role in the growth and progression of non-small cell lung cancer (NSCLC). We aimed to evaluate the influence of germline polymorphisms within genes involved in the VEGF

pathway on the prognosis of advanced NSCLC patients treated with first-line platinum-based chemotherapy.
Methods: We included 170 patients diagnosed with stage IV NSCLC between 2012 and 2016 in our hospital. A total of 28 polymorphisms in 12 genes involved in the angiogenesis process were genotyped by real-time PCR using the BioMark™ system.
Results: The median follow-up period was 15 (1–75) months, the median overall survival (OS) was 14.8 (12.0–17.6) months and the median progression-free survival (PFS) was 6.0 (4.7–7.2) months. In the univariable analysis, four polymorphisms located in three genes were significantly associated with OS: rs2010963 and rs3024997 (*VEGFA*), rs2071559 (*VEGFR2*) and rs10842513 (*KRAS*). In the multivariable analysis, two of them retained their statistical significance: rs2010963 [Hazard ratio (HR) = 0.676; $P = 0.026$] and rs2071559 [HR = 1.544; $P = 0.025$]. Regarding SLP, three variants showed a statistically significant association in the univariable analysis: rs9513070 (*VEGFR1*), rs35251833 (*ITGAV*) and rs2076139 (*MAPK11*). In the multivariate analysis, two of them remained significant: rs35251833 [HR = 0.772; $P = 0.002$] and rs2076139 [HR = 0.584; $P = 0.013$].
Conclusions: Germline polymorphisms in the VEGF pathway may be useful as prognostic biomarkers in advanced non-small cell lung cancer.

P155
NUDT15 RS116855232 AND TPMT RS1142345 POLYMORPHISMS AS GENETIC DETERMINANTS IN 6-MERCAPTOPURINE (MP) INTOLERANCE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

Silva-Rodríguez P.[1], *Paz E.N.*[1], *García A.R.*[2], *Nogueira S.F.*[2], *Freire S.R.*[2], *de Trocóniz L.L.F.*[1], *García J.M.C.*[2]

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Background: 6-Mercaptopurine is a widely used treatment in childhood ALL.[1,2] Thiopurines have narrow therapeutic indices due to frequent toxicity that could be explained partly by *TPMT* polymorphisms. But some patients who are wild-type homozygous for *TPMT* still develop toxicity. *NUDT15* variation is also related to thiopurine intolerance. Patients who develop toxicity need MP dose reduction or protocol interruption, so continuous efforts to optimize efficacy and safety treatment are needed.[3]
Objective: The aim is to genotype *TPMT*rs1142345 and *NUDT15*rs116855232 polymorphisms in patients with ALL during the maintenance therapy with 6-Mercaptopurine and Methotrexate.
Materials and Methods : Twenty-five ALL patients were evaluated for germline *TPMT* and *NUDT15* polymorphisms. Genotyping of *TPMT* (c.719A>G;rs1142345) and *NUDT15* (c.415C>T;rs116855232) was performed by Sanger sequencing. MP dose reached during the maintenance phase, necessary to maintain the leukocyte count above 1000/mm, and liver toxicity were evaluated in all patients.
Results: Our results showed that just one patient was heterozygous for the *NUDT15*rs116855232 polymorphism. Two patients showed the *TPMT*rs1142345 variant; one was homozygous for it and the other was heterozygous. Interestingly, the MP administered dose was lower than the 50% of the planned dose in two patients and between 15 to 60% in the one who carries the *NUDT15* polymorphism. Neither of them showed analytical manifestations of liver toxicity.
Conclusion : Inter-individual variability in thiopurine-related toxicity could not be explained just by *TPMT* polymorphisms. The *NUDT15* polymorphism is also associated with decreased thiopurine metabolism and leukopenia in ALL. Genotyping *TPMT* and *NUDT15* polymorphisms may inform personalized therapy for ALL patients.
Keywords: 6-Mercaptopurine (MP), acute lymphoblastic leukemia (ALL), intolerance, toxicity.

References:
[1] HL McLeod, EY Krynestski, MV Relling and WE Evans: "Genetic polymorphism of thiopurinemethyltransferase and its clinical relevance for childhood acute lymphoblastic leukemia". Leukemia (2000) 14, 567–572.
[2] Takaya Moriyama, Rina Nishii, Virginia Perez-Andreu et al. "*NUDT15* Polymorphisms alter Thiopurine metabolism and hematopoietic toxicity". Nat Genet. 2016 April; 48(4):367–373.
[3] Nathalie K. Zgheib, Reem Akika, Rami Mahfouz et al. "MUDT15 and TPMT genetic polymorphisms are related to 6-mercaptopurine intolerance in children treated for acute lymphoblastic leukemia at the Children's Cancer Center fof Lebanon". Pediatr Blood Cancer 2017; 64:146–150.

P156
IMPACT OF CYP2C19 GENETIC POLYMORPHISMS ON VORICONAZOLE EXPOSURE IN PATIENTS WITH INVASIVE FUNGAL INFECTIONS

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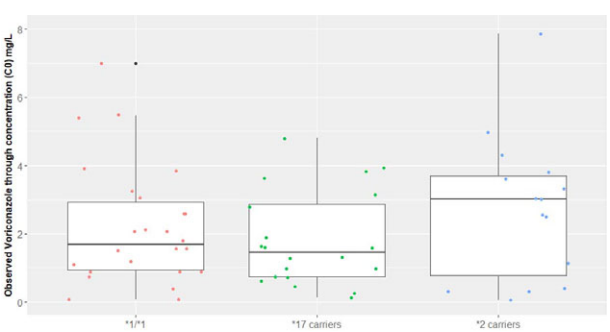
Introduction and Objective: The aim of this study is to analyse the impact of the CYP2C19 genotype on exposure to voriconazole.
Materials and Methods: A prospective analysis of data from patients receiving systemic voriconazole was performed. Voriconazole trough plasma levels (C_o) were determined using a high-performance liquid chromatography method.[1] Genotyping was performed on the QuantStudio ™ 12K Flex System platform (Applied Biosystem) with OpenArray® technology. Additional variables collected included age, sex and weight. Patients were classified according to the C_o in infratherapeutic, supratherapeutic or in range. A target range of 1–5 mg/L was considered based on literature reports.[2] The impact of CYP2C19 genotype on voriconazole exposure was investigated by R Statistics.
Results: A total of 61 patients (35 man; median age 65 years, range: 13–91; mean weigh 68.5 ± 14.2 kg) were included in this study. The mean C_o was 2.2 ± 1.7 mg/mL. C_o was subtherapeutic for 19 patients (30.1%), supratherapeutic for 4 (6.3%) and within the target range for 40 (63.5%). The allele frequencies of the CYP2C19*17 and *2 were 31.4% and 20.0% respectively. Observed voriconazole trough concentrations correlated with the CYP2C19 polymorphic alleles is represented in figure 1. Patients with the gain-of-function allele CYP2C19*17 presented more frequently subtherapeutic concentrations, while carriers of the allele * 2 had higher C_o.
Conclusion: Although the results were not statistically significant, there seems to be higher or lower concentrations of voriconazole depending



on the presence of certain alleles of CYP2C19. Optimal TDM (jointly with pharmacogenomics information could improve patient outcomes.

References:

[1] Cendejas-Bueno E, Cuenca-Estrella M, Gómez-López A. Clinical indications for therapeutic drug monitoring of antifungal agents. In the way for optimizing the treatment of fungal infection. *Rev Espanola Quimioter* r **2014**;*27(1)*:1–16.

[2] Dolton MJ, Ray JE, Chen SC-A, Ng K, Pont LG, McLachlan AJ. Multicenter Study of Voriconazole Pharmacokinetics and Therapeutic Drug Monitoring. Antimicrob Agents Chemother 2012;56 (9):4793–9.

Keywords

voriconazole, pharmacogenomics, CYP2C19, antifungals, drug monitoring.

P157
IDENTIFICATION OF ANTIPSYCHOTIC DRUG RESPONSE PHENOTYPES BY CLUSTERING LONGITUDINAL DATA: A CRITICAL STEP FOR PERSONALIZED MEDICINE

Mas S.[1,2,3], *Boloc D.*[4], *Torres T.*[1], *Martínez A.*[1], *Rodríguez N.*[1], *Parellada M.*[3,5], *Saiz-Ruiz J.*[3,6], *Cuesta M.J.*[3,7], *Gassó P.*[1], *Bernardo M.*[2,3,4,8], *Lafuente A.*[1,2,3]

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The application of personalized medicine requires tools for the classification of patients according to their response to treatment, considering both efficacy and toxicity. In this study, using longitudinal data, we present a clustering strategy to classify patients with a first episode of psychosis (FEP) treated with antipsychotics. 193 FEP with complete data were selected from the PEPs project.[1] The efficacy was assessed using PANSS, and adverse effects using UKU, during one year follow-up. We used the Klm3D method to cluster longitudinal data.[2] We identified four clusters: (A) patients with good efficacy and no toxicity; (B) patients with good efficacy who present adverse effects; (C) patients with low efficacy and no toxicity; and (C) patients with low efficacy who present persistent adverse effects. These groups significantly differ in baseline demographics (age, CI, cannabis use cocaine use, obstetric complications, trauma experiences, premorbid adjustment), clinical (diagnosis, severity, days of untreated psychosis) and neuropsychological characteristics (verbal memory, executive function, working memory). The type and dose of antipsychotic was not significantly different between clusters at baseline. However, a different evolution was observed in the dosage of antipsychotics. A genetic association study identified ten SNPs with nominal significance. All baseline variables associated with the clustering were included in an algorithm using machine learning. The algorithm is able to assign the correct cluster to 91% of patients. The results shows that these subgroups of patients can be identified before treatment assignment, making possible to apply personalized medicine, taking into account both the efficacy and the toxicity of the treatment.

Keywords: pharmacogenetics, personalized medicine, antipsychotics, clustering.

References:

[1] Bernardo M, Bioque M, Cabrera B, et al, *Schizophr. Res.*, **2017**, 189, 181–189.

[2] Genolini C, Sentenac M, Alacoque A et al, *J Statist Software*, **2015**, 65, 1–34.

P158
NOD2 AND ATG16 L1 ARE ASSOCIATED WITH ANTI-TNF TROUGH LEVELS IN A COHORT OF PATIENTS WITH CROHN'S DISEASE

Almenara S.[1], *Egoavil C.M.*[1], *Londoño A.C.*[1], *Gutiérrez A.*[2,3], *Sempere L.*[2,3], *Zapater P.*[1,2,3], *Cameo J.I.*[3], *Gonzalez-Navajas J.*[2], *Frances R.*[2]

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Background: Anti-TNF-alpha therapy is widely used in Crohn's Disease (CD). The quantification of serum anti-TNF levels to optimize treatment is controversial. The objective was to identify genetic, inflammatory, clinical and analytical parameters related with trough levels of anti-TNF in CD patients.

Methods: CD patients either on regular or intensified schedules with infliximab or adalimumab were included. Patients were genotyped for genes associated with inflammation and autophagy (NOD2, ATG16L1, PTPN2, TLR5 and IRGM). Serum cytokine levels and anti-TNF drugs were evaluated. The presence of circulating bacterial (bact)DNA was also determined. Linear regression was performed using the R software (v3.2.3).

Results: 112 CD patients with anti-TNF (62 on infliximab and 50 on adalimumab) were included. Fourteen patients on infliximab (22.5%) and 15 on adalimumab (30%) were receiving an intensified regimen ($P = 0.37$). No significant differences were found in clinical characteristics according to intensified *vs* non-intensified regimen, regardless of biological drug used. We didn't find significant differences in anti-TNF trough levels between infliximab (5414.4 ± 2336.6 ng/mL) and adalimumab (5612.9 ± 2116.8 ng/mL; $P = 0.64$) either (Fig. 1). In the univariate analysis controlled by weight, type of anti-TNF and regimen the presence of bactDNA in serum (Beta = −1296.93; $P = 0.006$), serum levels of IL-12 (Beta = −2.86; $P < 0.0001$), IFNγ (Beta = −5.41; $P < 0.0001$), TNFα (Beta = −40.84; $P < 0.0001$), varNOD2(Beta = −1866.77; $P < 0.0001$) and varATG16L1 (Beta = −3057.75; $P < 0.0001$) were associated with lower anti-TNF levels, on the contrary, serum IL-10 levels were directly related to anti-TNF levels (Beta = 90.14; $P < 0.0001$).

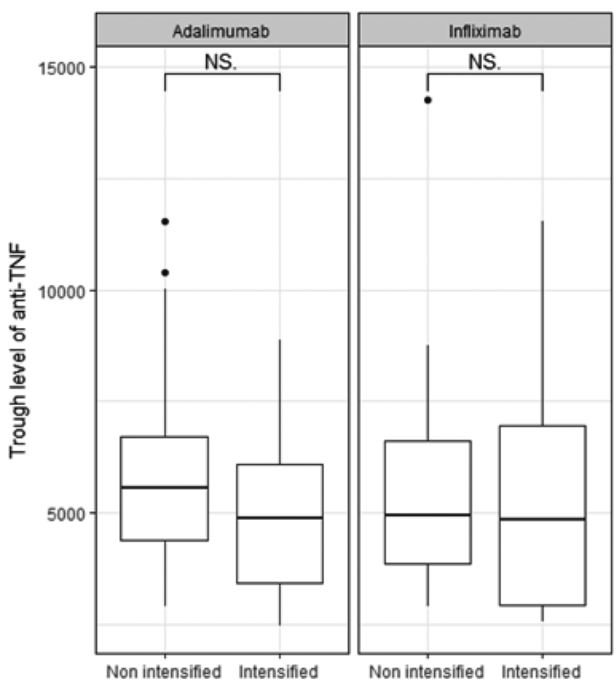

Figure 1

Conclusion: NOD2 and ATG16L1 variants are associated with lower anti-TNF levels (Fig. 2). The presence of bactDNA, TNF α, IL-12, IFNγ and especially IL-10 are also strongly associated with trough levels of anti-TNF.

Keywords: : anti-TNF levels, Crohn's disease, pharmacogenetics.

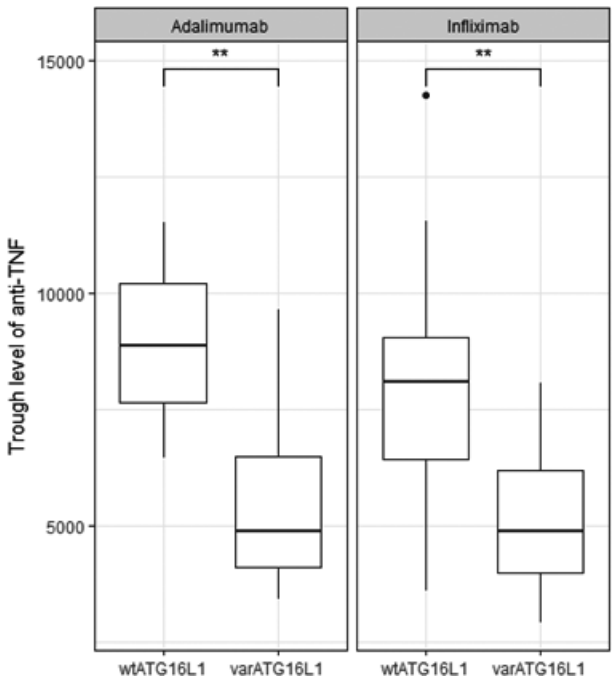

Figure 2.

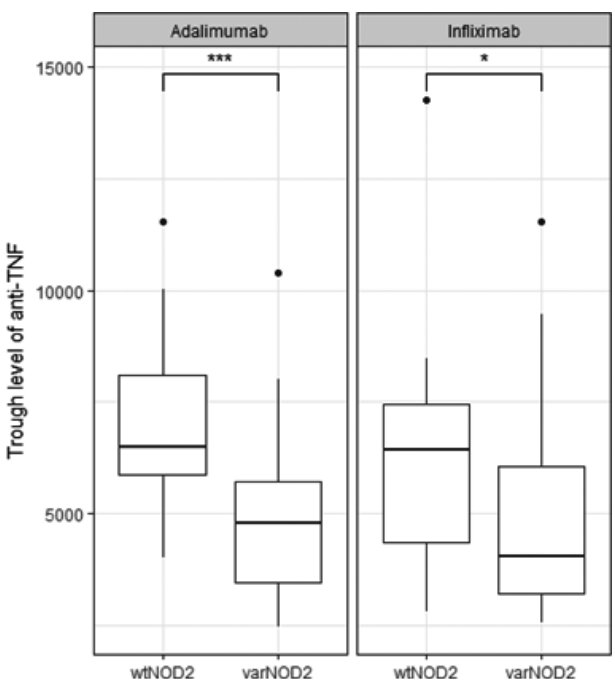

Figure 3.

O017
GENETIC POLYMORPHISMS ASSOCIATED WITH SUSTAINED RESPONSE TO ANTI-TNF DRUGS IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE

Salvador-Martín S.[1], *García M.I.*[1], *Blanco C.*[1], *Viada J.F.*[2], *Navas V.M.*[3], *Magallares L.*[4], *Moreno A.*[5], *Loverdos I.*[6], *Rodríguez A.*[7], *Merino V.*[8], *González R.*[9], *Blanca J.A.*[10], *García R.*[11], *Eizaguirre J.*[12], *Aznal E.*[13], *Sánchez C.*[1], *Tolín M.*[1], *Martínez-Fernández C.*[1], *Manrique S.*[1], *Gil D.*[14], *Sanjurjo M.*[1], *López-Fernández L.A.*[1]

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Introduction: In children, inflammatory bowel disease (IBD) has different and important connotations, such as delayed growth and having to live much longer with a disease that has no cure. However, we have a limited therapeutic arsenal. Thus, identifying markers to optimize treatment with biological therapy is a priority.

Objective: To identify biomarkers in children that predict response to anti-TNFs in the short, medium and long-term, as well as those associated with drug levels.

Material and Methods: Children (n = 107) from 14 hospitals diagnosed with IBD treated with anti-TNFs were incorporated. Eighteen polymorphisms were genotyped in 16 genes related to anti-TNF response in autoimmune diseases.

Results: A 92% of patients with CC genotype for rs1800872 (*IL10*) and a 100% with AA genotype for rs2569190 (*CD14*) responded to anti-TNF treatment after 12 months (P = 0.032 and P = 0.037* respectively). The SNPs *IL17A* rs2275913 and *IL10* rs1800872 were associated with response after 24 months (P = 0.014 and P = 0.049 respectively). The *TLR9* rs352139 (P = 0.026), *IL10* rs1800872 (P = 0.015) and *IL17A* rs2275913 (P = 0.03) were associated with sustained response, some even after more than 5 years of treatment (Fig. 1). SNP *TLR4* rs5030728 was associated with infra-therapeutic levels of anti-TNF drug (P = 0.031).

Conclusions: Markers of response to short, medium, and long-term anti-TNFs were first identified, as well as those associated with drug

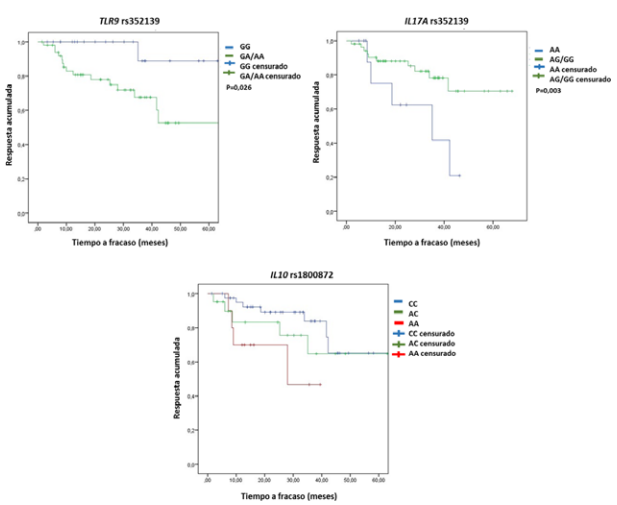

Figure 1. Association of SNPs with long-term response to anti-TNF treatment.

levels in children with IBD. Genotyping of these genetic variants in the pediatric IBD population could be included in clinical guidelines to select the most appropriate therapeutic regimen for these patients.
Keywords: pharmacogenetics, infliximab, adalimumab, crohn's disease, ulcerative colitis.

O018
WHOLE *DPYD* GENE SEQUENCING IN PATIENTS WITH SEVERE CAPECITABINE TOXICITY, IS CURRENT GENETIC SCREENING INSUFFICIENT TO PREVENT IT?

García-González X.[1], *García M.I.*[1], *Blanco C.*[1], *Salvador S.*[1], *Jarama M.*[1], *López-Tarruellas S.*[2], *García-Alfonso P.*[2], *Sanjurjo M.*[1], *Martín M.*[2], *López-Fernández L.A.*[1]

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Several clinical guidelines recommend dihydropyrimidine dehydrogenase genetic screening before starting treatment with capecitabine in order to prevent severe adverse events. However, commonly analysed variants often fail to explain toxicities we see in the clinical setting. In this work, we performed complete sequencing of the *DPYD* gene in 14 patients that developed severe toxicity during capecitabine treatment and did not have any of the 3 mutations we systematically test for in our clinical practice (rs3918290 [DPYD*2A], rs55886062 [DPYD*13], and rs67376798) in order to evaluate the potential impact of other variants in enzyme functionality. We found 15 polymorphisms: 5 potentially pathogenic and/or related to decreased enzyme activity, and 2 potentially affecting splicing. Each patient had a median off 4 polymorphisms (4–6). We discovered a new variant in a splicing site at the end of exon 19 (c.2442+1G>T) that generates a truncated protein with an altered sequence from amino acid 767 to the end (Figure 1). This region contains the fluoropyrimidine binding site and is therefore essential for protein activity. Our work demonstrates that whole *DPYD* gene sequencing, a technique increasingly accessible in clinical practice allows to obtain more complete information to explain the toxicity experienced by some patients. Also, this strategy led us to discover a new variant c.2442+1G>T that clearly alters enzyme activity.

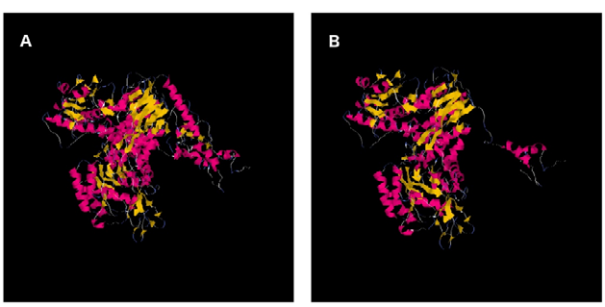

Figure 1 Structures of wild type DPYD (A) and truncated DPYD (B). The interpretation of mutation effect and the molecular modeling were performed by using Deep View Swiss-PDB viewer and Tasser. (A) Modeling of wild type DPYD; (B) Modeling of truncated DPYD.

O019
ABCB1 GENETIC VARIANT AS A RISK FACTOR FOR THE DEVELOPMENT OF DOCETAXEL-INDUCED PERMANENT ALOPECIA IN BREAST CANCER PATIENTS

Nunez-Torres R.[1], *Rodrigo-Faus M.*[1], *Pita G.*[1], *Millán M.M.*[2], *Herraez B.*[1], *Bermejo B.*[3], *Lluch A.*[3], *Martin M.*[2], *Gonzalez-Neira A.*[1]

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Background: Alopecia is a common toxicity of chemotherapy and is considered by the patients as the second worst physical side-effect after nausea and vomiting.[1] This toxicity is associated with conventional doses of chemotherapy and has been traditionally considered to be reversible in all cases upon cessation of therapy.[2] However, in the last years several studies have described a percentage of patients (about 5–10%) suffering permanent alopecia several years after the end of chemotherapy treatment, in particular docetaxel, which is a cause of anxiety and disturbances in these patients.[3] Efforts to understand the mechanism of follicle destruction and to identify strategies for possible prevention are mandatory.
Methods: We performed a genome-wide association study using Illumina Human OmniExpress Beadchip (738 432 SNPs) in 174 breast cancer patients treated for docetaxel, with independent replication in similarly treated cohort of 47 breast cancer patients. Statistical analysis were carried out with PLINK.
Results: Logistic regression analysis in the discovery cohort revealed significant association of a genetic variant of *ABCB1* to docetaxel-induced permanent alopecia (DIPA) phenotype (OR: 4.481, 95% CI: 2.447–8.225, P:1.183e-06). This association was successfully validated in the replication cohort (OR: 2.66, P: 0.023).
Discussion: To our knowledge, to date this is the first study exploring the role of genetic factors in the susceptibility to develop DIPA. ABCB1 is implicated in docetaxel transport outside the cell and it is expressed in human hair follicle stem cells. Further functional studies are necessary to explore the mechanism underline the relationship between *ABCB1* associated genetic variant and the risk of develop DIPA.
Keywords: Breast cancer, alopecia, docetaxel, toxicity, SNP.
References:
[1] Coates A, Abraham S, Kaye SB. *Eur J Cancer Clin oncol,* **1983**, 19 :203–208.
[2] Hood *AF. Med Clin North Am*, **1986**, 70:187–209.
[3] Kluger N, Jacot W, Frouin E. *Ann Oncol* **2012**; 23: 2879–84.