

CONFERENCES

THE HARNESSING OF GLYCOLYSIS AND GLUTAMINOLYSIS FOR CELL PROLIFERATION

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Cell proliferation is accompanied by an increase in the utilization of glucose and glutamine. The proliferative response is dependent on a decrease in the activity of the ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C)-Cdh1 which controls G1- to-S-phase transition by targeting degradation motifs, including the KEN box. This occurs not only in cell cycle proteins but also in the glycolysis-promoting enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isoform 3 (PFKFB3), as we have recently demonstrated in cells in culture as well as in proliferating human T lymphocytes. Moreover, we have found that glutaminase 1 is a substrate for this ubiquitin ligase and appears at the same time as PFKFB3 in proliferating cells. Glutaminase 1 is the first enzyme in glutaminolysis, which converts glutamine to lactate, yielding intermediates for cell proliferation. Thus APC/C-Cdh1 is responsible for the provision not only of glucose but also of glutamine and, as such, accounts for the critical step that links the cell cycle with the metabolic substrates essential for its progression.

TRANSLATIONAL RESEARCH AND DRUG DEVELOPMENT

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Despite the large resources invested by Pharmaceutical companies in R&D, the number of new drugs approved by the regulatory authorities is experiencing a continuous decline. More than ten years ago the deciphering of the human genome was predicted to lead to a therapeutic revolution, which unfortunately has not yet occurred. There are many possible explanations for this situation, but the key fact is that the progress in our scientific understanding has not been translated into an increase in therapeutic innovation. With the recent advancements in predictive early pharmacokinetics and safety, the lack of clinical efficacy has become the major cause of attrition during drug development. This dilemma is "particularly" dramatic for CNS diseases, especially Neurodegenerative diseases. In many therapeutic areas, there is a clear disconnect between the pharmacological activity observed in experimental animal models and lack of therapeutic efficacy in clinical trials. Simplistic interpretations for this poor translation between preclinical and clinical studies have included the lack of validated experimental animal models or poor clinical trial design. However, it is now recognized that the major culprit is the inability to make a scientifically driven translation from experimental animal data to clinical trials. Even the most sophisticated animal models only reflect some aspects of the causes and phenotype of the disease and for many therapeutic areas that are without effective treatments, it is impossible to ascertain the predictability of these models. Despite these limitations, it is important to continue to fully optimize the use of existing models by selecting experimental protocols and pharmacological endpoints that are as close as possible to those used in clinical trials. Moreover, these experimental studies should identify translatable biomarkers (BM) that can be measured in human to better validate the scientifically driven transition between experimental models and clinical trials.

Although, in most cases, activity on BM is not accepted as a proof of clinical efficacy from a regulatory standpoint, these markers are critical to make important drug development decisions. For example, they can be used to demonstrate that a drug is acting on the expected target or pathway (clinical proof of mechanism, POM), that drug engagement with this target modulates processes related to the disease (clinical proof of concept, POC) and for the evaluation of safety and dose selection. In

addition, diagnostic BM can be used to ensure the recruitment of patients that are most likely to benefit from a given therapy. An additional application of BM is to provide secondary endpoints for pivotal trials. These are qualified BM, a term recently coined by EMEA to cover a very limited number of BM for which a close relation with the pathological process has been established and validated measurement technologies are available. These combined applications of BM facilitate the design of clinical trials and provide indicators of drug efficacy/safety that ultimately enable early go/no go decisions, based on the confidence in the possibility of success (POS), before engaging in long lasting and costly late stage pivotal trials. It is noteworthy that the therapeutic areas for which BM have not been available are those for which little or no therapeutic advancement has been made. By contrast, other disease areas in which BM are easier to measure have generated huge therapeutic advancements. However the situation for Neurological diseases is changing thanks to a number of technologies, such as brain imaging and different 'omics' that are making it possible to access body compartments that could not be previously explored. Brain imaging has been successfully used to provide a POC for drugs for multiple sclerosis and is also being actively used for clinical trials in AD and Stroke. In addition, it has been increasingly recognized that the validation of BM requires a scale of effort that can only be realized through large public/private collaborations. In conclusion, in order to reduce the large attrition rate that plagues drug development it is essential to define very early during the drug discovery process a translational research strategy aimed to improve the transition from experimental models to clinical trials. This strategy should include the identification of translatable BM for the validation of experimental animal models and protocols and for use as decision making tools in clinical trials.

OLIVE POLYPHENOLS: FROM THE ALMAZARA TO THERAPEUTIC

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The excellence of virgin olive oil and its benefits for the Mediterranean diet are well known. A great part of its qualities comes from the presence of polyphenolic compounds in the fruit. Olive polyphenols comprise an immense group of compounds: derivatives of cinnamic and benzoic acids (caffeic, vanillic, syringic acids), phenyl acids (3,4-dihydroxyphenylacetic acid), phenyl ethanols (hydroxytyrosol, homovanillic alcohol, tyrosol), secoiridoid derivatives (oleuropein), and flavonoids, among others. These are of great interest, contributing to the organoleptic properties of the fruit and oil. Many of them have been the object of extensive research as disease-preventing agents. Of them, hydroxytyrosol, whether free or as a derivative, is found as a major component in the olive and its products. Specifically, in virgin olive oil (VOO), hydroxytyrosol is found either as the secoiridoid derivatives or as the acetate, appearing as the free form only in a very small amount, given its slight liposolubility. In any case, its presence contributes to the chemical stability of VOO.

In recent years, hydroxytyrosol has been shown to have important biological properties. *In vitro* studies have demonstrated its capacity to reduce atherosclerosis, and to inhibit the oxidation of low-density lipoproteins (LDL) rich in cholesterol. It is able to modulate cyclooxygenase, lipoxigenase, nitric oxide, or NO-synthetase, thereby contributing to palliating thrombogenic and inflammatory processes. In addition, it is able to reduce the production of free radicals, such as the superoxide anion, and therefore has an inhibitory effect on the induction of mutagenic and carcinogenic processes. Currently there are numerous studies attempting to demonstrate how this compound acts in the prevention and treatment of certain diseases. This background suggests the use of hydroxytyrosol as functional component of foodstuffs. This biophenol can be easily puri-

fied, in large amounts and low cost, from olive oil waste waters. However, as hydroxytyrosol is barely soluble in lipid matrices, its use in foodstuffs with high fat content is very limited. For this reason, the search for new lipophilic derivatives with enhanced properties is of great interest.

PHARMACODYNAMIC PROFILE OF HYDROXY-TYROSOL DERIVATIVES IN ISCHEMIA

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Hydroxytyrosol (HT) is the most important and abundant phenolic compound in virgin olive oil. It has been demonstrated that HT exerts antioxidant, antiatherogenic, antimutagenic, anticancer and microbial effects. HT has a powerful free radical scavenger effect of superoxide anion, hydrogen peroxide and hypochlorous acid. It also has a metal chelating effect such as iron and decreases the appearance of oxygen reactive species from derivate reactions with that metal. This feature is the main factor responsible of preventing atherosclerosis effect that is caused because of the entrance of oxidized LDL (oxLDL) within the endothelium. HT antiatherogenic effect seems to be related not only with the inhibition of oxLDL but also with the decrease of adhesion molecules (ICAM-1) expression in endothelial cells. It has been demonstrated an antithrombotic effect because inhibits platelet thromboxane B2 production and stimulates endothelial nitric oxide production. HT probably has anti-inflammatory effect due to an inhibition in COX-2 and iNOS expression in human monocyte cells.

Most of Spanish olive oils, depending on geography areas and olive variety, contain important amounts of HT from oleuropein hydrolysis which is originated of olive ripening procedure, oil storage and preparation procedure of table olives. The relative problem of HT is its high polar nature that cause important amounts of HT in olive mill waste water. This fact together with the rise of functional food has led the exploitation of this natural residue, causing by olive industry with HT purification procedures and synthesis of new derivative compounds, such as esters and ethers, with different alkyl chains with the purpose of increasing HT bioavailability.

We have been demonstrated that HT and HT acetate inhibit antiplatelet activation, cyclooxygenase activity, inflammatory mediators and increase nitric oxide production in human blood samples. We observed the same effect given the treatment by oral administration to experimental animals. Moreover these compounds showed neuroprotective effect in rat brain slices under a hypoxia-reoxygenation model, both in 'in vitro' and after oral administration for 7 days.

Nowadays we are studying HT-ethers effects which do not suffer from hydrolysis and in this way they will accede to the plasma unaltered. With this, they will have access to the organ lipid part having here their effects against oxidative stress. Ethers have shown an antiplatelet effect in *ex vivo* and *in vitro* experiments which action mechanism seems to be the modulating effect of cyclooxygenase inducible activity whether it is directly or regulated by anti-inflammatory mediators. These compounds also have neuroprotective effect in rat brain slices subjected to a hypoxia-reoxygenation model both in 'in vitro' and after oral administration for 7 days. Their neuroprotective mechanism seems to be the sum of their antioxidant and anti-inflammatory effects. It seems to be logical to study the effects that these new compounds have in ischemic process, known their toxicity, bioavailable, the value of their alkyl chains, etc. Nowadays we are studying the possible effects of other HT derivate compounds as nitrocatecols on these mechanisms.

We cannot forget that exist other phenols with bigger molecular weight contained in this residue that shown much more antioxidant capacity in 'in vitro' assays without toxic effects. We will get compounds with biological properties contained in olive mill waste water which can be used in food industries as natural antioxidant compounds in addition to decreasing what until now has been considered a major environmental problem.

THE EFFECT OF RICH-POLYPHENOL OLIVE OIL CONSUMPTION ON CARDIOVASCULAR RISK FACTORS

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Olive oil is the primary source of fat in the Mediterranean Diet. Although a high degree of adherence to the traditional Mediterranean diet has been associated with a reduction in overall and cancer mortality and other diseases such as neurodegenerative, the most impressive benefits of this diet are related to cardiovascular morbidity and mortality. In spite of this, data concerning olive oil consumption and primary end points for disease are scarce. However, there is a large body of knowledge providing evidence of the benefits of olive oil consumption on secondary end points for cardiovascular disease (CVD). According to this, on November 2004, the FDA (Federal Drug Administration, USA) permitted a claim on olive oil labels concerning the benefits on the risk of cardiovascular disease of eating about two tablespoons (23 g) of olive oil daily, due to the mono-unsaturated fat (MUFA) content in olive oil [1]. However, if this effect of olive oil could be attributed solely to its MUFA content, any type of olive oil, rapeseed/canola oil, or a MUFA-enriched fat should provide the same health benefits. So it is doubtful these beneficial effects can be due exclusively to oleic acid. The *minor* components of olive oil, particularly the antioxidant phenolic compounds, can explain part of the benefits associated to virgin olive oil (VOO) consumption. Among olive oils usually present on the market, VOOs produced by direct-press or centrifugation methods have higher phenolic content (150–350 mg/kg of olive oil) compared with other olive oils such as ordinary or pomace olive oils.

Current evidence indicates oxidative damage as a promoter of pathophysiological changes occurring in oxidative stress-associated diseases, such as CVD, cancer, and neurodegenerative disorders, and also in ageing. Oxidized low-density lipoprotein (oxLDL) is currently thought to be more damaging to the arterial wall than native LDL cholesterol. So, oxLDL, commonly used as a marker for oxidative damage, may play a major role in atherosclerosis and CVD [2]. The *in vivo* antioxidant effect of olive oil phenolic compounds were controversial in the human studies performed until 2004 [3]. The results of the EUROLIVE study clarified the point. The EUROLIVE (The effect of olive oil on oxidative damage in European populations) was a randomized, crossover, controlled trial aimed to assess the effect on lipids and oxidative damage of sustained daily doses of similar olive oils, but with differences in their polyphenol content. The study covered 200 healthy male volunteers, aged 20–60 years, recruited in six research centres from five European countries [4]. Participants were randomly assigned to three sequences of daily administration of 25 ml of three olive oils. Olive oils had *low* (2.7 mg/kg of olive oil), *medium* (164 mg/kg), or *high* (366 mg/kg) polyphenol content but were otherwise similar. Intervention periods were 3 weeks preceded by 2-week washout periods. Results of the EUROLIVE study showed a linear increase in high-density lipoprotein (HDL) cholesterol levels and a linear decrease in the total cholesterol/HDL and LDL/HDL ratios, as well as in the lipid oxidative damage with the polyphenol content of the olive oil. Results of the EUROLIVE confirmed that olive oil is more than a MUFA fat. The EUROLIVE results provided evidence to recommend the use of polyphenol-rich olive oil, like VOO, as a source of fat in order to achieve additional benefits against cardiovascular risk factors. On the basis of the EUROLIVE and other of our previous results (e.g. concerning the bioavailability of olive oil polyphenols in humans) the EFSA (European Food Safety Authority) has recently released a claim concerning the benefits of olive oil polyphenols in protecting LDL from oxidation [5].

Anti-inflammatory properties, improvements in endothelial dysfunction and in blood pressure have been also reported for dietary olive oil polyphenols in human studies. Our recent transcriptomic data suggest that one of the mechanisms by which olive oil polyphenols could exert their health benefits is by modulating the expression of atherosclerosis-related genes toward a protective mode.

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MULTIPLE SCLEROSIS: A DISEASE IN TWO PHASES

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Multiple sclerosis (MS) is an autoimmune disease that occurs in genetically predisposed persons over which an environmental agent, of probable infectious nature, induces an altered immune response against myelin antigens, originating a disease characterized by inflammation, demyelination and neurodegeneration at the central nervous system (CNS).

Lesions are located at the perivenous space and distributed typically in some regions (optic nerve, periventricular, subcortical, infratentorial and spinal cord). In those lesions, the damage of the axon isolating myelin sheet, produce a slowing of the conduction of the nerve impulses and eventually a conduction block, originating the clinical symptoms of the disease.

Along the course of the disease, it can be determined a preclinical phase, that is diagnosed occasionally in asymptomatic patients that get performed for another reason a magnetic resonance study (MR). This is called Radiological Isolated Syndrome (RIS).

Later on, the patients have a first manifestation of clinical symptoms typical of a demyelinating disease or Clinically Isolated Syndrome (CIS). Afterwards there are new clinical episodes, entering in a phase called relapsing-remitting MS (RRMS) in which there are clinical exacerbations, or attacks, characterized by neurological symptoms typical of the disease. After a mean of 10 years, at least 50% of the patients evolve to a phase in which there is neurological deterioration, with or without exacerbations, called secondary progressive MS (SPMS). The majority of patients, if untreated, will progress along their lives to this phase, sooner or later.

What happens clinically has its counterpart both pathogenically and pathologically: there is an initial or compensatory period, during the RRMS phase in which the more prominent role is played by the adaptative immune system. This phase is characterized by an altered blood brain barrier (BBB) and the existence of restricted, focal lesions (plaques), localized perivascularly. In this phase there is a good differentiation of oligodendrocyte precursor cells (OPCs) and an elevated remyelinating capability.

When the disease evolves into the more advanced SPMS, there is a phenomenon of compartmentalization of inflammation, and patients enter in a non compensatory phase. In this period the main player is the innate immune system, being very prominent the role of microglial cells. There are two co-existing processes, 'trapped' inflammation surrounding the vessels of the piamater and out of a closed BBB, forming lymphoid follicles of B cells at the Virchow-Robin spaces, and at the same time, there is a diffuse inflammation of the CNS, in which there are many microglial nodules distributed all throughout the CNS, causing the neurodegeneration found in this period.

Hence, the pathological processes occur sequentially as follows: Initially, coincident with the RRMS phase, there is a prominent inflammation, followed by remyelination; at the same time, there is a severe acute axonal loss due to inflammation; both processes diminish along the time. In a posterior period, coincident with the SPMS phase, there is a diffuse axonal loss and cortical demyelination, and both processes increase along the time [1–3].

As the lesions can affect different brain or spinal cord areas, the disease is characterized by its clinical variability. To make a diagnosis of MS there may be dissemination in space (more than one lesion in the CNS)

and dissemination on time (more than a clinical episode). There are new criteria making possible to diagnose the disease in very early periods with the help of MR, which also permit to exclude other confounding conditions.

MS treatment has been very much improved in the last decade. Probably the main advance has been the combination of earlier diagnosis and treatment initiation. Acute episodes (exacerbations, attacks) can be limited in severity and duration with corticosteroids in high dosages, an exceptionally with plasma exchange.

But the most important advance has been the introduction of disease modifying drugs, immunomodulators (interferon beta 1-b, interferon beta 1-a and glatiramer acetate) and immunosuppressants (azathioprine, mitoxantrone, cyclophosphamide, natalizumab, bone marrow transplantation) that can reduce the activity of the disease (number of exacerbations or acute MR lesions) and slow the progression of disability at a statistically significant and clinically relevant level [4].

In the very next future, we will have available new therapies that have finished the period of development in phase III or are close to it. In particular we are expecting to use the new oral agents (fingolimod, laquinimod, teriflunomide) or the new monoclonal antibodies (alemtuzumab, daclizumab, ocrelizumab, ofatumumab) [5].

Nowadays MS can be satisfactorily treated during the first, more inflammatory phase of the disease, because all the available drugs have anti-inflammatory effects. But, as the secondary phase of MS is mostly degenerative, the majority of drugs have little or no efficacy at all. Very much needed are, therefore, therapeutic agents that can induce neuroprotection or even neuroregeneration. Some new drugs are very promising in this respect.

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FINGOLIMOD: A NEW DRUG WITH A NEW MECHANISM OF ACTION

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Multiple Sclerosis (MS) is a chronic autoimmune disabling disease of the central nervous system (CNS) with inflammatory and neurodegenerative components [1]. In the last years, immune-modulatory treatment options of patients with relapsing-remitting MS have improved considerably due to approval of Natalizumab (Tysabri[®]; BiogenIdec/Elan), a recombinant humanized monoclonal antibody directed against the $\alpha 4$ chain of VLA-4 (very late antigen) on the surface of lymphocytes, and most recently of FTY720 (Gilenya[®]; Novartis), a functional antagonist of the sphingosine 1-phosphate (S1P) receptor [2, 3]. These new therapeutics have been proven to be superior to standard immune-modulatory treatment with interferon- $\beta 1a$ in terms of relapse rate, disease progression, and MRI lesion development (AFFIRM, SENTINEL, TRANSFORMS) [4–6]. However, in the case of natalizumab, superiority in efficacy is associated with an increased risk of severe opportunistic infections, especially progressive multifocal leucencephalopathy (PML), which is influenced by the duration of treatment among other factors, suggesting that superiority in efficacy correlates with compromised immune surveillance and hence increased risk of opportunistic infections [7, 8].

Fingolimod (FTY720, Gilenya®), a functional antagonist of the sphingosine 1-phosphate (S1P) receptor family, is the first oral therapy approved for immune-modulatory treatment of highly active multiple sclerosis (MS). The mechanism of action of fingolimod is to retain lymphocytes within peripheral lymphoid organs, thus sequestering rather than destroying immune cells. This sequestration predominantly affects central memory T cells (T_{cm}) and naive (T_n) cells, while effector memory T cells (T_{em}), which are critically involved in immune surveillance are less affected. However, it is not clear whether these gradual effects on immune cell trafficking might also affect effective antiviral immunity during systemic infections, either newly acquired infections or control of latent virus persistence. Moreover, several animal studies suggested that interference with S1P signalling is also involved in the regulation of lymphocyte differentiation and function, which might on the one hand contribute to the desired effects in suppression of autoimmunity, but might on the other hand enhance the immuno-suppressive potential of FTY720 treatment in humans. Hence, identifying changes of immune cell function and monitoring of T cell-specific antiviral responses under FTY720 therapy thus represent an important challenge, which will help to further elucidate the mechanisms of FTY720-mediated immune-modulation during autoimmunity, and to assess immune competence during treatment with FTY720.

FTY720 is a derivative of myriocin, a metabolite of the fungus ascomycete *Isaria sinclairii* [10]. Upon phosphorylation into its active form, it binds to specific S1P receptors, which belong to the family of G protein-coupled receptors [11]. The binding of phosphorylated FTY720 to S1P₁ on lymphocytes results in internalization and degradation of the receptor as well as a reduction in S1P₁ mRNA levels, resulting in functional antagonism [12]. Due to the resulting imbalance between lymphoid homing signals mediated by CCR7 and CD62L, and S1P signalling which is important for immune cell egress from the lymphatic tissue, these cells are trapped in the lymph nodes [13,14]. Hence, functional antagonism of S1P signalling by FTY720 results in a reduction in peripheral lymphocyte counts by inhibiting egress from lymphatic tissue in the peripheral circulation, including potentially encephalitogenic T cells. It is assumed that this reduction in encephalitogenic T cells in the periphery does in turn limit auto-reactive T cell invasion into the CNS as crucial mechanism-of-action of FTY720 in MS patients. Accordingly, in the animal model of MS, experimental autoimmune encephalomyelitis (EAE), FTY720 treatment not only ameliorated clinical and histopathological features of the disease, but also interfered with immune cell infiltration into the CNS of these animals [15]. In mice, during the last years several other effects of S1P signalling on immune cell functions have been characterized: For example, positive S1P signalling induces antigen-specific IL-17 producing helper T (T_H17) cells, whereas FTY720 interfered with T_H17 differentiation [16,17]. Another group has demonstrated that treatment of mice with FTY720 results in enhanced differentiation of regulatory T cells while restraining the development of T_H1 cells. These studies are especially interesting in light of the current view that regulatory T cells are important for control of autoimmunity, whereas both T_H1 cells and T_H17 cells are crucial for development of autoimmunity [18]. In humans, an effect on the frequency of T_H17 central memory T cells in the peripheral blood has been recently described; however, it is completely unclear whether FTY720 treatment in humans alters the capability of naive CD4⁺ T cells to differentiate into distinct T helper cell subsets, as the decrease in T_H17 central memory cells in the circulation might be due to pronounced retention of this population in the lymphatic tissue [19].

Further to this, FTY720 is considered to exert significant direct actions within the CNS, thereby influencing the neurobiological components of MS (putatively independent of the immunoregulatory effects).

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ALTERACIONES VASCULARES EN LA DIABETES Y SU PREVENCIÓN POR FÁRMACOS

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Diabetes is associated with a perturbation of signalling pathways in vascular tissue, which causes vasomotor dysfunction such as hypertension, accelerated development of atherosclerosis, and microcirculatory disorders. The latter conditions may lead to retinopathy, nephropathy, and gangrene, which are common complications of diabetes. Although blood vessels are not classic targets of insulin signaling, insulin is known as a vasodilator and an apoptosis and mitogenic regulator. Insulin signals through the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, which increases activity and expression of endothelial nitric oxide synthase (eNOS) and regulates a variety of cellular activities in endothelial and smooth muscle cells [1]. For this reason, chronic insulin PI3K/Akt signaling deficiency could affect vasomotor mechanisms of blood vessels. During the last 10–15 years, the investigation of vasomotor dysfunction in diabetes has been carried out extensively in animal models, primarily streptozotocin-treated rats and alloxan-treated rabbits. Although the data

on contractile responsiveness are controversial and depend on the type and duration of the disease, endothelial dysfunction based on nitric oxide (NO) deficiency has been well established. Exaggerated production of vasoconstrictors and elevated myogenic response of small arteries have also been shown. Also, an emerging body of evidence suggests that vascular remodeling in diabetic patients involves a perturbation of the balance between cell proliferation and cell death and this misbalance could contribute to the vasomotor dysfunction. The results obtained in animal studies, however, need to be validated in humans, especially since the recent discovery of significant divergence in vasomotor mechanisms between human and animal blood vessels

Inflammatory products have been implicated in the pathogenesis of several inflammatory diseases. However, their role in diabetic vascular disease is unclear. Overproduction of COX-2 in tumour cells has been implicated in the resistance to apoptosis as it is produced simultaneously with the anti-apoptotic protein B cell lymphoma 2 (Bcl2). We first evaluated the role of COX-2 in CRP-induced apoptosis in vascular smooth muscle cells (VSMC) from non-diabetic patients and subsequently we investigated whether overproduction of basal levels of COX-2 might be implicated in the resistance to induced apoptosis in VSMC from diabetic patients.

We investigated the effects of pioglitazone, a PPAR- γ agonist, on the apoptosis and expression of COX-2 on diabetic patients.

Internal mammary arteries (IMAs) were collected from a total of 176 patients (aged 35–80 years) undergoing coronary artery bypass grafting at St. Paul's Hospital (Vancouver) and Cardiac Surgery Service, Hospital Clínico Universitario San Carlos, Madrid, Spain

Our data show that diabetes adversely affects multiple cellular mechanisms in human arteries, thus causing vasomotor dysfunction (failure of relaxation is the attenuation of the overall NO production due to reduction in eNOS mRNA and protein expression). Stimulation of diabetic arteries resulted in augmented vasoconstriction, due to both endothelial dysfunction and increased contractility of smooth muscle cells. Increased contractile activity may be related to abnormal intracellular Ca^{2+} metabolism, which is associated with the diabetic state. Alternatively, sensitization to Ca^{2+} through a Rho-kinase pathway, which is a feature of excitation-contraction coupling in human vessels, may contribute to elevated vasoconstriction in diabetes [2].

In isolated arteries and (VSMCs) of diabetic patients are upregulation of the antiapoptotic protein Bcl-2, increased resistance to apoptosis, increased VSMC proliferation, and enhanced media thickness. We have found an increase in Bcl-2 expression in the media layer as well as in VSMCs obtained from diabetic patients

Overproduction of cyclo-oxygenase-2 (COX-2) is involved in the resistance to apoptosis in vascular smooth muscle cells from diabetic patients: a link between inflammation (COX-2) and apoptosis (Bcl2). This relationship is not causative and the common production of these two proteins may be co-regulated by shared regulatory elements in diabetes [3].

Pioglitazone (100 μ M) induced apoptosis in human VSMC from diabetic and nondiabetic patients, analyzed by DNA fragmentation and by degradation of Bcl-2, in high-glucose-containing medium (15, 25 mM). This effect of pioglitazone might contribute in the treatment of alterations of vascular remodeling in DMII [4].

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NEW TREATMENTS FOR TYPE 2 DIABETES: FROM PIPELINE TO PATIENT SIDE

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Despite treatment with current oral glucose-lowering agents, most patients with type 2 diabetes experience a gradual loss of glycaemic control, finally leading to treatment failure. Irrespective of the degree of insulin resistance, the progressive loss of beta-cell function and mass are the ultimately responsible for the development of hyperglycaemia. The cause of beta-cell dysfunction are multifactorial, including functional and reversible toxicity caused by elevated glucose and/or lipid levels, increased secretory demand because of insulin resistance, amyloid deposition, altered levels of cytokines, increased beta-cell apoptosis which at the end encompasses a loss of beta-cell mass and lately recognized, a defective incretin action. Pharmacologic research in the last years and currently is trying to cover all or most of well characterized functional defects relevant for beta-cell failure.

From a clinical point of view, the ideal antidiabetic drug would require to have the following characteristics: (i) long durability of glucose control (efficacy); (ii) to delay or prevent loss of beta-cell function; (iii) to increase beta-cell mass and function; (iv) to provide an optimized control of postprandial glucose state; (v) do not cause facilitate weight gain, and (vi) to decrease cardiovascular risk by acting upon metabolic syndrome risk factors; and least but not last an ideal antidiabetic compound would also have actions that directly affect the progression of diabetic complications.

The efforts towards obtaining an ideal and single antidiabetic agent are elusive as the mechanisms by which the type 2 diabetic syndrome develops are multiple and the possibility that a single compound could cover all the biological targets by which this syndromic entity could be controlled seems not possible. Thus, it is warranted that the therapeutic design for a given patient would require a combination of drugs acting through the mechanisms responsible for defective beta-cell function in this given patient. Ideally, when the biological basis of type 2 diabetes would be recognised for a specific patient and the genetic markers would also be available, a la carte treatment may be possible. This may require some more years of research, and by now no genetic markers are enough potent to be useful on an individual basis. In the meanwhile, research aiming at improving the diabetologist armamentarium for our patients is pointing towards the following fields of interest: (i) ligands to G-protein-coupled receptors improving glucose-stimulated insulin secretion; (ii) fatty acid-binding receptors; (iii) glucokinase activators; (iv) gluconeogenesis inhibition: fructose-1,6-bisphosphatase inhibitors; (v) promotion of glucose excretion into the urine: sodium glucose transport inhibitors; (vi) glucagon antagonists and glucagon receptor blockers; (vii) dual GLP-1/glucagon agonists; (viii) diacylglycerol acyltransferase inhibitors, and finally (ix) new insulins with longer acting action that those currently available.

Emerging evidence from interventional trials suggests that both intensive lifestyle changes and pharmacotherapy can delay or possibly prevent the onset of type 2 diabetes in high-risk individuals. For patients newly diagnosed with type 2 diabetes, early and intensive intervention strategies that combine maximal glucose-lowering efficacy alongside potential beta-cell preserving properties may provide an opportunity to delay or prevent progression of the disease. Moreover, as shown with the long time reevaluation of individuals included in intensive glucose control trials, the earlier glucose control is achieved after diagnosis, the higher are the benefits in terms of development of vascular complications at long term, indicating a metabolic memory or legacy effect of this early metabolic control. Preservation of beta-cell function is now gaining recognition as a critical target in the management of type 2 diabetes. For patients with overt type 2 diabetes, preservation of beta-cell function has the potential to reduce or stabilise the progression towards the necessity of implementing insulin substitution treatment. In this regard, very promising data from the long term follow-up of individuals treated with GLP-1 receptors agonists and GLP-1 based therapies are now coming to the light.

Beyond glucose control: It should be noted that despite all the preventative therapies available, numerous patients will still develop some of the disabling and immensely expensive end-stage complications of diabetes. Owing to advances in genomics and translational medicine, new targets for retinopathy, nephropathy and neuropathy will be validated and investigated in the near future. We forecast that exciting pharmacological targets will become validated and agents will enter early human proof-of-concept trials.

A BRIEF HISTORICAL REVIEW OF NEW TECHNOLOGIES IN THE TEACHING OF PHARMACOLOGY

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Educational technology is the art or science of applying scientific knowledge to education practical problems.

New technologies have been used in the teaching of Pharmacology throughout the ages. Although we have no-exact knowledge about it, the very early Pharmacology's teaching tools were the pre-writing symbols petroglyphs drawn on caves walls by prehistoric peoples. The oldest post-Palaeolithic primitive drugs-related cave-painting is in Bullon cave (Villar del Humo, Cuenca, Spain), a Psilocybe-hispanica (hallucinogenic mushroom) representation, and the oldest cave-paintings depicting a primitive Neolithic-Pharmacology teacher, a Shaman, is in Bradshaw cave (North Kimberley coast, Australia, 10,000 BC).

The ideographs, pictographs and hieroglyphics written on the temples and pantheons' walls developed by Sumerian, Egyptian, Chinese and Pre-Columbian allowed transmitting and preserving knowledge about drugs for the student. But these 'help-class materials' were fixed on large structures and student may only access to them with their teachers and they must study them inside hidden and religious enclosures. The development of new writing-surfaces such as clay tablets, papyrus, bones, tortoise shell, animal tanned skin, bamboo slips or silk in which were possible to drawn with a pen (sharpened reed stylus, feather) made the writing quicker and easier for teachers and students and allowed the knowledge diffusion to other schools and countries. Pictographs, hieroglyphics and cuneiform signs were replaced by sonograms and syllabic alphabet signs making the Teaching-Learning process much easier to understand and allow the Pharmacology's-knowledge translation to different languages. The Code of Hammurabi (Babylon, 2000 BC), the Edwin Smith papyrus (Egypt, 2700 BC), the Corpus Hippocraticum (Hippocrates, Greece, 460–370 BC), the Shang Han Bin Lun (Zhang Zhong Jing, 115–218 BC), De Universa Medicina (Dioscorides, Rome, 40–90 AC) and Contraria Contrariis Curantur (Galeno, Rome, 135–201 AC) are some of Pharmacology treatises used to Teach-Learn Pharmacology in the old ages.

The 9th to 19th Centuries brought us new teaching scenarios changing the setting to teach Pharmacology: the firsts Universities were born (Bologna, 1088, Oxford, 1096, Salamanca, 1134); appeared the teaching hospitals (El Hôtel-Dieu, Paris, 651). New technologies were developed to facilitate research, diagnosis, drugs' use and new drugs development. But the Pharmacology teaching was still based on direct knowledge transmission by the teacher to the student and the books' use.

The paper invention (Tsi Lun, China, 105 BC) allows making books thin, much more easily to copy and transport, and lower cost. Books made of paper were the Canon (Avicenna, 980–1037 AC), the Treatise on Poisons and Their Antidotes and the Glossary of Drug Names (Maimonides, 1135–1204 AC). A crucial milestone was the invention of metal types printing press (Gutenberg, Germany, 1450) accelerating the copying process, and greatly reducing costs allowing closer to a larger number of students. The Pharmacology treatises firsts press printed (1537) were the Syruporum Universa Ratio (Miguel Servet) and the Dioscorides (Ruel's translation).

Traditional ideas dominated long after that and the Pharmacology teaching remained longitudinal and based on face-to-face teaching and books' use. But voice like those of Paracelsus (1493–1541) try to change the

system denouncing that all medical teaching must be based on experiment and experience (evidence-based medicine).

In the 20th to 21th centuries teaching technology has grown exponentially. Problem-based learning became a Pharmacology teaching method in the mid-1960s (Mc-Master University, Ontario). In the 1970s appeared the computer-based learning (CBL) as a simplified form of the actual e-Learning. The preferred technologies were smaller chunks of text augmented with graphics and multimedia presentation, and hypertext through the World-Wide-Web (www) and e-mail. CBL focused on student/computer drills interaction and usually means self-study learning. Popular-movies became a Pharmacology teaching method in 1993 (Koren, Awakenings, 1993).

The idea that people can learn through aural and visual reception prevailing nowadays. It involves the learning based in computer-mediated students-teachers communication. New teaching supports are PC, CD-ROM, DVDs, Web-based platform (Moodle, Neighborhood, Web-CT), video clips, PDAs, iPhones, or tablets. And new teaching methodologies including the Information and Communication Technologies (ICTs) as an important complement to traditional teaching model, or even as unique Teaching-Learning methodology. ICTs involves power-point presentations with voice-over or hypertext, internet (Google, PubMed, YouTube), blogs, pharmacology e-books, class 2.0 (files, podcast, wikis, forum, chat, e-files, seminary, questioner...), computers generated imageries, video modelling, virtual simulations, games, scientific movies and animations, high-fidelity simulations, avatars, second life, between others.

Traditional teaching-model based on the teacher just directing the students through the lecture has been changed to a new much more versatile model in which the teacher directs-accompanies to the students in the learning-way and students play a key-role and constantly interact with their teachers and peers. This allows: rapid transmission of knowledge and permit update and assessment and self-assessment almost instantly, classroom enhancement, develop self-directed learning habits, identify the own knowledge gaps, permit no-risk practices, full-time distance teaching, cooperative and integrated learning using blended learning-in-context scenarios integrating school and authentic settings, and achieve and ensure the student high-quality learning evidence-based.

TEACHING AND LEARNING WITH VIRTUAL CAMPUS TOOLS: THE VIRTUAL PHARMACOLOGY CLASSROOM 2.0

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Virtual learning environments, as those available in many virtual universities, permit us to explore new educational strategies which are non-strictly based in a face-to-face relation between teachers and students. From 2008, we have adopted a blended learning method with small groups of volunteer students enrolled in our 'Pharmacology, Pharmacy and Therapeutics' subject with the support of a *Virtual Classroom of Pharmacology* developed using Moodle [1]. Blended Learning o b-learning strategy combines face-to-face instruction with computer-mediated activities.

One of the pillars of our system is to promote a more active role of the students in their own learning. For it, different activities are proposed each year to create original materials inspired by a collaborative learning. Thus, students are actively involved in the creation of educational materials to be employed during the b-learning experience. These materials are stored for future students creating an educational resources repository hosted in our institutional web under the *Virtual Classroom of Pharmacology*.

A natural evolution of this tool, pursuing to reinforce this collaborative teaching philosophy is to adopt new tools corresponding to the known as web 2.0. Different web-based tools (as wikis, blogs or podcasts) have permitted that web pages becoming transformed in platforms where collaborative work and active communication is possible allowing everyday users to add, edit or delete materials in a web space without specialized technical skills.

The pros and cons of these 2.0 tools for educational purposes next to our experience in the teaching of Pharmacology will be presented.

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HIGH-FIDELITY SIMULATION TECHNOLOGIES FOR PHARMACOLOGY EDUCATION

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Healthcare professional education in the last 10–15 years has witnessed a significant increase in the use of simulation technology for teaching and assessment. Multiple factors likely have contributed to this evolution, including: changes in healthcare delivery and academic environments that limit not only patient availability as learning opportunities, but also trainee work hours and time for clinical faculty to teach; worldwide attention focused on the problem of medical errors and the need to improve patient safety; and the paradigm shift to outcomes-based education with its requirements for evaluation and demonstration of competence. The use of simulators addresses many of these issues: they can be readily available at any time and can reproduce a wide variety of clinical conditions and situations on demand. In lieu of the customary ‘see one, do one’ approach, whereby novices carry out the practice required to master various techniques – including medication administration – on real patients (where the consequences of mistakes can be grave), simulation-based education allows trainees to hone their skills, including those needed to manage rare and/or critical situations, without risk of punishment or harm to patients. For the assessment of professional competence, simulators – by virtue of their fidelity, reproducibility, and programmability – provide a standardized testing experience, which is especially important when utilized for high-stakes determinations, such as specialty certification or licensing.

Medical simulations, in general, aim to imitate real patients, anatomic regions, or clinical tasks, and/or to mirror the real life circumstances in which medical services are rendered. Our discussion here may use the term *simulation*, which in its broad sense includes any approximation of actual clinical situations (such as standardized patient [SP] encounters), but we will focus more narrowly on *simulators*, referring to particular simulation devices. These can take many forms and span the range from low- to high-fidelity, including part task trainers, virtual reality simulators, and computer-enhanced mannequins. Part task trainers consist of 3-D representations of body parts/regions with functional anatomy for teaching and evaluating particular skills, such as plastic arms for venipuncture or suturing. In most cases, the interface with the user is passive (i.e. the device is examined, or procedures are performed on it, with little more than rudimentary responses from the simulator). Although more sophisticated part task trainers may contain computerized or even virtual reality components, we nonetheless distinguish them from computer-enhanced mannequins (CEMs) because the latter reproduce not only the anatomy, but also normal and pathophysiologic functions. For this reason, to the extent that pharmacology education has embraced use of these technologies, it has chiefly involved this last category of simulators. With CEMs the interface with the user is more often active or even interactive, such that simulator responses will vary according to user actions (for example, heart rate and blood pressure will change appropriately depending on the dose of a particular drug administered intravenously). Moreover, high-fidelity mannequins are commercially available that connect to actual anesthesia machines, and vital signs and other clinical parameters will vary appropriately as the concentration of anesthetic gases are adjusted or other medications are administered.

The programming of these simulators obviously involves sophisticated computer algorithms and pharmaco-physiologic modeling, but when incorporated into hands-on activity with the mannequins in a simulation scenario, learning of these otherwise ‘dry concepts’ is no longer passive (as in the lecture hall), but becomes a highly interactive process. More-

over, the increased relevance of seeing these pharmacology principles ‘come to life’ in the context of (simulated) patient care enhances learner satisfaction and knowledge retention. This is a fine example of the recent trend toward vertical curriculum integration: breaking down the traditional separation between the (earlier) basic science and (later) clinical phases of medical school training. Earlier attempts to increase interactivity of pharmacology education with ‘wet lab’ experiences using animals as patient substitutes faced challenges in terms of realism (imperfect modeling of human physiology and obvious differences in physical appearance) as well as ethical issues (animal welfare); the use of simulators largely circumvents these problems. As a result, these technologies are coming into greater use for pharmacology education not only in medical schools, but also across multiple healthcare professions (e.g. nursing and veterinary medicine) and at postgraduate and higher learner levels.

THE USE OF MEDICAL AVATARS IN PHARMACOLOGY EDUCATION

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Medical avatars are digital representations of patients or professionals for health care purposes. In learning environments, these animated, life-like digital characters can be embedded in computer-based instructional systems to facilitate social interaction and learning. The successful use of avatars in computer instruction is largely explained by the media equation theory, which suggests that people interface with and respond to media as if they were human and capable of social interaction. Medical avatars can be used to create an animated, personal, and more engaging encounter for both health care professionals and patients. The addition of avatars to health care education over the Internet introduces advantages in terms of better motivation and persuasion for behavior change. Medical avatars embedded in online training offers an alternative to face-to-face interventions, merging ease of access and lower long-term cost while engaging participants and sustaining interest. For health care professionals, time and logistic constraints often preclude them from receiving useful feedback and adequate supervision. It is difficult to expose the learner to an adequate number and case mix of actual patients for achieving an acceptable level of competence. A strategy that incorporates online simulations of patient encounters and targeted feedback from an avatar mentor built into the simulation can help physicians develop competency in pharmacological management through deliberate practice. For patients, medications are the most common therapies and components of chronic disease self-management. Using avatar coaches to help patients achieve competence in medication management may improve their long-term adherence to pharmacological management and result in better clinical outcomes. Dr Ruiz will present two applications of medical avatars in pharmacology education – one for the health care professional and the other for the patient.

Educating primary care physicians in pharmacological management of smoking cessation – an Avatar-Mediated CME program: Traditional CME improves physicians’ knowledge, but has failed to change physicians’ behaviors. New CME modalities created with innovative educational technologies foster active learning and may be educationally efficient and effective approaches for improving the smoking cessation practices of the primary care physician (PCP). Dr Ruiz will present the design and development of a CME program for PCPs consisting of tutorials, interactive problem-solving exercises, and game-based simulations with debriefing conducted by pedagogical avatars. The goal of this program is to improve PCPs’ skills in encouraging smoking cessation with special emphasis on pharmacologic therapies. A pedagogical avatar enables the PCP learner to review his or her interactions with virtual patients, evaluating the PCP’s actions and receiving feedback on how to improve future real-world clinical experiences with smokers. A needs assessment to ascertain existing PCP knowledge and practices in smok-

ing cessation will provide an educational foundation for the training of PCPs. We have defined specific goals and objectives (competencies) for PCP education in smoking cessation, in a process that matches educational strategy to the identified PCP competencies. Evaluation of the educational impact of the avatar-mediated CME program will occur within the Moore's framework for educational outcomes. PCPs will first complete online pretests and then complete the avatar-mediated CME program over an eight-week period. Immediately after finishing the CME program and at 12 weeks later, the PCPs will complete online posttests.

Educating patients about medications for diabetes mellitus – the avatar-based virtual teach-back: The 'live' teach-back technique confirms that a health message given to an individual is understood by the individual. In this method, the clinician explains a new concept to the patient via an interactive communication loop. After the explanation, the professional verifies the patient's comprehension by asking the patient to repeat the explanation in his or her own words. As currently implemented, the teach-back technique is usually executed 'live' by a health care professional during a face-to-face encounter. The evidence for the efficacy of teach-back comes from a few studies. We have developed a 'virtual' teach-back technique (vT-B) that overcomes many of the limitations of the 'live' form, such as lack of standardization and excessive demand on staff time. The vT-B takes advantage of developments in computer science, gaming, multimedia e-learning, and communications research. In the vT-B we automate the process by replacing two elements of the live technique: we replace the health care professional's explanation with a computer-delivered software explanation or, in some instances, with a static or animated human avatar; for the patient's verbal, open-ended explanation, we substitute a menu of options that includes a single best answer, an incorrect option, and an option requesting that the information be repeated. All statements are written at a 6th-grade reading level. Dr. Ruiz will present data on the efficacy of different features of the vT-B (text, voice, static and dynamic avatars) for improving patients' recall of drug information (metformin and sitagliptin). He will also present correlations of recall data with psychological constructs of attention, emotional valence, cognitive load, and arousal obtained with psychophysiological measurements (heart variability, electrodermal response, facial electromyography, and pupillary dilatation).

NEW CONCEPTS IN ARTHROSIS

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Osteoarthritis (OA) is characterized by degeneration of the hyaline cartilage, modifications in the synovial tissue and subchondral bone and bony overgrowth, osteophytes. The structural and biomechanical modifications of the OA joint may have an impact on the behaviour and possibly on the structure of the periarticular tissues such as ligament or tendon that can be considered as a part of the disease. This entire joint disease finally produce pain and functional impairment.

A challenge in the OA clinical trials is to know what is the most relevant feature as well sensitive to change, to monitor and how to do it. Serum biomarkers have not been established to follow up patients with OA. Until now, clinical signs and symptoms, conventional radiography (CR) to confirm the structural changes, and algofunctional scores has been used to evaluate patients in clinical trials with varying results. It is important to consider that the cartilage is not well seen in CR and the has not pain fibers.

High resolution Muskuloskeletal Ultrasound (US) is an emerging image technique that inform about soft tissues including cartilage, fibrocartilage, synovial tissue, tendon, ligament and muscle.

Degeneration of the cartilage is the most representative structural change in the OA joint. This abnormality can be demonstrated by US. Healthy cartilage is homogeneous and anechoic or hypoechoic with pristine edges. The US appearance of the upper chondro-synovial interface is thinner than the osteochondral interface. The most relevant US features

of degeneration include loss of a defined chondro-synovial and osteochondral interface, loss of uniformity of the anechoic or hypoechoic cartilage band and asymmetric narrowing of the cartilage. Although no single feature of degeneration of OA cartilage is more significant than the others for the evaluation of cartilage damage by US, blurring of the chondro-synovial interface is considered an early sign of OA, whereas, narrowing of the cartilage as well as the irregularities of the osteochondral interface are indicators of long-standing disease.

A number of studies have addressed the validity of the measurement of the cartilage of the femoral condyle. Using cadaveric or perioperative cartilage as a gold standard, there is a good correlation between the actual thickness measured and the US measurement. In comparison with magnified CR, one group found US to be a valid tool for measuring cartilage thickness and with their scoring system they were able to show that reduced cartilage thickness by US allowed discrimination of early symptomatic OA versus early RA and healthy joints.

Inflammation of the synovium is another relevant feature in patients with OA. The histologic appearance of synovitis differs in the early and late stages. US has been shown to be more sensitive and specific in detecting knee synovitis when compared to clinical examination and is equivalent to magnetic resonance imaging (MRI). The OMERACT consensus US definitions of the two components of synovitis, synovial effusion and synovial hypertrophy, which were devised for the metacarpophalangeal joint in rheumatoid arthritis has been applied to other joints and other forms of arthritis including OA. Synovial hypertrophy is defined as the presence of thickened intra-articular tissue that is non displaceable and poorly compressible and that may exhibit a Doppler signal (indicative of increased blood flow in the microvasculature), whereas, synovial effusion is defined as an abnormal hypoechoic or anechoic intra-articular material that is displaceable and compressible and does not exhibit a Doppler signal.

The synovitis of OA appears to differ from that of RA in some important aspects. Although less frequent than in rheumatoid arthritis, OA shows evidence of angiogenesis and inflammation. Furthermore, the relationship between hypoxia in the joint cavity and US-detected synovial proliferation (SP) was investigated in both OA and in RA patients by analysing intra-articular IL-8 which is known to be up-regulated by hypoxia, pO₂ and white cell counts. RA patients showed significant higher levels of IL-8 and white blood cell counts and decreased pO₂ than in patients with OA.

In US, as with other imaging techniques, the knee has been the most commonly evaluated joint in OA patients, with joint effusion and synovial hypertrophy being the most commonly evaluated pathologies. Synovitis detected with US is a common finding in patients with knee OA ranging from 50% to 100% depending on the study.

The utilization of Power Doppler (PD) in the diagnosis of synovitis is well known. In OA of the knee and hip, the validity of PD was studied using histology as a gold standard and was shown to be a valid and reliable diagnostic method for qualitative grading of the vascularisation of the synovial tissue.

The synovitis both of erosive and non-erosive OA of the hand has been the focus of a number of recent studies. Although a number of limitations such as the small size of the distal interphalangeal joint, the complex anatomy of this region and multiple potential vascular and non-vascular artifacts complicates these investigations.

There is evidence that synovitis detected either by US or contrast enhanced MRI can be correlated with pain severity in patients with knee OA. Since cartilage, the primary location of pathology in OA, lacks pain fibers, US is potential a valuable tool for further evaluation of the pathophysiology of OA pain.

Knee effusion, as seen by US, appears be an independent predictor of the need of joint replacement in OA patients linking synovitis to progressive structural damage. Thus, there is a compelling rationale to assess the role of US in detecting inflammatory activity in the longitudinal evaluation of OA.

With US is also possible to visualize the bony cortex and its modifications. US can be consider as a promising technique to evaluate the OA joints, facilitating us repeated follow up examinations thus, monitoring disease activity in OA patients. Cost and availability, that can limit the use of magnetic resonance imaging (MRI) and guide procedures are additional advantages of the US technique.

TREATMENT OF ARTHROSIS: THE PHARMACOLOGIST'S POINT OF VIEW

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Osteoarthritis (OA) of the knee is a very frequent disorder involving more than 50% of the individuals with more than 65 years. According to the 2010 OARSI recommendations, optimal management of OA requires the combination of non-pharmacological and pharmacological measures. Non-pharmacological measures comprise information and education, loss of weight, exercises and/or physical therapy, walking aids, including appropriate footwear and insole, thermal modalities, transcutaneous electrical nerve stimulation and acupuncture.

Pharmacological measures include paracetamol, up to 4 g/day for mild to moderate pain, chondroitin sulfate and/or glucosamine, nonsteroidal anti-inflammatory drugs, non-specific (NSAIDs) and COX2 selective, at the lowest effective dose and for the shortest period, topical NSAIDs and capsaicin as adjunctives, and weak and strong opioids. Intraarticular injections of corticosteroids and hyaluronate may be used in patients with moderate to severe pain not responding to oral treatment.

The incidence of OA increases with aging, gender (especially in women), overweight, heritability and genetics (the heritable proportion of knee OA is $\leq 30\%$ associated with six genes on chromosome 7q220), occupational use, leisure time physical activities, smoking habits, and presence of limb malalignment across the joint. Among these risk factors, aging and overweight are also associated with other pathologies, such as cardiovascular diseases, e.g. hypertension, coronary artery disease, stroke, and congestive heart failure, metabolic diseases, e.g. metabolic syndrome, type 2 diabetes, dyslipidemia, hyperuricemia and gout, and cancer, e.g. of the colon, breast and uterus. Moreover, with aging, renal function may decrease. As a consequence, patients with OA are commonly exposed to many drugs, among which, acidic drugs are frequent. In elderly patients with OA and other diseases, the benefit over risk ratio of the treatment of OA is low because the risks are elevated. Effectively, in these patients, the incidence of drug adverse effects is increased, such as peptic ulcer disease (PUD) and bleeding. The use of NSAIDs will increase the morbidity of gastrointestinal adverse effects ranging from nausea and dyspepsia (50–60% of patients) to endoscopy-documented peptic ulceration (15–30% of individuals), complicated by bleeding or perforation in as many of 1.5% users per year. The association of proton pump inhibitors shall reduce the incidence of NSAIDs-induced PUD but will introduce other risks such as bone fragility, infections and may increase the risk for cardiovascular death, myocardial infarction, and stroke [1].

Intake of paracetamol and NSAIDs for more than 22 days/month increase the relative risk of myocardial infarction and stroke by 35% and 44%, respectively [2]. NSAIDs can raise blood pressure, antagonize the effect of antihypertensive drugs, and increase hypertension-related morbidity, e.g. NSAIDs are linked to approximately 20% of hospital admissions for congestive heart failure exacerbation. Renal effects of NSAIDs include hyperkalemia, sodium retention, decreased glomerular filtration rate, acute renal failure, nephrotic syndrome with acute interstitial nephritis, and renal papillary necrosis. However, serious renal adverse effects associated with the use of NSAIDs is estimated to be 1–5% of exposed patients, but it can reach 20% for patients at risk due to co-morbidities [3].

In addition, the risks of treating patients with OA are increased by kinetic and dynamic drug-drug interactions. For instance, being acidic drugs, NSAIDs may affect the kinetics of oral hypoglycemic agents at the level of membrane protein carriers, plasma protein binding, and drug biotransformation by CYP2C9. Paracetamol administered to patients treated with warfarin enhances the INR and may increase the risk of bleeding. Compared with the risk of gastrointestinal bleeding of clopidogrel or warfarin alone (adjusted ratio rates (aRR) of 1.67 and 1.94, respectively), concomitant administration of clopidogrel or warfarin and NSAIDs increases the aRR of gastrointestinal bleeding to 2.93 and 4.60, respectively [4].

In order to optimize the benefit to risk ratio in patients with OA, the treatment needs to be individualized, not only taking into account the pharmacological properties of the drugs to be used, but also the specific

conditions of the patients, e.g. age, gender, other pathologies associated and the drugs taken. As a general principle, for patients with OA and other risk factors, it may be wise to start the treatment of OA with measures entailing low risks, such as non-pharmacological approaches, and chondroitin sulfate and glucosamine. The addition of paracetamol and/or NSAIDs will depend upon the severity of the pain and always at the lowest doses possible and for the shortest periods of time. In presence of increased cardiovascular risk, naproxen may be safer than other NSAIDs, but this drug may raise blood pressure. Opiates and intraarticular treatments may be used, when the former steps are insufficient to improve knee function and quality of life. Finally, when all these approaches fail, surgery has to be considered.

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OSTEOARTHRITIS AND MUSCULOSKELETAL PAIN: NEW TARGETS, NEW CANDIDATES?

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Chronic pain treatment represents a continuous challenge due to the important physiological changes that occur during the evolution of the disease. Those changes affect to the autonomous system, the hypothalamo-pituitary-adrenal axis or the immune system among others. Most of the latest discoveries deal with the immune system (central and peripheral) and its involvement in neuropathic pain, although most of these changes have been recently described also in other forms of chronic pain, since chronic pain induces changes similar to those described in neuropathic situations.

Osteoarthritis (OA) has been classically described as an example of nociceptive pain. However recent publications have shown that among patients with chronic symptomatic OA, around one-quarter had neuropathic pain symptoms [1]. Indeed, in a rat model of OA, electrophysiological changes in A β non-nociceptive primary sensory neurons, consistent with observations in models of peripheral neuropathy but not models of peripheral inflammation, have been reported [2].

Even though the participation of the immune system in chronic pain (neuropathic and nociceptive) is known for more than a decade [3], much of the recent work has focused in the role played by glial cells (mainly microglia and astroglia) and receptors present in their membranes, for example TLR4 [4] or P2X/Y [5] among others. It has been shown that these two families of receptors are involved in osteoarthritis and musculoskeletal pain [6,7].

Not only membrane receptors are of interest in this search for a treatment of chronic musculoskeletal pain; the modulation in the expression of different proteins could also play a role in the regulation or even the development of pain. Again these proteins are related with the immune system. One example could be resolvins, a family of lipids, derived from omega-3 fatty acids, involved in the resolution of acute inflammation. Recent work [8] has shown that these proteins can act not only in acute situations, but also in experimental chronic pain, reducing neutrophil infiltration, paw edema and proinflammatory cytokine expression and, more interestingly, blocking TRPV1- and NMDA-receptor activity and, at least theoretically, normalizing the spinal plasticity.

Another example of pain modulation, in this case via interfering with intracellular activity is acting on enzymes. Among an ever-growing number of candidates, we will focus on Rho-kinases, a family of enzymes involved in cellular activation and whose pharmacological modulation has demonstrate to induce 'pain' relief, even 'pain' abolishment, in animal models of nociceptive and musculoskeletal [9,10].

Although the real, therapeutic potential of all these strategies (among others) to treat chronic musculoskeletal remains to be confirmed in clinical situation, the involvement of immune system in chronic pain is nowadays fully accepted, and, since an increasing amount of research in this area is assured, more and more targets will hopefully be identified to alleviate pain or, at least, to improve quality of life in patients suffering from chronic pain.

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WHAT IS NEW ON THE PHARMACOLOGY OF THE ANTITHROMBOTIC AGENTS

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Most investigations on new antithrombotic drugs are based on the nature of thrombus: fibrin and platelets. Despite thrombolytic therapy maintains its role in clinical practice, antithrombotic strategies involve anticoagulants and antiplatelets. Traditionally, unfractionated heparin has been the most commonly used parenteral anticoagulant, but owing to its variable dose response and narrow therapeutic indices, it is being replaced by low molecular weight heparin, fondaparinux, and bivalirudin. New oral factor Xa inhibitors and the introduction of dabigatran, an oral direct thrombin inhibitor has been changed the scene of the thromboprophylaxis. The antiplatelet field has also expanded by the addition of two new agents, prasugrel and ticagrelor.

Dabigatran. Generation of thrombin is a pivotal step in hemostasis. Thrombin not only cleaves fibrinogen into fibrin to form a thrombosis, but it also activates platelets and many procoagulant factors including V, VIII, XI, and XIII. Thus, it represents a potent target for antithrombotic agents. It is a direct thrombin inhibitor that has been tested in phase III trials for stroke prevention in atrial fibrillation, and for venous disease prophylaxis and prevention. It is approved for use in the United States for stroke prevention in atrial fibrillation and many other countries for prevention of venous thrombosis after orthopedic surgery.

Rivaroxaban. Stopping hemostasis at this point halts the generation of potent procoagulant thrombin and is another proven method of anticoagulation. Rivaroxaban is the first and the most studied of these agents but many other factor Xa inhibitors are in clinical trials or development.

Apixaban. It is the third new anticoagulant to have published phase III trial data. It is also a factor Xa inhibitor.

Edoxaban. Is another factor Xa inhibitor in clinical trials. Currently, the 30 and 60 mg daily dose is being compared to warfarin in an atrial fibrillation stroke prevention phase III trial [28, 29].

Betrixaban. This factor Xa inhibitor is currently in phase III clinical trials. If effective, its lack of renal clearance may make it an appropriate agent for many older patients with renal insufficiency.

Respect to platelet inhibitory drugs, aspirin, of which the primary anti-thrombotic mechanism is to inhibit the biosynthesis of thromboxane by inactivation of platelet cyclo-oxygenase-1, affords a 22% risk reduction for nonfatal myocardial infarction (MI). However, aspirin is not very effective alone. Recurrent vascular events in patients taking aspirin have many possible causes and aspirin resistance has emerged as a relevant issue in clinical practice. Thus, there was a need for new antiplatelet agents. The P2Y₁₂ receptor that mediates ADP-induced platelet aggregation has become a favored target for the inhibition of platelet aggregation. Clopidogrel is the most widely used P2Y₁₂ inhibitor. Despite its proven efficacy, safety, and advantages over ticlopidine, clopidogrel has its own drawbacks including limited potency and high interpatient variability in pharmacological response. These limitations have led to the search for alternative P2Y₁₂ inhibitors.

Clopidogrel and *prasugrel* are thienopyridine prodrugs acting on the P2Y₁₂ receptor by almost identical active metabolites which irreversibly bind to the receptor. The thienopyridine prasugrel has a more efficient active metabolite generation than clopidogrel with a rapid onset of action, more pronounced platelet inhibition, and no clinically important variability in response. *Ticagrelor*, the first reversibly binding oral P2Y₁₂ receptor antagonist, does not require metabolic activation, has a rapid onset of action, and can disassociate from the receptor permitting restoration of platelet function without the need for production of new platelets. In pharmacodynamics studies, ticagrelor has demonstrated greater, more rapid, and more consistent ADP-induced platelet inhibition as compared with clopidogrel and more rapid offset of action following cessation of therapy. *Elinogrel*, a novel, potent, reversibly binding competitive P2Y₁₂ receptor antagonist, useful both for intravenous and oral administration is currently under evaluation. It has potential advantages over thienopyridines, such as clopidogrel and prasugrel, because it is a direct-acting drug (non-prodrug) with reversible platelet inhibition and competitive P2Y₁₂ receptor binding. In particular, the reversible and competitive nature of P2Y₁₂ receptor binding of elinogrel could provide additional antithrombotic protection beyond that of aspirin alone with the potential for less impact on bleeding.

Thrombin might be a good target for platelet inhibition because it is the most potent activator of platelets during the thrombosis process. As a result, a protease-activated receptor 1 (PAR1) inhibitor, a member of a potentially new class of drugs called thrombin receptor antagonists, is under investigation. Pre-clinical and phase II studies suggest that consistent and high levels of PAR1 inhibition may have a beneficial antithrombotic effect with a minimal increase in bleeding. Phase III studies of the selective PAR1 inhibitor *vorapaxar* are currently underway.

Within the next few years, more treatment alternatives might be available to further improve outcomes of the large population of patients with cardiovascular events. The use of these new antiplatelet agents in combination with other new oral anticoagulants should provide important insights about the efficacy and safety of triple therapy in patients.

ANTIPLATELET AGENTS AND ANESTHESIA

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Cardiovascular disease is a medical and social problem of the importance. Moreover, that dubious honor is expected to continue to keep under 2030 years in what is likely to double to deaths from cancer in the population younger than 70 years. To curb this rapid development, have developed a series of medical and surgical strategies include prophylactic use of antiplatelet drugs. These drugs have declined significantly both morbidity and mortality of these diseases.

Moreover, many papers have shown the benefits of the practice of spinal locoregional techniques regarding the general anesthetic technique. Thus, in a meta-analysis on 141 studies which examined the influence of the performance of spinal locoregional techniques in the development of postoperative complications was observed that overall mortality was reduced by almost a third in patients with neuraxial blockade. Neuraxial blockade reduced the likelihood of deep vein thrombosis (44%), pulmonary embolism (55%), transfusion requirement (50%), pneumonia (39%), and respiratory depression (59%). Besides locoregional technique appears to provide specific advantages in certain surgical procedures: carotid endarterectomy (a reduction of almost 50% in the probability of ischemic stroke, death, AMI or respiratory complications after surgery), peripheral vascular (improving graft patency), the short-term mortality in hip fracture (Parker *et al.* 2001), decreasing the incidence of postoperative myocardial infarction if sustained analgesia epidural chest postoperatively.

However, drugs that inhibit the interaction of hemostasis and locoregional spinal anesthesia technique can promote the development of spinal bleeding complications with the appearance of a rare but serious complication: spinal hematoma. For this reason, the locoregional anesthesia-related drug has wide debate in our society in recent years has led to the development of recommendations for various companies including the Spanish anesthesiology.

These limits have been a source of constant controversy since it generally is considered to be practicing safe spinal anesthesia in the presence of a functional platelet population of only 25% (which in most patients coincides with a number between 50 and 75,000 platelets than is usually reached after 4–5 days to suspend to suspend treatment with aspirin or clopidogrel).

Parallel to the binomial antiplatelet-spinal hematoma, another source of controversy has been the relationship antiplatelet drugs and surgical bleeding. The anesthesiologist as perioperative medicine expert, has been implicated in the maintenance or withdrawal of antiplatelet medication for its potential for bleeding. It is a fact that these drugs predispose to spontaneous bleeding. In this regard, it has shown that the recent interruption of medication antiagregante is associated with increased probability of death from cardiogenic in the course of AMI, suggesting that during the period of interruption there is a greater predisposition to serious thrombotic events. Burger *al* have analyzed this phenomenon even to be coded in days median time to onset of thrombotic event (between 7 and 14 days after the removal of the medication). These data should be of concern for anesthesiologists since it coincides in many cases with the perioperative period implying an increased thrombotic risk superimposed generated by a situation (anesthetic-surgical process) which in itself and is thrombogenic. This hypothetical risk is significantly increased if the patient wears a coronary stent. The so-called conventional stent or bare metal (bare metal stent, BMS) has joined the so-called drug-coated or (drug eluting stent, DES) which emits drug antiproliferative features that most effectively prevent restenosis of the vessel. However, this advantage adicional is accompanied by a drawback: the endothelialization of the stent is slower, making it more susceptible to thrombosis. This requires maintaining dual antiplatelet therapy (aspirin + clopidogrel) for longer (12 months with DES compared with BMS month) to prevent the most serious complication of stent: PT. Furthermore, stent patency is very dependent on the maintenance of antiplatelet medication since his abrupt withdrawal increases by a factor of 100 the probability of coronary thrombotic events in both AAS makers as clopidogrel which justifies maintain at all times this medication. However, as was the case

with the dual antiplatelet-bleeding, also occurs frequently seen as non-consensual withdrawal of medication during the perioperative process, for the particular characteristics of these patients, is an attitude that goes beyond recklessness to achieve the irresponsibility: Riddell *et al.* have reported that the perioperative mortality noncardiac surgery in these circumstances can reach a mortality rate of 2.5–21.4% for stent thrombosis. Fortunately, this dreaded complication decreases as the time of surgery away from the date of stent placement (peak incidence in the period <35 days) which favors the establishment of a plan of action.

In recent years, several authors have proposed protocols with more or less accepted that trying to bring order to the chaotic situation following general guidelines have been identified and embodied in our country by Pilar Sierra in a brilliant review and basically can be summarized in maintaining the AAS whenever possible and to delay elective surgery if only after the safety time from implant: BMS > 6 weeks and DES > 1 year.

RESISTANCE TO ANTIPLATELET THERAPY

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The studies refer to the resistance to antiplatelet effect of aspirin and/or clopidogrel are varied, both in the dosage used and the number of patients analyzed or concomitant pathologies present. In fact, aspirin has been described with a margin of strength 5–60% of all patients taking aspirin chronically at antithrombotic doses (75–330 mg/day), clopidogrel this margin is 4–34%. These margins so wide not only depend on the variability of patients and members of the doses of drugs used, but also the definition of resistance arising in each study, as some define it as an antiplatelet response <10% and others raise this to 30% range, which seriously hampers the joint interpretation of the results reported to the present.

Clearly, if a cardiovascular event occurs in a patient receiving an antiplatelet agent for secondary prevention, treatment has not been effective. However, the explanation of why it can be difficult.

Catteano, defines 'resistance' as the inability of the drug to reach its therapeutic target. In the case of aspirin would be associated with inhibition of production of thromboxane A₂ (TxA₂) dependent of cyclooxygenase-1 (COX-1). While for the thienopyridines, inhibition of P2Y₁₂ receptor for adenosine diphosphate (ADP). So for Catteano, 'resistance to antiplatelet therapy' should be restricted to situations where the impossibility of reaching the therapeutic target has been established by specific laboratory tests.

There are certain factors that may modify the response to ASA. Can be classified into factors leading to insufficient levels are reached the AAS in plasma (pharmacokinetic mechanisms) and factors that induce a change in drug targets through which the ASA exerts its antiplatelet effect (pharmacodynamic mechanisms).

Drug interactions are another important factor in reducing the effectiveness of treatment. He has found a higher percentage of resistance among patients taking statins. Other groups of drugs such as NSAIDs compete with ASA and block access to its binding site on the enzyme COX-1, reducing its activity (shown for ibuprofen). It has also been shown that the intake of inhibitors of proton pump reduces the bioavailability of aspirin for increased esterases in the gastrointestinal mucosa, which results in less absorption of this (although there are studies that do not observe this effect).

Alternative routes have been described for the formation of TxA₂ by the enzyme COX-2. COX-2 enzyme is primarily synthesized in nucleated cells such as monocytes, macrophages and new platelets formed in response to inflammatory stimuli, which are quickly able to synthesize this enzyme. In these cases, require higher doses of aspirin and more frequent administration to inhibit this isoenzyme and thus produce the effect.

It was also noted an induction of the isoform of the platelet COX-2 in patients who have undergone coronary artery bypass. Also, there is evidence that increased platelet turnover after bypass may explain the ineffectiveness of low-dose aspirin (platelet TxA2 synthesis should be blocked at least 10% to achieve efficient platelet inhibition). Therefore, aspirin, which has a short plasma half-life of elimination, it would not be able to inhibit the synthesis of new platelets formed.

They could also be related to aspirin resistance, elevated blood levels of von Willebrand factor, P-selectin or ADP.

The presence of elevated levels of 8-isoprostanes, which act as a vasoconstrictor and platelet aggregating, would relate to greater resistance to treatment with ASA.

Factors are not modifiable, relating to the ineffectiveness of antiplatelet therapy with ASA: female gender and age.

Despite the obvious and proven cardiovascular protective effect of aspirin and clopidogrel is known that this effect is not complete in patients with diabetes (primarily shown for ASA). In addition, at present, the use of antiplatelet drugs for primary prevention of cardiovascular events in patients with diabetes, is seriously discussed.

The smoking has also been suggested, although not without controversy.

Among the clinical conditions that are associated with resistance are: hypertension, dyslipidemia, obesity, diabetes, ischemic heart disease and congestive heart failure.

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