



“40 years with the Argentine Science”

**ARGENTINE SOCIETY OF EXPERIMENTAL
PHARMACOLOGY**

SOCIEDAD ARGENTINA DE FARMACOLOGÍA EXPERIMENTAL

Abstracts from the

XL ANNUAL SCIENTIFIC MEETING

December 2 - 5, 2008

TANDIL, ARGENTINA

The abstracts from XL Annual Meeting have been revised and evaluated
for the scientific committee

BOARD OF DE ARGENTINE SOCIETY OF EXPERIMENTAL PHARMACOLOGY

President	Regional representatives
Carlos Lanusse	María Victoria Aguirre (Corrientes)
Vicepresident	Otto Orsingher (Córdoba)
Carlos María Baratti	Alejandra María (San Luis)
Secretary	Ana Evangelista (Rosario)
Gabriela Acosta	Ignacio Alvarez (Tandil)
Treasurer	Ricardo Cabrera (Mendoza)
Sergio Sanchez Bruni	Roberto Rule (La Plata)
Members of the board	Gabriel Orce (Tucumán)
Ana Genaro	
Nora Brandan	Local Committee
Lilian Pelzer	Carlos Lanusse
Accounting Committee	Sergio Sánchez Bruni
Carlos Reyes Toso	Hugo Solana
Damasia Becú	Luis Alvarez
Accounting Committee Substitutes	Juan Sallovitz
Marcela Rebuelto	Adrián Lifschitz
Gabriel Cuadra	Guillermo Virkel
	Mónica Sparo
Representative before the	Alejandro Díaz
a) <u>Phorum of Scientific Societies</u>	Germán Chavez
b) <u>A.A.P.C.</u>	Soledad Sazatornil
Carlos María Baratti	

ACKNOWLEDGEMENTS

CONICET
 ANPCYT
 CICPBA
 UNCPBA
 COLEGIO FARMACÉUTICO DE TANDIL
 COSMOBIO SRL, AD INSTRUMENTS
 JENCK SA
 ROCHE ARGENTINA
 Laboratorios BAGÓ SA
 SEASING SRL
 AGROVET
 EDGARDO CAMILION, Publicaciones Médicas
 GE HEALTHCARE
 Laboratorios BACON
 Laboratorios ALLIGNANI Hnos. SRL
 MICROLAT SRL
 CHEMICAL CENTER
 IMAGENES TANDIL SA

Address

Junín 956, 5° piso. (1113 AAD). Buenos Aires - Argentina
 Tel: (54-11)-4964-8266. FAX: (54-11)-4964-8266
 Email: safe@canopus.com.ar
congreso2008@safe-digital.org
www.safe-digital.org

C01.**CANNABIS AND THE HEART***Adler-Graschinsky E.**ININFA – CONICET. Junín 956, 5° Piso. (1113) Buenos Aires, Argentina. E-mail: adlere@infovia.com.ar*

Evidence of the medical use of cannabis can be found as early as 5000 BC in China. Nevertheless, it was not until the 1960s that the main active compound of marijuana, delta 9-tetrahydrocannabinol, was isolated and identified. Later on, specific CB1 and CB2 receptors were identified and an endogenous cannabinoid named anandamide, on the basis of the Sanskrit word ananda that means bringer of inner bliss, was isolated from pig brain. Experimental work performed at our Institute led to demonstrate that anandamide plays a complex role in the cardiovascular system. In the isolated rat mesenteric arteries anandamide elicits a concentration-dependent reduction of the contractile responses to noradrenaline, that was mediated through the interaction with vanilloid VR1 receptors, whose responses are mediated by the release of calcitonin gene related peptide. The anandamide-induced relaxations were potentiated after long-term inhibition of the nitric oxide synthesis, suggesting a possible compensatory role for endocannabinoids in the vascular function. Relaxations induced by anandamide were higher in mesenteric beds from female compared to male rats, as well as in a model of septic shock caused by lipopolysaccharide administration. Higher responses to anandamide were linked to the overexpression of vanilloid VR1 receptors, that was constitutive in the case of female rats and inducible in the case of septic shock. Anandamide also caused a central decrease in blood pressure through the activation of VR1 and CB1 receptors. This central effect was potentiated by endogenous lipidic compounds present in the brain, such as palmitoylethanolamide. It is concluded that the pharmacological manipulation of the endocannabinoid system may offer novel therapeutic approaches in a variety of cardiovascular disorders.

C2.**MELATONIN AT ITS FIFTY ANNIVERSARY***Cardinali DP.**Dep. de Fisiología, Fac. Medicina, Univ. Buenos Aires. Web site: www.daniel-cardinali.medem.com*

It has been a great privilege to witness how the perception of melatonin has evolved since the early 1970's, from a molecule-in search of a function to the prototype of the new family of therapeutically valuable, chronobiotic drugs. The entrance of melatonin into canonical endocrinology of receptors was anticipated by kinetics studies employing ³H-melatonin in the early 1970s [Cardinali *et al.*, *Neuroendocrinology* 12: 30, 1973]. The administration to rats of nonlabeled melatonin before the intracisternal injection of ³H-melatonin decreased hypothalamic and brain melatonin uptake. The first experiments on brain melatonin receptor sites were carried out in the late 1970s by employing ³H-melatonin as a ligand. Presumptive melatonin acceptor sites were identified in membranes of bovine hypothalamus and cerebral and cerebellar cortexes [Cardinali *et al.*, *Endocrinology* 105: 437, 1979]. Membrane binding sites were maximally concentrated at late evening in rat and hamster brains [Vacas and Cardinali, *Neurosci Lett* 15: 259, 1979]. Pineal denervation, or continuous exposure to light, reportedly eliminated the morning-evening differences in ³H-melatonin binding sites. The introduction of the 2-[¹²⁵I]-iodomelatonin analog [Vakkuri *et al.*, *Anal. Biochem.* 142: 284, 1984] heralded a new era in the melatonin receptor field. By using this radioactive probe, melatonin binding was detected in several brain areas as well as in peripheral organs and eventually this versatile ligand allowed the cloning of the MT₁ and MT₂ melatonin receptors, a major achievement in the field [Reppert *et al.*, *FEBS. Lett.* 386: 219; 1996]. Eventually all this effort leads to the development of several melatonin analogs with very important therapeutic effects in human and veterinary medicine.

C3.**POSSIBLE THERAPEUTIC TARGETS TO MODULATE THE IMMUNE RESPONSE***Chuluyan E.**Pharmacology Department, Chair III, Faculty of Medicine, University of Buenos Aires. Paraguay 2155 9°floor, Ciudad de Buenos Aires, Argentina (C1121ABG). E-mail: echuluyan@fmed.uba.ar*

Advances in molecular technology have facilitated identification of novel therapeutic targets including cell subsets, cytokines and other molecules. Several new therapeutic agents are protein-based drugs termed 'biologicals' and target TNF (infliximab, adalimumab, etanercept), IL-1 (anakinra), IL-6 (Tocilizumab), CD20 (rituximab), CTLA-4/Ig (abatacept), CD4 (keliximab), ICAM-1 (alefacept), among others. Evidence about the benefits of these drugs is accumulating. However, they are not risk-free.

C4.**IMMUNE RESPONSES IN PARASITIC INFECTIONS***Jones D.**Moredun Research Institute, Edinburgh, Scotland, UK.*

A characteristic feature of the inflammatory immune response to both endo- and ectoparasitic infections is the development of tissue eosinophilia. The conventional view has been that eosinophils act as protective effector cells which kill and remove invading parasites. However, there is becoming increasingly apparent that, in many situations, eosinophils have little or no effect on parasite viability. Moreover, in non-parasitic inflammatory eosinophilic conditions such as asthma, there is now compelling evidence that the associated lung pathology is a direct result of eosinophil-mediated tissue damage. Our current hypothesis is that parasites, such as endoparasitic nematodes and ectoparasitic mites, may directly influence eosinophil recruitment and metabolism in order to facilitate their own establishment and survival. Thus, eosinophil-mediated mucosal damage could provide a permissive local microenvironment for gut nematodes, whilst serous exudates from the eosinophil-rich skin lesions associated with mite infestations appear to provide a primary nutrient source for the parasites.

The research described in this paper has provided support for this contention by initially demonstrating that extracts from parasitic (but not free living) nematodes, and *Psoroptes spp.* mites contained factors which were potently chemokinetic for eosinophils *in vitro*. Further *in vitro* studies suggest that this biological activity is due to factors which are actively secreted by the live organisms. Subsequent purification and examination of the physicochemical and mass spectrometric properties has characterised them as a component of a mixture of galectin-like molecules produced by the parasites. Nematode-specific galectins 1 & 2 have been identified as major constituents of this mixture but neither of these possess chemokinetic activity. Work is now focussing on identifying parasitic analogues of mammalian galectin-9, which is a known eosinophil chemokine. In addition, also like galectin-9, the parasite-derived factors inhibit pharmacologically-induced eosinophil apoptosis. Ultimately, the aim will be to assess the impact of parasite-derived galectins *in vivo* and explore the possibility of utilising parasite galectins as vaccine candidates and/or immunotherapeutic targets.

**C5.
UPDATE CONCEPTS ON TREATMENT OF RHEUMATOID ARTHRITIS***Scublinsky D.**Pharmacology Department, Chair I, Faculty of Medicine, University of Buenos Aires. Paraguay 2155 15 P, Buenos Aires City, Argentina (C1121ABG). E-mail: dscublinsky@fmed.uba.ar*

In the last years, the significant improvement of knowledge about the cytokine network made possible to understand better the physiopathology of many autoimmune diseases as Rheumatoid arthritis (RA). Since the first treatments, therapeutics was addressed to add drugs or replace the older ones but a new way of thinking the disease changed the concepts of morbidity and mortality and overturned the natural evolution of RA. The discovery of new target molecules made possible to develop new drugs that improve the disease activity, disabilities and prognosis of RA. Three first line and two second line treatments are now available in Argentina where the experience with these drugs is increasingly relevant.

**C6.
STATINS AND DIABETES: EFFECTS OF ATORVASTATIN IN PANCREATIC REGENERATION***Arany E, Weese K, Hill DJ.**Lawson Research Institute, University of Western Ontario, London, Canada. 268 Grosvenor St. Room F4-114, N6A 4V2. E-mail: earany@lti.sjhc.london.on.ca*

Several studies demonstrated a novel function for statins. In addition to their cholesterol lowering properties, they promote angiogenesis and re-endothelialization by inducing the mobilization and differentiation of EPCs (endothelial progenitor cells) *in vivo* and *in vitro*. It had been shown that patients treated with statins had a better recovery after MI (myocardial infarction) as it improved regeneration in the area of injury by stimulating angiogenesis and allowing EPCs differentiation into cardiomyogenic cells.

Our laboratory is interested in the mechanisms by which type 2 diabetes develops. Many patients with diabetes have high cholesterol and are medicated with statins therefore we thought that this would be a good model to investigate the impact of statins on pancreatic regeneration. In diabetes there is period of beta cell plasticity, where beta cell mass can be recovered. Statins could allow an appropriate niche to maintain the beta cell mass. Many potential mechanisms have been proposed to be involved in determining beta cell mass such as replication of pre-existing cells, appearance of new beta-cells from potential precursor cells or by trans-differentiation of mature cells from the islets, the ducts or the exocrine tissue. All these potential mechanisms require a rich microvascular environment which provides the inductive signals needed for recovery. EPCs are known to mature into ECs, which will signal to the pancreas through VEGF in order for the vasculature to supply the metabolic stimulus for growth. Our hypothesis was that statins will recruit endothelial progenitor cells (EPC), which readily home to sites of injury and will contribute to new blood vessel formation and deliver appropriate signals for beta cell regeneration. The model that we used to examine the role of Atorvastatin (AT) was the well known streptozotocin (STZ)-induced beta cell injury. We used two different doses of statins in order to determine which dose was the most effective. We treated the animals for three weeks during the last week of gestation and the first two weeks of postnatal life. The offspring were studied to determine the effects of the statin after STZ injury in the pancreas. The 20 mg/kg AT dose showed an increased beta cell mass in the animals treated with STZ at postnatal day 44. These animals also showed improved glucose tolerance at this time. We found that the treatment with AT caused an increase in islet endothelial cells (EC) which may be indicative of an increase in EPC, as well as neovascularization. It also affected the growth and regeneration of beta cell mass both during normal development and during regeneration. Our findings suggest that AT is involved in beta cell regeneration and suggest that may have a positive effect on the maintenance of beta cell mass in patients with diabetes.

**C7.
P-GLYCOPROTEIN INTESTINAL ACTIVITY AND THE VARIABILITY IN THE ORAL BIOAVAILABILITY***Rubio MC, Bramuglia G.**Instituto de Investigaciones Farmacológicas UBA-CONICET. E-mail: mcr@ffyb.uba.ar*

In mammals, the ATP-binding cassette (ABC) superfamily was first described as responsible for the multidrug resistance phenotype. Nowadays, these biological pumps have been identified as having a major impact on the pharmacological behavior of most drugs in use. ABC transporters are present in all cells of all organisms and use the energy of ATP binding/hydrolysis to transport substrates across cell membranes. The first member described, and by now the most studied, was P glycoprotein (P-gp), codified in the ABCB1 gene, which received its name due to its effect in reducing plasmatic membrane drug permeability. The most striking property of P-gp is its ability to transport an incredibly diverse range of compounds which do not share obvious structural characteristics. P-gp has been involved in relevant clinical drug transport such as antibiotics, antimalarials, analgesics, antiretrovirals, chemotherapeutic drugs, and also fluorescent dyes. This efflux pump has a ubiquitous expression in tissues including intestine, liver, kidney, blood brain barrier, placenta and cells from the hematological compartment. P-gp is expressed and active human peripheral blood lymphocytes. In our work, we investigated P-gp activity in lymphocytes of healthy volunteers. The variability observed in the population studied was important, with some volunteers with very scarce activity and some with a fourfold higher activity. The analysis of P-gp activity distribution showed a bimodal model, finding no relationship between the subjects' age and P-gp activity. Herein, results of our laboratory showed an inverse correlation between P-gp lymphocytic activity and the bioavailability of an oral Indinavir formulation.

Twenty-one ambulatory pediatric patients HIV infected receiving indinavir plus zidovudine were included, in which indinavir plasma levels were determined, as well as MDR1 genotypes on exon 26 (C3435T). Nine of the 21 patients initially receiving 250 mg/m² yielded trough levels below 0.10 µg/ml, (median: 0.21, range: 0.04-1.31 µg/ml). When the dosage was increased to 400 mg/m² indinavir plus 100 mg/m² zidovudine every 12 hours, all, except one patient, achieved levels above 0.10 µg/ml. The pharmacokinetic analysis showed higher volume of distribution (V/F) median values related to C/C genotype in comparison with C/T or T/T genotypes. These results may be explained by an incomplete absorption of the drug, related with lower plasma indinavir levels in those patients carrying the C/C genotype in exon 26.

C8.**PATHWAYS OF INTESTINAL BIOTRANSFORMATION AND DRUG EXCRETION IN DOMESTIC ANIMALS***Virkel G.**Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, UNCPBA, (7000) Tandil, Buenos Aires, Argentina. CONICET (Argentina). E-mail: gvirkel@vet.unicen.edu.ar*

Livestock animals are exposed to a variety of xenobiotics during their production cycles. These compounds are likely to be metabolized by different enzymatic systems from both hepatic and extra-hepatic tissues. As a result of these enzyme reactions (biotransformations), the metabolic product is generally less lipophilic and more polar (hydrophilic). Generally, the metabolites formed are readily eliminated by normal excretion routes. Metabolism may also give rise to the formation of toxic metabolites. In addition, the metabolic activity of xenobiotic metabolizing enzymes play a major role in determining the persistence of therapeutically or illegally used compounds in livestock, which may additionally impose a risk to the consumers as a consequence of the permanence of drug residue levels in edible tissues. Undoubtedly, xenobiotic biotransformation takes place predominantly in the liver, although metabolic activity is apparent in extra-hepatic tissues such as the intestinal mucosa. This tissue constitutes an absorptive barrier in the uptake of toxic compounds or orally administered drugs. In addition, it also has the ability to metabolize a great number of xenobiotics by both phase 1 (oxidative, reductive and/or hydrolytic) and phase 2 (conjugative) reactions. It is well known that the bioavailability of an enterally administered drug can be reduced by both intestinal and hepatic first pass metabolism. Besides, both tissues play a role in protecting the organism from toxic compounds. Cytochrome P450 (CYP) 3A4 is the major isoenzyme involved in the biotransformation of many xenobiotics in the small intestinal mucosa in humans and largely contributes to the first pass metabolism of many drugs (i.e.: midazolam and cyclosporine). In addition, the transport protein P-glycoprotein (P-gp) limits the absorption of xenobiotics but also is involved in their active intestinal secretion. P-gp is located within the brush border on the apical (luminal) surface of the enterocytes and is able to extrude a broad range of compounds. Moreover, it has been shown that both CYP 3A4 and P-gp act coordinately as an absorption barrier for enteral administered drugs. Most of the published investigations on the intestinal metabolism and excretion of xenobiotics have been carried out in laboratory animals and man. An overview of the available information on the relevance of xenobiotic metabolizing enzymes and transport proteins in livestock species is presented here. The activity and expression of several phase 1 and phase 2 drug metabolizing enzymes was characterized in the small intestine of sheep and cattle. For example, microsomal preparations obtained from both sheep and cattle small intestine showed CYP2C, 2B, 3A activities and FMO-mediated oxidative metabolism. Intestinal microsomes obtained from these species were also able to conjugate 1-naphthol (a glucuronosyltransferase probe) and 1-cloro,2,4-dinitrobenzene (a glutathione transferase substrate). The transport protein P-gp may be involved in the intestinal secretion of ivermectin (an endectocide antiparasitic drug) in ruminant species. Future research efforts should be directed to establish the relevance of the intestinal biotransformation and excretion pathways in domestic animals.

C9.**ALTERED LOCALIZATION OF CANALICULAR TRANSPORTERS AS A NOVEL COMPONENT IN CHOLESTASIS***Mottino A, Crocenzi F, Sánchez Pozzi E, Roma M.**Instituto de Fisiología Experimental. CONICET-UNR. Facultad de Ciencias Bioquímicas y Farmacéuticas, Rosario, Argentina. E-mail: amottino@unr.edu.ar*

Bile flow formation is dependent upon normal localization and function of two major canalicular ATP-dependent export pumps: Bile salt export pump (Bsep) and Multidrug resistance-associated protein 2 (Mrp2). They transport bile salts and glutathione, respectively, the main osmotically active solutes secreted into the canalicular space that account for bile formation. Recent evidence in experimental animals indicates that normal localization of these pumps at the canalicular membrane can be affected in cholestasis of different etiologies, either obstructive or induced by drugs. Among the latter, estradiol-17 beta-glucuronide (E2-17G), an endogenous derivative of estradiol, was found to induce an acute, reversible, and dose-dependent decrease of bile flow in rats, which is accompanied by internalization of Bsep and Mrp2 from the canalicular membrane to an intracellular, subapical domain. This process affects their transport activity, and is microtubule-independent and partially mediated by activation of calcium-dependent PKC isoforms. Indeed, E2-17G promotes translocation of PKC alpha, one of most relevant calcium-dependent PKCs, from the cytosol of the hepatocyte to intracellular or plasma membranes, thus increasing its phosphorylating activity. The targets for PKC mediated phosphorylation are still unknown, but could be the transporters themselves, tight junction structures, or membrane anchoring proteins involved in retention of Bsep or Mrp2 at its normal localization in the canalculus. Sustained administration of E2-17G additionally produces miss-localization of Mrp2 to the basolateral membrane of the hepatocyte and alteration of the normal pattern of localization of the tight junction proteins zonula occludens 1 and occludin. This finding correlates well with an opening of the paracellular route, a phenomenon also associated with cholestasis. Bsep and Mrp2 were also found to be internalized after acute treatment of rats with the cholestatic bile salt tauro lithocholic acid, as well as under cholestatic conditions induced by oxidative stress in the hepatocyte. Very recently, evidence has been provided that cholestasis in humans is also associated with miss-localization of canalicular transporters. For example, Mrp2 was found to be internalized in human cholestatic liver diseases, such as drug-induced liver injury, obstructive jaundice, primary sclerosing cholangitis and autoimmune hepatitis. Thus, miss-localization of canalicular transporters constitutes a novel mechanism of relevance to explain liver secretory dysfunction in experimental and human cholestatic disease.

C10.**TRANSPORT OF BILIRUBIN, DRUGS AND GLUCOSE IN THE BLOOD-BRAIN BARRIER: INTERACTIONS AND THERAPEUTIC IMPLICATIONS**

Kapitulnik J, Sasson S.

School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, P.O.Box 12065, Jerusalem 91120, Israel. E-mail: jaimek@savion.huji.ac.il

The blood-brain barrier (BBB) is a physical and functional barrier that prevents the entrance of many drugs and chemicals into brain cells regardless of their molecular size. Carrier-mediated transport systems in the BBB, that are active in efflux of drugs, include the P-glycoprotein pump (Pgp/MDR1/ABCB1) and non-Pgp multidrug-resistance proteins (MRPs). The human **MDR1-Pgp** is located at the luminal site of BBB endothelial cells. It is an amphipatic cationic efflux pump, and limits the influx and diffusion of many drugs and chemicals, thus protecting the brain from the toxic effects of high concentrations of these compounds. Very importantly, Pgp handles also the efflux of endogenous compounds, as is the case with bilirubin (BR), a neurotoxic catabolite of heme. Thus, BR-drug competition for the Pgp in the BBB may affect the entrance of both BR and drugs into the brain. Since Pgp is expressed only after birth, newborn infants are very sensitive to this endogenous neurotoxin, which can diffuse into the brain and precipitate in discrete areas such as the basal ganglia (kernicterus). Pgp can have a pronounced influence on the pharmacodynamic effects of drugs in the brain. Many CNS-active drugs are Pgp substrates (e.g., morphine, phenytoin, anti-brain-cancer drugs, anti-HIV agents, etc.), and consequently inhibition of Pgp at the BBB increases the therapeutic effects of these drugs by increasing their concentrations in the brain. However, *in vivo* drug-drug interactions at the BBB Pgp can also lead to side effects (e.g., for diltiazem and tacrolimus) and even to CNS toxicity. The **MRPs** are non-Pgp, amphipatic anion efflux pumps that can also transport cations and neutral compounds (cotransported with GSH). Several of the identified members of the MRP family are expressed at the BBB.

Diabetes causes changes in BBB transport which may affect drug and glucose delivery to the brain. Glucose transporter 1 (GLUT1) is the major transporter responsible for the entry of glucose into the brain. We have recently examined the effect of BR on the regulation of GLUT1-mediated glucose transport in vascular endothelial cells (VEC). BR significantly augmented the rate of glucose transport, GLUT1 expression, and plasma membrane localization of GLUT1 in these cells. BR also reversed the high glucose-induced down-regulation of the glucose transport system in VEC. Pathological concentrations of BR in the vascular compartment (jaundice) may thus influence the cellular handling of glucose in diabetes, including its delivery to the brain.

C11.**HALF CENTURY OF FREE RADICAL RESEARCH IN BIOLOGICAL SYSTEMS**

Boveris A.

Instituto de Biología de Radicales Libres, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. E-mail: aboveris@ffyb.uba.ar

Organic free radical chemistry was well developed by 1954 when Gerschman postulated that oxygen free radicals were responsible of the molecular mechanism of oxygen toxicity. The discovery of superoxide dismutase (1969) and the recognition that mitochondria produce superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) in physiological conditions (1972-1976) opened the way to the knowledge on free radical and hydroperoxide metabolism. The observation of a mitochondrial production of nitric oxide (NO) by a specialized mtNOS, increased the interest in free radical metabolism in biological systems. The current view is that oxidative and nitrosative stresses are common cellular phenomena that lead to apoptosis, autophagia and necrosis and that such processes are involved in a series of pathological and clinical situations.

C12.**RENAL SODIUM HANDLING IN ESSENTIAL HYPERTENSION**

Ramírez AJ, Sánchez RA.

Sección Hipertensión Arterial y Unidad Metabólica, Fundación Favaloro, Belgrano 1782, P 4. (C1093AAS) Buenos Aires, Argentina. E-mail: aramirez@favaloro.edu.ar

Non-modulators (NM), are a subset of essential hypertensives with strong family history of hypertension (40-45%) characterized by high renin values, failure to increase the effective renal plasma flow (ERPF) and adequately excrete Na in response to high Na intake (Williams GH, Hollenberg NK: *Hypertension* 1991, 17 (Suppl I): 81-85). We have shown that these individuals are also sodium sensitive in as much that they respond to Na load with a BP increase. (Sánchez R *et al.* *Hypertension* 2003, 41: 919-924). This altered renal hemodynamic response to Na load is explained by either a local over activity of angiotensin II (AII) or an altered AII receptor sensitivity (Moore T *et al.* *Circ Res* 1977, 41: 167). In addition, it has been shown that non-modulation is also associated with development of early cardiovascular alterations such as endothelial dysfunction and microalbuminuria (Sánchez R *et al.* *Hypertension* 2003, 41: 919-924). Furthermore, non modulators show a high prevalence of insulin resistance. (Gaboury CL *et al.*: *Am J Hypertens* 1995; 8: 870-875) and since the intrarenal effects of Ang II are modulated by NO, it was proposed that an imbalance may induce a specific renal dysfunction and salt sensitivity and renal endothelial dysfunction. Although the mechanism by which Angiotensin II causes production of superoxide has not been entirely elucidated it is known that the infusion of Ang II increases NADPH oxidase activity via PKC mechanisms. In hypertensive patients, independent of the effects of salt on blood pressure, salt sensitivity may be a marker for susceptibility to cardiovascular and renovascular injury (Bigazzi R *et al.*: *Hypertension* 1994, 23: 195-199). Similarly insulin resistance and diabetes are strong cardiovascular risk factors characterized by increased prevalence of hypertension, salt sensitivity, and decreased EDR-mediated by NO.

We will provide further evidencies supporting the idea that this non-modulating salt sensitive hypertensives have a renal reduced activity of the kinin-kallikrein system that favours an increased AII activity. This leads to an increase in the intrarenal oxidative stress with the consequent decrease in NO bioavailability. Basic and clinical research data will be shown to support these evidencies.

C13.**PARKINSON'S DISEASE: PRESENT AND FUTURE CHALLENGES**

Gershanik O, Gomez Arévalo G.

Instituto de Neurociencias, Fundación Favaloro. Solís 416, Capital Federal, Argentina. E-mail: gersha@gmail.com

Diagnosing Parkinson's disease as early as possible is one of the major challenges we are presently facing as evidences strongly indicate that early medical intervention significantly changes the prognosis and course of the disease. Detecting subtle clinical changes, identifying at risk populations through genetic testing, and using modern functional imaging techniques in combination are some of the approaches we are implementing. Results from recent clinical trials, using conventional or novel designs are highly suggestive of the presence of disease modifying effects whenever medical intervention was done early. Claims about neuroprotection have been made based on these results. However, the scientific community remains skeptical and is requiring more objective and stringent indicators to substantiate such a claim. It has been suggested that the positive clinical outcomes of early intervention do not necessarily mean that the drug in question is exerting true neuroprotective effects but allowing for a more benign course of the disease through compensatory mechanisms.

Another major therapeutic hurdle that we are presently trying to overcome is how to manage or prevent the host of motor and non motor symptoms that plague the latter stages of the disease in association with the long-term side effects of medication. Gait and balance disturbances, deglutition and speech disorders, cognitive changes and dementia, together with motor fluctuations, dyskinesia, impulse control disorders and psychosis are examples of the problems for which we have no definitive solution at present.

The development of novel pharmacological targets for intervention aside from the dopaminergic system has been one way of tackling these problems. However, the results obtained so far with this approach have been disappointing.

The ultimate challenge in addition to prevention, neuroprotection, disease modification and compensation is the development of pharmacological tools that can effectively afford a measure of restoration to the damaged nigrostriatal and associated circuits. Cell based therapies, and genetic manipulations with the help of novel delivery techniques are some of the avenues that are presently being explored. Unfortunately a substantial number of hurdles and roadblocks have to be overcome before these therapies come of age.

C14.**UPDATE OF PHARMACOLOGY AND THERAPEUTICS IN ADDICTION**

Kalina E.

Brain Center, Buenos Aires.

The pharmacological treatments of addictions are relatively recent. However, they are critical in "the multiple treatment models". These models are the most appropriate for the pathology of addictions. I will discuss relevant aspects of this topic using for this purpose several clinical examples.

O-01.**Na⁺/H⁺ EXCHANGER-1 INHIBITORS (NHE-1_{inh}) DECREASE MYOCARDIAL SUPEROXIDE PRODUCTION BY A DIRECT MITOCHONDRIAL ACTION**

Garciarena C, Caldiz C, Correa V, Schinella G, Mosca S, Chiappe de Cingolani G, Cingolani H, Ennis I.

Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. 60 y 120. (1900) La Plata, Argentina. E-mail: cgarciarena@atlas.med.unlp.edu.ar

A direct mitochondrial action of NHE-1_{inh} decreasing reactive oxygen species (ROS) production was explored in cat cardiac slices. Angiotensin II induced after 30 min a NADPH oxidase (NOX)-dependent increase in O₂⁻ production (50±9; apocynin: -8±17) that was prevented by three different NHE-1_{inh} without scavenger activity: cariporide (Car: 7±6), BIIB723 (6±14) and EMD87580 (-2±12). The source of the NOX-dependent O₂⁻ released seemed to be the mitochondria since it was blunted by the mitochondrial K_{ATP} channel (mK_{ATP}) blockers 5HD (3±14) and glibenclamide (-21±5), by inhibition of complex I of the electron transport chain with rotenone (-12±24) and the mitochondrial permeability transition pore by cyclosporin A (CsA, -9±9). Car also prevented O₂⁻ production induced by the opening of mK_{ATP} with diazoxide (53±9; Car 12±6) and decreased Ca²⁺ induced-mitochondrial swelling by 37±3%, similarly to CsA (41±4) and bongkreikic acid (34±2). Ang II-increased O₂⁻ induced ERK1/2 and p90^{RSK} phosphorylation (315±7% and 173±11%), and this was also prevented by Car (116±25% and 111±13%). These data support a direct mitochondrial action of NHE-1_{inh} preventing ROS release that may explain their beneficial effect in cardiac hypertrophy and failure and in ischemia/reperfusion injury.

O-02.**SERUM INTERLEUKIN -6 AND TUMOR NECROSIS FACTOR-ALPHA LEVELS AFTER ROSUVASTATINA THERAPY IN PATIENTS WITH ACUTE CORONARY SYNDROME**

Brizuela N, Heredia D, Demurtas S.

Departamento de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. Santa Rosa 1085, (5000) Córdoba, Argentina. E-mail: nilda.brizuela@gmail.com

The purpose of this study was to evaluate the anti-inflammatory effect and the effect on the lipids profile of Rosuvastatin in patients with acute myocardial infarction.

Aims: in patients with acute myocardial infarction treated with Rosuvastatin. 1- To determine the variation of the inflammatory markers: TNF- α and IL-1 values. 2- To quantified LDL-cholesterol. Material and Methods: plasma samples were obtained from 14 patients admitted at the cardiac coronary unit with acute myocardial infarction and during the following 4 weeks of treatment with 10 mg of Rosuvastatin. The results were compared with a "control group" without the treatment of the statin. Patients who had previously received treatment with statins were excluded.

The use of rosuvastatin resulted in significant ($P < 0.001$) reductions for: LDL-cholesterol (39.9% versus 4.4%), TNF (21.4% versus 2.9%), IL-1 (16.4% versus 2.7%).

Thus, statin-induced inhibition of inflammatory markers may play an important role in the markers pharmacological and clinical effects of statins seen in cardiovascular diseases.

O-03.**ERYTHROPOIETIN CYTOPROTECTIVE EFFECT AGAINST DOXORUBICIN MYOCARDIAL INJURY IN MICE**

Reyes M, Stemberg E, Stoyanoff T, Todaro J, Ruiz Diaz D, Aguirre M, Brandan N.

Cátedra de Bioquímica. Facultad de Medicina. UNNE. Moreno 1240. (3400) Corrientes. E-mail: nbrandan@med.unne.edu.ar

Doxorubicin (DOX) is a potent chemotherapeutic agent, associated with severe cardiotoxicity. Erythropoietin (EPO) has been shown to exhibit several non-hematopoietic properties including tissue protective effects.

In order to evaluate the potential cytoprotective effects of EPO on murine myocardium, cardiomyopathy was induced in adult female CF-1 mice by a single intraperitoneal injection of doxorubicin (15 mg/kg). Control group was treated with saline. EPO (5000 U/kg) was administered simultaneously and 15 days post-DOX administration in two mice groups (early and late regimens).

Ultrastructural myocardial features (scanning electron microscopy, SEM) upon DOX were correlated with HIF- α , Epo-R, Fas and Bax expressions (western blotting). SEM studies showed that DOX induced myocardial degenerative changes in a time dependant manner. DOX (15 D) caused an overexpression of HIF- α and a decrease of Epo-R levels, without changes in Fas and Bax expressions. Only the early regimen of EPO administration was associated with a significant myocardial protective effect. Bax and Fas invariable levels suggested that apoptotic pathways are not involved in DOX-induced cardiotoxicity.

O-04.**OAT5 AND NADC1 URINARY EXCRETION IN ISCHEMIC ACUTE RENAL FAILURE (iARF)**

Di Giusto G, Torres AM.

Area Farmacología. Facultad de Cs. Bioquímicas y Farmacéuticas. UNR. CONICET. E-mail: giseladg@hotmail.com

The urinary abundance of the organic anion transporter 5 (Oat5) and the sodium-dicarboxylate cotransporter 1 (NaDC1) was studied in male Wistar rats with iARF. iARF was induced by occlusion of both renal pedicles for 0 (S), 5 (I5), 15 (I15), 30 (I30) or 60 (I60) min followed by 60 min of reperfusion. The abundance of Oat5 (%) and NaDC1 (%) was evaluated by Western blotting and related to urinary creatinine levels. Urinary Alkaline phosphatase activity (AP, mUI/mg Creatinine) and plasma creatinine levels (Crp, mg/L) were determined. Data were analysed with ANOVA plus Newman-Keuls $P < 0.05$: [a] vs S, [b] vs I5, [c] vs I15, [d] vs I30.

(n)	S (6)	I5(4)	I15(4)	I30(4)	I60(4)
Crp	4.9±0.3	5.5±0.6	6.0±0.6	8.0±0.9 ^a	9.8±1.3 ^{a,b,c}
Oat5	100±5	138±6 ^a	142±7 ^a	166±13 ^a	161±12 ^a
NaDC1	100±6	113±5	112±7	126±12	165±17 ^{a,b,c,d}
AP	359±53	312±11	299±9	463±50	847±163 ^{a,b,c,d}

An increase of Oat5 and NaDC1 urinary excretion was observed in I60 as well as alterations of other widely used parameters for renal dysfunction (AP, Crp). Oat5 excretion was the only parameter that showed a premature increase since group I5. This is the first report for Oat5 and NaDC1 detection in urine. These results suggest that urinary excretion of Oat5 might be an early biomarker of iARF.

O-05.

EVALUATION OF THE BINDING SITE OF THE 99mTc DURING THE LABELING OF THE RED BLOOD CELLS

Collia N¹, Arnoldi S¹, Kaliski MA¹, Merelli A², Salgueiro MJ¹, Lazarowski A², Zubillaga A¹

¹Laboratorio de Radioisótopos, FFyB, UBA. ²Dpto. Bioquímica Clínica-Hematología, Hospital de Clínicas "José de San Martín", FFyB, UBA. Junín 956 PB, 1113 CABA, Argentina.

E-mail: ncollia@ffyb.uba.ar

For many years it has been postulated that the labeling of red blood cells (RBC) with 99mTc depends on the binding of the radioisotope to the beta chain of the globin. Objective: To study the binding site of the 99mTc during the labeling of the RBC. Materials and methods: The samples with citrate as anticoagulant were labeled by the *in vitro* method and the biological labeling efficiency (BLE) was determined. The RBC were lysed and the samples were centrifuged to separate membranes and hemolysate. Activity was measured in both fractions. Hemoglobin (Hb) and globin chains electrophoresis were made with the hemolysate to determine the site of binding of 99mTc. Results: Preliminary results show a BLE higher than 98%. The membranes and the hemolysates of the RBC presented the same activity distribution (approximately 50% in each fraction). The 99mTc labeling on Hb fraction was observed for Hb A ($\alpha_2\beta_2$) and HbA2 ($\alpha_2\delta_2$). Conclusions: During the labeling of RBC with 99mTc, the radioisotope was incorporated not only to the Hb but also to the membrane. It will be useful to analyze to which component of the membrane was bound the 99mTc. The activity in the Hb was distributed in both Hb (A and A2) and the location of the label in the Hb (α , β and/or δ chain) will be evaluated.

O-06.

ENHANCEMENT OF IMMUNOLOGICAL ACTIVITY OF CPG-ODN VEHICULIZED IN LYOTROPIC LIQUID CRYSTAL CARRIERS

Maletto B¹, Alemandi DA², Morón G¹, Pistoiresi-Palencia MC¹, Palma SD².

¹Dep. de Bioquímica Clínica-CIBICI, ²Dep. de Farmacia, Fac. de Ciencias Químicas, U.N.C. Cdad. Universitaria, 5016 Córdoba, Argentina. CONICET. E-mail: sdpalma@fcq.unc.edu.ar

CpG-ODN (oligonucleotides containing unmethylated CpG in particular base context) is a new adjuvant for vaccines that unfortunately has shown reduced bioavailability owed mainly to lack of chemical stability. In this work, we evaluated the adjuvant capacity of CpG-ODN incorporated into lamellar mesophases of lyotropic liquid crystals (coagels). For that, BALB/c mice were immunized (s.c.) three times during fifteen days (schedule: 0, 7 and 15) with a protein antigen (OVA, 60 ug/mice/dose) plus CpG-ODN (50ug/mice/dose) or OVA/CpG-ODN/coagel. On day 21, we measured specific antibodies in plasma and specific cytokines in cell culture supernatants (ELISA). The measured titles (log10) were:

	IgG	IgG1	IgG2a
OVA/CpG-ODN	3,9±0,0	3,4±0,1	2,7±0,2
OVA/CpG-ODN/coagel	5,1±0,0	4,4±0,1	3,8±0,2

The levels of IFN- γ were higher in animals immunized with OVA/CpG-ODN/coagel than in those immunized with OVA/CpG-ODN (p<0,005). These results indicate that CpG-ODN intensified the specific immune response when administered in coagels.

O-07.

MODULATION OF M2 MUSCARINIC RECEPTOR-RECEPTOR INTERACTION BY IgG ANTIBODIES FROM CHAGAS' DISEASE PATIENTS

Beltrame SP¹, Auger SR², Bilder CR³, Goin JC¹.

¹CEFYO-CONICET-UBA, ²Htal. Santojanni, ³Fundación Favaloro. ¹(1121) Buenos Aires, Argentina.

E-mail: juangoin@fmed.uba.ar

In previous reports we showed that the M2 muscarinic acetylcholine receptor (M2R) forms constitutive homodimers in live cells. In this work we tested the ability of serum IgG antibodies against M2R from chronic Chagas' disease patients (ChD-IgG) to modulate M2 receptor-receptor interaction by bioluminescence resonance energy transfer (BRET). HEK 293 cells cotransfected with fusion proteins M2R-RLuc and M2R-YFP were exposed to ChD-IgG or the IgG fraction from normal subjects (N-IgG) during 60 min at 25°C, and the BRET between M2-RLuc and M2R-YFP was assessed by luminometry. ChD-IgG promoted a concentration-dependent increase in the BRET signal reaching a maximum at 50 μ M (23.4±6.6 mBRET, n=7), which was significantly higher than the effect induced by N-IgG (10.1±2.9 mBRET, n=5) (p<0.002). Pretreatment of cotransfected cells with 1 μ M atropine failed to inhibit the increased energy transfer induced by ChD-IgG, suggesting that the observed effect is not a consequence of receptor activation. BRET signals obtained by treating cells coexpressing M2R-RLuc and M3R-YFP or M3R-RLuc and M2R-YFP or M3R-RLuc and M3R-YFP with ChD-IgG were not different from that induced by N-IgG, indicating that the effect of ChD-IgG is strictly subtype-specific. Our data show that serum IgG antibodies from ChD patients can enhance M2 receptor-receptor interaction in live cells, defining a new differential pharmacological property for these anti-M2R antibodies as compared with conventional muscarinic ligands.

O-08.

CANCER IMMUNOTHERAPY BASED ON SALMONELLA ADMINISTRATION INDUCES ANTITUMOR EFFECT. ROLE OF NEUTROPHILS, EFFECTOR AND REGULATORY T CELLS IN SALMONELLA INDUCED TUMOR IMMUNITY

Vendrell A, Gravisaco MJ, Croci M, Mongini C, Waldner C.

CEFYO, CONICET-UBA. Paraguay 2155, P16 (CP 1121). Buenos Aires. E-mail: avendrell@fvet.uba.ar

We have previously demonstrated that a novel *Salmonella* immunotherapy induced a significant reduction in tumor size and prolonged survival in mice bearing the mammary adenocarcinoma LM3. The aim of this study is to elucidate the immune mechanisms regulated by *Salmonella* to achieve this antitumor response. BALB/c mice were subcutaneously inoculated with LM3 tumor cells. When tumors were palpable, groups of animals were immunized twice with bacteria or PBS, either intratumoral or in the peritumoral and in the tumor-draining lymph nodes (TDLNs) area. After 14 days of the first immunization, mice were sacrificed to study antitumor response in TDLNs and tumors. Our results indicate that *Salmonella* promotes activation of CD4⁺ and CD8⁺ effector T cells producing IFN- γ , and reduction of T regulatory cells in TDLNs respect to PBS-treated mice (p < 0.05). Moreover, we found that neutrophils are the most important cells infiltrating bacteria-infected tumors, exerting antitumor effect probably by IFN- γ and TNF- α secretion. In conclusion, we demonstrated that the anticancer therapeutic properties of *Salmonella* are mediated by induction of both innate and adaptive immune mechanisms to overcome immune tolerance to tumors.

O-09.**CONTRIBUTION OF BALTERGIN TO THE BACTERICIDAL ACTIVITY EXHIBITED BY *BOTHRUPS ALTERNATUS* VENOM**

Rodríguez J¹, Bustillo S¹, Gay C¹, Leiva L¹, Acosta O².

¹Facultad de Ciencias Exactas y Naturales y Agrimensura. UNNE. Av. Libertad 5400, (3400) Corrientes, Argentina. E-mail: rodriguezcasco@yahoo.com.ar; ²Fac. de Ciencias Veterinarias, UNNE. Sto. Cabral 2139 (3400) Corrientes, Argentina.

We have previously demonstrated the bactericidal activity of *B. alternatus* venom against Gram (-) and Gram (+) strains. Baltergin is a metalloproteinase enzyme isolated from *B. alternatus* venom, with an extensive biological toxicity.

In this work, we examined the microbicidal role of baltergin and its contribution to the antimicrobial activity exhibited by the whole venom against *Staphylococcus aureus* (ATCC 25923).

A turbidimetric method was employed to measure the bactericidal activity of the enzyme. Inhibitory concentration 50 (IC₅₀) was defined as the concentration of toxin needed to reduce the 50% of bacterial growth. To evaluate the contribution of this toxin, antibodies against baltergin were produced in rabbits and neutralization was developed using inhibitory halos test.

The enzyme showed a high bactericidal activity (IC₅₀ 70 µg/ml) and the neutralization assay demonstrated that this hemorrhagin contributed to 45% of the total activity exhibited by the venom.

These results show that baltergin, would be a microbicidal protein emerging as a promising therapeutic alternative to cope with the increasing rates of antibiotic resistance encountered worldwide.

O-10.**BIOLOGICAL CONTROL OF PATHOGEN BACTERIA IN GROUND MEAT BY *Enterococcus faecalis* CECT7121**

Ranno G¹, Sparo M¹, Ceci M¹, Sánchez Bruni S^{2,3}.

¹Centro de Estudios Bioquímicos, Tandil. ²Lab. de Farmacología, Facultad de Ciencias Veterinarias, UNCPBA, (B7000APA) Tandil. ³CONICET. E-mail: ssanchez@vet.unicen.edu.ar

The use of biological controllers in meat is an interesting choice for the control of infectious diseases in risk populations. The ingestion of contaminated semi-raw ground meat (burgers) is involved on the transmission of pathogens such as *Salmonella* spp, enterohaemorrhagic *Escherichia coli* (ECEH) and *Clostridium perfringens* (Cp). The aim of this study was to evaluate the bactericide efficacy of the biological controller (BC) *E. faecalis* CECT7121 against pathogens artificially inoculated in raw ground meat. A hundred grams of ground meat were inoculated with 10³ UFC/g of *S. Typhimurium* (STm), ECEH, and Cp; whilst the BC was added at 10⁴ UFC/g. Three experiments were undertaken as follows: **Study I**: each pathogen and BC were simultaneously inoculated. **Study II**, The BC was inoculated 24 h before the inoculation of the pathogen and **Study III**, the pathogen was inoculated 24 h before the BC. Bacterial counts by the killing curve method were performed at 0, 24 h, 48 h y 72 h post-incubation. Results obtained in Study I, indicated no viable counts perceived from 48 h for ECEH and Cp, and from 72 h for STm. The Study II has shown no viable counts at 24 h for ECEH and, 48 h for Cp and STm. The Study III, revealed no viable counts of all pathogens were found at 48 h post-BC inoculation. *E. faecalis* CECT7121 showed a fast bactericide activity against all the pathogens assayed, being it used as potential biological tool to prevent infectious diseases from semi raw ground meat.

O-11.**CIPROFLOXACIN AND CIPROFLOXACIN-LIDOCAINE SUSTAINED RELEASE HYDROGELS FOR TOPICAL ADMINISTRATION**

Breda S, Manzo R, Olivera M*.

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000, Córdoba, Argentina. *E-mail: meoliver@fcq.unc.edu.ar

The objective was to design hydrogels for the controlled release of Ciprofloxacin, (CIP, *antibiotic*) alone or in combination with Lidocaine, LIDO (*analgesic*). A set of hydrogels were prepared from partial neutralization of an acidic polyelectrolyte carrier with the basic groups of CIP and LIDO, to achieve the doses commonly indicated for their topical administration. Stability after one sterilization cycle (autoclave) was determined. The irritation potential was evaluated through Dreize test in rabbit skin. Release (0.9% NaCl solution) and rheology (32, 35 and 37°C) were assayed. The systems allow adjusting doses in the hydrogels with final pHs between 6-7. They are physically and chemically stable after sterilization. The products were regarded as non-irritant with non-tixotropic plastic flow. They behave as intelligent systems since both drugs are released upon contact with biological fluids. Delivery is sustained and at time ≥ 30 min CIP concentration is above 3 x CIM (determined for main strains responsible of skin infections). The formulations could be useful to improve available pharmacotherapy for the topical treatment and prophylaxis of infections caused by CIP-sensitive isolates such as endometriosis, bloody wounds and/or varicose ulcers. Also, the systems can be valid for other fluoroquinolones having the free basic group.

O-12.**FLUBENDAZOLE AND ALBENDAZOLE CAPACITY TO ACCUMULATE INTO HYDATID CYSTS IN MICE**

Ceballos L^{1,2}, Elissondo C^{1,3}, Alvarez L^{1,2}, Sánchez Bruni S^{1,2}, Denegri G^{1,3}, Lanusse C^{1,2}.

¹Lab. Farmacología, FCV, UNCPBA; ²CONICET; ³Lab. Zoonosis Parasitarias, FCEyN, UNMdP. E-mail: ceballos@vet.unicen.edu.ar

Few chemotherapeutic agents are available for the medical management of hydatid disease. The aim of this work was to evaluate the plasma pharmacokinetic (PK) behaviour of albendazole (ABZ) and flubendazole (FLBZ) and their ability to accumulate into hydatid cyst of infected mice. BalbC mice (n= 88) infected with *E. granulosus* (eight months of infection) were orally treated with a FLBZ or ABZ solution (5 mg/kg). Blood and cysts samples were collected between 0 and 12 h post-treatment and analysed for FLBZ-ABZ/metabolites by HPLC. FLBZ parent drug was detected in plasma (AUC=1.8 ± 0.16 µg.h/mL) and cysts (AUC=0.35 ± 0.13 µg.h/mL) collected from treated animals, conversely the ABZ parent drug do not was detected neither in plasma nor cysts of animals treated with ABZ solution, instead of that its metabolite albendazole sulphoxide was the main molecule detected either in plasma (AUC= 4.4 ± 0.42 µg.h/mL) or cysts (AUC= 1.50 ± 0.14 µg.h/mL). The excellent efficacy observed for FLBZ (murine model) may be related with its ability to reach the cyst. In contrast after ABZ administration, the effect against hydatid cyst depend on ABZSO, a metabolite with lower anthelmintic potency and reduced capacity to diffused through the cysts wall.

O-13.**EFFECT OF pH ON THE ANTIBACTERIAL ACTIVITY OF AZITHROMYCIN AND PENICILLIN AGAINST *Staphylococcus aureus***

Moncada Cárdenas A, Marchetti L, Daniele M, Lambertini A, Errecalde J, Mestorino N.

Cátedra de Farmacología. Facultad de Cs Veterinarias. Universidad Nacional de La Plata. CC 296, 1900, La Plata. E-mail: noram@fcv.unlp.edu.ar

S. aureus survives in acidic media, including phagolysosomes. Controversial *in vitro/in vivo* data exist on its susceptibility to antibiotics in such environments. The purpose of this study was to evaluate the effect of the pH variation on the antibacterial activity of azithromycin (AZT) and penicillin (PEN) against strains of *S. aureus* isolated of mastitic quarters. *S. aureus* strains isolated (N = 10) and *S. aureus* ATCC 25923 were tested by macrodilution method at pH 7.4, 6.5 and 5.0, in order to simulate the conditions of acidity of subcellular structures which are commonly associated with *S. aureus* intracellular persistence. The results at pH 7.4, for both antimicrobials were consistent with those reported by CLSI 2007 (AZT MIC: 1 µg/mL, PEN MIC: 0.125 µg/mL). The AZT MIC was approximately 16 times higher at pH 5.0 than at pH 7.4. In contrast, lowering the pH over the same range markedly and almost linearly increased the activity of PEN (~20 fold decrease in MIC). The understanding of these contrasting effects of pH on the antibacterial activity may prove their potential usefulness in the treatment of intracellular pathogens.

O-14.**PRENYLATED FLAVONOID 6PP SHOWS DUAL ACTION: ANTIFUNGAL EFFECT AND RHODAMINE 6G EFFLUX INHIBITION**

Peralta M¹, Calise M², Ortega G¹, Cabrera JL¹, Diez RA², Pérez C³
¹Farmacognosia-IMBIV, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba; ²Farmacología, Facultad de Medicina; ³Farmacología, Facultad de Odontología, Universidad de Buenos Aires. E-mail: cperez@farmaco.odon.uba.ar

Some compounds of vegetal origin show antimicrobial synergism. When **2',4'-dihydroxy-5'-(1''',1''''-dimethylallyl)-6-prenylpinocembrin (6PP)**, isolated from roots of *Dalea elegans* and fluconazole (Flz) were incorporated to the incubation medium, inhibited the efflux of rhodamine 6G (Rh 6G) in *Candida albicans* resistant to azoles (*CaR*). This strain, isolated from the oral cavity, was obtained from Dr. T.White (University of Washington, Seattle, EE.UU) and expresses transporters of CDR1, CDR2 and MDR1 type. Our parameters were for 6PP and Flz respectively, maximum inhibition: 45.46% and 41.42%; apparent K_i 42.95 µM and 107.81 µM. The addition of Flz in culture medium of *CaR* was studied on the cellular growth (A), Rh 6G efflux (B) and antifungal activity (C). At concentrations 0; 3,2; 6,5 and 13 µM of Flz, respectively, were detected the following % values: 100, 114, 114 and 109, for A (absorbance to 580 nm); 70, 48, 52 and 68, for B (retention of fluorescence, 100 in efflux reversion by 1,000 µM 6PP). In antifungal activity evaluation (C), 20 mM 6PP inhibited fungal growth at 0 (23 mm diameter) and 13 µM Flz (13 mm). The results are consistent with the expression of constitutive efflux pumps and a dual action of 6PP: inhibition of azole transporters and antifungal effect on *CaR*.

O-15.**EVIDENCE FOR A THALAMOCORTICAL DYSRHYTHMIA IN MICE TREATED WITH ACUTE COCAINE BINGE**

Bisagno V, Peskin V, Wikinski SI, Urbano FJ.

ININFA-UBA-CONICET, Junín 956, 5to. C1113 Buenos Aires, IFIBYNE-UBA-CONICET Ciudad Universitaria, C1428 Buenos Aires. E-mail: vbisagno@ffyb.uba.ar

Cocaine intake might induce brain anomalies with broad behavioral consequences. Altered thalamocortical dynamics are in the basis for several types of both neurological and neuropsychiatric diseases, currently grouped under the name *thalamocortical dysrhythmia syndrome* (TCDs; Llinas *et al.*, 1999). Abnormal activity of thalamic relay neurons is suggested to be related to an increase of low frequency oscillatory activity due to protracted activation of the T-type calcium channels related to a hyperpolarization due to excess GABAergic inhibition. In our experiments using an acute cocaine binge protocol (3 X 15mg/kg, i.p. inj., 1 hour apart), *in vivo*, EEG recordings showed a significant increment of *delta*-, *theta*- and *alpha*-band activity in the experimental group that was partially reversed after a 24 hour washout period. We measured the enzyme involved in GABA synthesis, GAD, by immunocytochemistry in thalamic nuclei and parietal cortex. No significant differences were found between groups. Preliminary results showed comparable spine density in cortical and thalamic nuclei (using Golgi staining techniques) between saline and cocaine treated mice. Our results suggest that acute cocaine binge induced a transitory TCD. Future experiments will further explore the role of GABA on this phenomenon.

O-16.**OMEGA-3 FATTY ACID POTENTIATION OF FLUOXETINE AND MIRTAZAPINE ANTIDEPRESSANT EFFECTS**

Laino C, Fonseca C, Sterin-Speziale N, Reinés A.

Instituto de Investigaciones en Ciencias de la Salud Humana (IICSHUM)-UNLaR, Departamento de Ciencias Exactas, Físicas y Naturales - UNLaR, Cátedra de Biología, Instituto de Química y Físicoquímica Biológicas, Fac. de Farmacia y Bioquímica-UBA e ININFA (CONICET-UBA). E-mail: carloslaino2001@yahoo.ca

Epidemiological studies indicate an association between depression and low dietary intake of omega-3 (ω-3) fatty acids. However, robust preclinical characterization of the ω-3 antidepressant effect is still lacking. The aim of this study was to examine in rats the antidepressant effects of ω-3 supplementation alone as well as in combined chronic treatments with antidepressants fluoxetine (FLX) or mirtazapine (MTZ) in the forced swimming test. We found that compared to control diet, ω-3 supplementation dose-dependently increased behaviors of swimming, indicative of the ω-3 antidepressant-like effect. Co-administration of FLX (1 or 10 mg/kg/day) or MTZ (20 mg/kg/day) and ω-3 fatty acids (0.72 g/kg/day) revealed higher antidepressant efficacy than the individual treatments. Biochemical studies showed that compared to control diet, ω-3 supplementation increased docosahexaenoic acid (DHA) levels in brain membranes without modifying the composition of phospholipid classes. It can be suggested that ω-3 may potentiate antidepressant drug actions possibly by increasing the DHA proportion in brain membrane phospholipids.

O-17.
TOPOTECAN VITREOUS LEVELS AFTER PERIOCCULAR OR INTRAVITREOUS INJECTION

Buitrago E¹, Hocht C¹, Opezzo J¹, Fandiño A², Abramson D³, Chantada G⁴, Bramuglia GF¹.

¹Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, UBA; ²Servicio de Oftalmología, ⁴Hemato-Oncología Hospital de Pediatría JP Garrahan, Buenos Aires, ³Memorial Sloan Kettering Cancer Center, New York.

Chemoreduction is now the standard treatment of intraocular retinoblastoma. Topotecan (TPT) is active for retinoblastoma and it has good penetration to the vitreous when given intravenously. The periocular (PO) or intravitreous (IVT) route may be preferable due to its favorable toxicity profile.

PO or IVT TPT was given to each eye to non tumor-bearing rabbits. Vitreous and plasma samples were obtained at different times. Total and lactone TPT was measured by HPLC. Pharmacokinetic models were developed using NONMEM.

After the PO injection of TPT (1 mg) vitreous lactone levels in the range of 5 to 10 ng/mL were achieved in the vitreous and maintained until 4 hours post-administration. The contralateral eye presented a similar concentration profile. High TPT plasma exposures were observed because of absorption from the PO depot. IVT injection of TPT (0.2 mg and 0.05 mg) achieved higher lactone TPT vitreous levels up to 1.5 hours after the injection (TPT 0.05 mg Cmax: 10.4 ug/ml-1.5 h: 0.9 ug/ml). Low TPT plasma levels were observed after IVT injection. Scleral diffusion of topotecan occurs after PO administration to a rabbit model reaching active levels in the vitreous. IVT injection showed higher intravitreous levels with no plasma exposure. Both administration schedules may represent an alternative for retinoblastoma treatment.

O-18.
EFFECTS OF ILICIC ALDEHYDE ON GASTRIC SECRETION IN PYLORUS-LIGATED RATS

María A¹, Wendel G¹, Donadel O², Tonn C², Pelzer L¹.

Cátedras de ¹Farmacología y ²Química Orgánica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. Chacabuco y Pedernera. (5700) San Luis. Argentina. E-mail: alemaria@unsl.edu.ar

Ilicic aldehyde (IA), obtained from *Fluorensia oolepis*, prevents the formation of gastric and duodenal lesions induced by various necrotising agents in rats and mice. The gastroprotective mechanism of IA depends on PG and NO, by the increment of mucus production.

In this study, the effects of IA on gastric secretion in acute treatment or at a repetitive doses were investigated.

Male Wistar rats were used after 24 hours fasting period. A mid-line incision was performed and the pylorus was ligated. After this, IA (40 mg/kg), saline or ranitidine (30 mg/kg) were administered intraduodenally. Four hours after pyloric ligation, the rats were sacrificed, the stomach removed and the gastric contents collected and centrifuged. The volume of supernatant was measured and the acid concentration estimated by titration with NaOH 0.1 N. In other experiment, IA was administered at a dosis of 40 mg/kg for 11 days. Ranitidine inhibit acid secretion and was used as a control (p<0.001). IA increase the values of pH in acute and repeated dose treatments (p<0.001 vs. saline control). However, the values of volume and titratable acid concentration were not modified by treatment with IA in both cases. In conclusion, the gastroprotective mechanism of IA does not depend on its inhibitory effect of gastric secretion in rats.

O-19.
STUDY OF THE INTESTINAL TRANSPORT OF THE ANTI HIV DRUG ZIDOVUDINE

Quevedo MA, Mariani E, Briñón MC.

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Ciudad Universitaria, 5000, Córdoba, Argentina. E-mail: alfredoq@fcq.unc.edu.ar

Zidovudine (AZT), the first drug approved for the treatment of AIDS, has been reported to induce drug efflux transporter systems in different tissues. Over-expression of P-gp and multidrug resistance proteins (MRPs) has been reported in the immune and central nervous system cells when exposed to AZT. In this work we studied the transport of AZT through rat small intestine segments using the everted gut sac technique, evaluating the amount of drug transported, flux and apparent permeability coefficient at different concentrations and times of exposure. We observed a constant transport ratio from the mucosal to the serosal side of the segments corresponding to jejunum at all concentrations, while for ileum the ratio was constant at low concentrations. A decrease in the amount of drug transported with the increase of time exposure was observed at higher concentrations in ileum. Serosal to mucosal transport assay evidenced an increase in the ratio of drug transported with respect to time at high drug concentration. These results allow us to conclude that the expression of efflux transporters may occur for AZT in rat small intestine, although further assays using inhibitors are needed to identify the system involved.

O-20.
DURABILITY OF INDUCTION OF RAT INTESTINAL P-GLYCOPROTEIN (Pgp) ACTIVITY BY ACETAMINOPHEN (AP)

Ghanem CI^{1,2}, Dellicarpini G², Novak A², Filia MF¹, Mottino AD³, Rubio MC¹.

¹ININFA, CONICET-UBA. ²Cát. de Fisiopatología (FFyB). ³IFISE, CONICET-UNR. E-mail: cghanem@ffyb.uba.ar

A toxic dose of AP induced Pgp expression and activity in rat liver and intestine. Repeated doses of AP also induced the expression of Pgp protein in rat intestine. We here evaluated the effect of repeated AP treatment on intestinal Pgp activity and the permanence of the changes registered. **Material and Methods:** Male Wistar rats were injected i.p. for 3 consecutive days with 0.2; 0.3 and 0.6 g/kg bw of AP (AP group) or vehicle (C group) (n=4). P-gp activity was determined using everted intestinal sacs at 24; 48; 72; 144 and 192 hr after the last injection. The last 10-cm portion (next to the ileocecal valve) was filled with rhodamine 123 (Rho, 15 uM, serosal side) and incubated in Krebs-Henseleit buffer with or without the Pgp inhibitor verapamil (100 uM, mucosal side) for 40 min. **Results:** Intestinal Pgp activity (nmol/min/g, means±SD) was increased at 24 hr in AP (38.9±7.1) vs C (20.4±5.0) and at 48 hr in AP (38.6±2.7) vs C (26.4±7.1) (p<0.05). At 72, 144 and 192 hr there were no significant differences. Verapamil inhibited Rho transport in AP and C at all times tested, confirming Pgp participation. **Conclusion:** induction of Pgp by AP at distal intestine is reversible and returns to basal values within 72 hr.

O-21.**ASSESSMENT OF INTESTINAL DRUG TRANSPORT: DEVELOPMENT AND APPLICATION OF THE USSING CHAMBER TECHNIQUE**

Ballent M^{1,2}, Lifschitz A^{1,2}, Virkel G^{1,2}, Sallovitz J¹, Maté L^{1,2}, Lanusse C^{1,2}

¹Lab. Farmacología, FCV, UNCPBA. ²CONICET.

E-mail: mballent@vet.unicen.edu.ar

The role of the transport protein P-glycoprotein (P-gp) in the pharmacokinetics of different drugs used in veterinary medicine has been demonstrated. The goal of the current work was to develop the Ussing chamber technique to characterize the intestinal P-gp-mediated drug transport in rats and sheep. The flat sheets of intestinal mucosa (ileum) were mounted into Ussing chambers. Digoxin (DGX) (200 μ M) and Albendazole sulfóxido (ABZSO) (30 μ M) were added to mucosal (M) and serosal (S) sides. Samples were taken between 30 and 240 min. DGX and ABZSO were analyzed by HPLC. The efflux rate (P_{eff}) was calculated. The intestinal transport of DGX and ABZSO was corroborated. In rats, the $P_{\text{eff S-M}}$ was significantly higher than $P_{\text{eff M-S}}$ for both compounds. The $\text{Peff}_{\text{S-M}}/\text{Peff}_{\text{M-S}}$ ratio ranged between 1.76 and 2.37 for both drugs, indicating a transport process to the intestinal lumen. In sheep, the $\text{Peff}_{\text{S-M}}/\text{Peff}_{\text{M-S}}$ ratio for ABZSO was 1.49. The presence of PSC833 enhanced the efflux M-S in both species. The $\text{Peff}_{\text{S-M}}/\text{Peff}_{\text{M-S}}$ ratio decreased to 1.23 (rats) and 1.30 (sheep). The use of the Ussing chamber technique is a useful tool to improve the comprehension of the absorption/excretion mechanisms involved in the transport of drugs therapeutically used in veterinary medicine.

O-22.**REGULATION BY ESTROGENS OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) IN CACO-2 CELLS**

Arias A, Ruiz ML, Villanueva SSM, Pellegrino JM, Catania VA, Mottino AD.

IFISE-CONICET, Fac. Cs. Bioq. y Farm., UNR, Suipacha 570, 2000 Rosario, Argentina. E-mail: agoarias@yahoo.com.ar

In previous studies we observed that repeated administration of the synthetic estrogen ethynylestradiol (EE) to rats reduces the expression of Mrp2 in intestine. Here, we studied whether EE treatment affects MRP2 expression in Caco-2 colon cancer cell line. The cells were treated for 48 hs with EE (5, 30 and 100 μ M, n=3), or solvent (DMSO) for the controls (C). When DMEM+10% FBS (medium A) was used as culture medium, EE significantly decreased MRP2 expression at 100 μ M, as detected by western blotting. In contrast, the same treatment but using a modified low estrogen medium (phenol red-free, DMEM+10% charcoal-dextran treated FBS) (medium B), showed a significant increase in MRP2 levels by EE (5 and 30 μ M). Messenger RNA levels, quantified by Real Time PCR, remain unchanged, indicating post-transcriptional regulation. To confirm that the presence of natural estrogens in the medium conditions EE response, we further treated cells in medium B with estradiol (0.5, 5, and 30 μ M, n=3) for 48 hs. The data indicated increased MRP2 levels by estradiol at 5 and 30 μ M. **Conclusion:** Estrogens composition of the incubation medium conditions MRP2 expression in Caco-2 cells. Any extrapolation to the pharmacological effects of EE on human intestinal MRP2 has to be cautiously done.

BI-01.**REYE'S SYNDROME ENCEPHALOPATHY, HYPERAMMONEMIA AND ACETYL SALICYLIC ACID INGESTION**

Fernández MA¹, Lemberg A¹, Romay S¹, Perazzo JC¹, Filingher EJ^{2,3}.
¹Cátedra de Fisiopatología, ²Cátedra de Farmacia Clínica, Facultad de Farmacia y Bioquímica, U.B.A., Junín 956, 1113, ³CONICET, Buenos Aires, Argentina. E-mail:efilin@ffyb.uba.ar

Twelve cases of Reye's syndrome (RS) are presented with encephalopathy and hyperammonemia; associated to acetyl salicylic acid (ASA) ingestion. The aim of this retrospective study was to describe our experience in patients with RS associated to the ASA ingestion and the influence of hyperammonemia on Reye's encephalopathy. All the cases presented moderate hyperbilirrubinemia, elevated ALAT and ASAT with an average of 302±205 UI/L and 285±149 UI/L respectively. Arterial blood ammonia averaged 172.4±71.3 µmol/L and glycemia 35.2±17.0 mg/dl. Considering that encephalopathy was the leading syndrome, the influence of ammonia in brain tissue, the alteration in the glutamine/glutamate cycle and glutamate metabolism were studied. The presence of ASA could decrease glucose production and lipid metabolism, increase ketone bodies concentration, and could cause the mitochondrial permeability transition in astrocytes. In RS, hyperammonemia and perhaps the increase of glutamate are the principal factors in the mechanism of encephalopathy.

BI-02.**THE DISPOSITION OF FREE AND NIOSOMALLY ENCAPSULATED RAC- FLURBIPROFEN IN DAIRY BOVINES**

Confalonieri O¹, Soraci A, Denzoin L, Becaluba M¹, Tapia O.
¹Area de Clínica, Area de Toxicología, Dpto. Fisiopatología, FCV-UNCPBA. Campus Universitario Paraje Arroyo seco s/n Tandil, Argentina. E-mail: oconfa@vet.unicen.edu.ar

Racemic flurbiprofen (Rac-FBP) is a powerful non-steroidal anti-inflammatory-analgesic compound. The distribution of free Rac-FBP in bovines is rapid with a relatively short half-life. Different studies show that drug-niosome formulations exhibit a different pharmacokinetic behavior from that of free drug molecules. Niosomes of Rac-FBP were prepared in different molar concentrations of surfactant/ cholesterol. The Rac-FBP-niosome formulation showed a drug loading of 92.0 ± 0.9%. Twelve clinically normal Argentine Holstein cattle were divided into two groups. One group (n=6) received the niosomal preparation (92% entrapment) of Rac-FBP at a dose of 0.5 mg/Kg by IV administration. The other group (n=6) received a Rac-FBP solution prepared in phosphate buffer pH 7.4 and DMSO at the same IV dose. The HPLC analyses of plasma concentration of Rac-FBP demonstrated that niosomal Rac-FBP formulation predominated (24 h) over Rac-FBP in solutions (8 h). The niosomal Rac-FBP increased in the MRT, AUC₀₋₂₄ and t_{1/2} values and decreased in the CL_B value. These results indicate that the niosomal formulations represent a promising drug delivery module in veterinary medicine for molecules that are rapidly eliminated from the organism.

BI-03.**EVALUATION OF DIFFERENT RADIOPHARMACEUTICALS FOR THE LABELING OF RED BLOOD CELLS**

Collia N¹, Arnoldi S¹, Kaliski MA¹, Leonardi N¹, Goldman C¹, Salgueiro MJ¹, Boccio J², Zubillaga M¹.
¹Laboratorio de Radioisótopos, FFyB, UBA. ²Cátedra de Física, FFyB, UBA. Junín 956 PB, 1113 CABA, Argentina. E-mail: ncollia@ffyb.uba.ar

Objective: To evaluate if the use of different radiopharmaceuticals (Fitate (F), Pyrophosphate (P), Stannous Chloride (SC)) as source of Sn²⁺ ions for the labeling of red blood cells (RBC) with ^{99m}Tc modifies the biological labeling efficiency (BLE) and biodistribution of ^{99m}Tc-RBC in normal and anemic conditions. Materials and methods: 120 female Sprague-Dawley rats were separated in 2 groups and fed with 2 different diets: control (Iron content: 100ppm) and anemic (Iron content: 18ppm). Both groups were subdivided in 3 groups, where F, P and SC were used as Sn²⁺ ions source for the labeling of RBC with ^{99m}Tc. The same quantity of Sn²⁺ ions was administered in the 3 groups for the *in vivo* labeling. In all cases the BLE and the biodistribution were evaluated at 30 min and 24 hs. Results: The BLE was higher than 96% in all groups. At 30 min in F and P, both in the control and anemic group, the CA% in blood was statistically different from SC. At 24 hs a decrease in blood CA% and an increase in kidney CA% was observed in all groups compared to 30 min. In all cases the blood CA% was statistically higher for controls than anemics. Conclusions: No differences were observed in BLE or in the biodistribution at 24 hs for any studied group. However the biodistribution of each radiopharmaceutical showed differences between 30 min and 24 hs. In all the cases the anemic group showed a decrease in the blood CA% for each radiopharmaceutical compared with controls.

BI-04.**EFFECT OF RUMINAL UREA INFUSIONS ON ACTIVITY OF LIVER UREA CYCLE ENZYMES IN WETHERS**

Recavarren M, Milano G.
 CONICET. Facultad de Ciencias Veterinarias, UNCPBA, Campus Universitario, 7000, Tandil, Argentina. E-mail: mireca@vet.unicen.edu.ar

In ruminants, diets with asynchronous release of rumen available energy and N, increase rumen ammonia concentration and liver ammonia uptake, and may stimulate the activity of key urea cycle enzymes responsible for ammonia conversion to urea. The objective of this experiment was to quantify the effect of changes in the rate of N release in rumen, through controlled ruminal infusions of urea, on the activity of carbamylphosphate synthetase I (CPS1) and ornithine carbamoyltransferase (OCT), liver weight and liver protein, RNA and DNA content. Eight Corriedale wethers (36±1.6 BW), fitted with permanent catheters in the rumen, were fed 753 g DM/animal/d of lucerne hay (600 kJ ME/kg^{0.75}/d; 22 g N/d; 7 g soluble N/d), in 2 equal meals, using automatic feeders. A completely randomized design, with 1 experimental period of 14 days and final sacrifice, was used. Ruminal infusion of urea (8 g/d) was either continuous (CT; 2.5 mg urea-N/min; N=4), using peristaltic pumps, or discontinuous (BT; 2 doses/d of 1 g urea-N after each meal; N=4). These contrasting rates of urea infusion in rumen did not change the activities (U, µmoles of formed product/min/g fresh tissue) of CPS1 (0.026±0.02 U) and OCT (36.5±15 U), fresh liver weight (443±36 g), liver protein (224±77 mg/g fresh tissue), DNA (1.31±0.75 mg/g fresh tissue) and RNA (3±0.6 mg/g fresh tissue) content, or RNA/DNA and RNA/protein ratios.

BI-05.**COMPARED BILIAR DISPOSITION OF SUPROFEN ENANTIOMERS IN DOMESTIC CARNIVORES**

Castro E, Soraci A, Tapia O, Fogel F, Franci R, Denzoin L, Ortega I.

Departamentos de Fisiopatología y Clínica, FCV, UNCPBA, Tandil, Argentina, Campus Universitario, Pje Arroyo Seco s/n.

E-mail: edcast@vet.unicen.edu.ar

Suprofen (SPF) is a non-steroidal anti-inflammatory drug (NSAID) who belongs to the 2-arylpropionic acids subclass. As result of its chiral characteristics, this compounds have shown a marked enantioselective behaviour [i.e, chiral inversion (CH.I) of (R) to (S) enantiomer, with a high degree of interspecies variation. They are mainly eliminated by glucuronidation. Previous data shown that SPF is poorly inverted or not in all the species that it had been tested. Our data about rac- SPF disposition in the cat are accordingly with that (Castro *et al.*, 2001). Biliar and urine disposition of racemic SPF was investigated to evaluate the contribution of the glucuronidation pathway to the detoxification of racemic SPF. The total amount of glucuronides excreted in the bile represented 1% of the total dose administered. No SPF glucuronides were found in urine. Racemic SPF showed an enantioselective disposition in none of the biological matrix investigated. This results, compared with those obtained in a similar experiment in dogs (Soraci *et al.*, 1995) suggest that the biological fate of SPF in the cat is not influenced by enantioselective physiological processes and that other pathways different from glucuronidation and CH.I. may be responsible for the clearance of SPF.

BI-06.**2,4-DICHLOROPHOENOXYACETIC ACID EFFECT ON RAT GRANULOSA CELLS IN CULTURE**

Madariaga MJ, Ghersevich S, Duffard R, Evangelista de Duffard AM.

Laboratorio de Toxicología Experimental (LATOEX), Fac. de Cs. Químicas y Farmacéuticas. UNR. Suipacha 531 (2000), Rosario. E-mail: aevangel@fbioyf.unr.edu.ar

2,4-Dichlorophenoxyacetic acid (2,4-D) and its derivatives are herbicides widely used to control the growth of broadleaf. Exposure of rats to this herbicides would have adverse effects on reproduction. The aim of the present study was to investigate if the ovarian Granulosa cell (GC) viability was affected by 2,4-D *in vitro*. Ovaries of immature 24 to 25-day-old Wistar rats were excised 3 days after the animals were implanted sc with diethylstilbestrol. GC were harvested from de ovaries by follicular puncture using a fine needle. The cells were resuspended in McCoy's 5A medium and cultured in 24 well plastic plates (100,000 viable cells/well in 0.4 ml of culture medium) in McCoy's 5A medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) and L-glutamine 2 mmo/l, in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Cells were incubated in the presence or absence of 0.2 mM, 0.5 mM, 1 mM or 2 mM 2,4-D concentrations. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used as a quantitative colorimetric measurement of cell death. Mean cell viability was decreased significantly by 2,4-D in a dose depending way. This observation was according to *in vivo* studies where we demonstrated a decrease in the number of ovarian follicles by the herbicide.

BI-07.**EFFECT OF EXPERIMENTAL INTOXICATION WITH METALLIC MERCURY ON BONE TISSUE**

De Lucca R^{2*}, Ubios A², Zalazar A¹, López E¹, Radenti J¹, Aromando R¹, Funosas E¹, Maestri L¹, Martínez A¹.

¹Department of Pharmacology FOUNR, ²Department of Histology and Embryology, FOUBA, Santa Fe 3160 7° piso. (2000) Rosario, Santa Fe, Argentina. E-mail: adrianabmartinez@yahoo.com.ar

At present, metallic mercury (mm) is one of the mayor environmental contaminants. There are no reports in the literature about its effects on bone tissue. The objective was to study the effect of mercury intoxication on bone volume (BV). We evaluated bones presenting endochondral ossification (femur) and endomembranous bone (maxillae) in mice. Adult male BALB/c mice, (25 and 30 g body weight) were divided into groups and treated as follows: Group A (n=8): absolute control, and Group B (n=8): treated with a single intraperitoneal 0.1ml dose of mm. All the animals were euthanized 48 hours after the onset of the experiment. Group C (n=8) and Group D (n=8) received the same treatment as Groups A and B respectively, but were euthanized at 14 days. All femurs and mandibles were resected to perform histologic and histomorphometric studies. The specimens were fixed in buffered formalin. The sections were stained with hematoxylin - eosin. Digital photographs were obtained and analyzed using Image Pro Plus software. Our results allow stating that the alterations in bone volume observed following administration of the dose of mm used in this study (0.1 ml) are not only associated with the experimental time points but also with the type of bone tissue.

BI-08.**COMPARATIVE ACTIVITIES OF XENOBIOTIC METABOLIZING ENZYMES IN SHEEP LIVER AND SMALL INTESTINE MUCOSAS**

Maté L¹, Virkel G¹, Lifschitz A¹, Sallovitz J^{1,2}, Ballent M¹, Lanusse C¹.

¹Laboratorio Farmacología, FCV-UNCPBA. ²CICPBA. ³CONICET. Argentina. E-mail: mmlmate@vet.unicen.edu.ar

Intestinal mucosa has the ability to metabolize a great number of xenobiotics by both phase 1 and phase 2 reactions. The objective of this work was to evaluate xenobiotic metabolizing enzyme activities in cytosolic and microsomal fractions obtained from sheep liver and small intestine mucosas (duodenum, jejunum and ileum). Oxidative (cytochrome P450-dependent), reductive (carbonyl reductase) and enzymatic conjugative activities were measured by using known marker substrates. Metabolic activities measured in liver subcellular fractions were used as reference values. Intestinal N-demethylase P450-dependent activities ranged between 11.0-46.1% (P450 3A) and 3.0-7.1% (P450 2C) than those measured in the liver. These metabolic activities did not decrease nor increase along the small intestine. Conversely, microsomal O-deethylase P450-dependent activities (CYP 1A) were not detected in sheep small intestine mucosas. Intestinal carbonyl reductase activities were 14.3-23.4% (microsomes) and 18.7-33.1% (cytosol) than those measured in the liver. Intestinal microsomes were able to conjugate 1-naphthol, (an UGT-mediated reaction) and 1-cloro,2,4-dinitrobenzene (a GST probe). Metabolic reactions, taking place in the intestinal mucosa, may contribute to the pre-systemic metabolism of orally administered drugs. These results contribute to further current knowledge on the extra-hepatic biotransformation pathways in ruminants.

BI-09.**P-GLYCOPROTEIN (P-gp) IS INDUCED BY BENZNI-DAZOLE (BZL) IN MURINE MACROPHAGE CELL LINE RAW 264.7.**

Echenique CG¹, Luquita MG², Ruiz ML², Rigalli JP², Pérez AR³, Revelli SS³, Mottino AD³, Catania VA².

¹Parasitología, Fac. Cs. Bioq. y Farm. (UNR), ²IFISE-CONICET, Suipacha 570, ³Inst. Inmunología, Fac. Cs. Médicas (UNR), Santa Fe 3100. 2000, Rosario, Argentina.

E-mail: vcatania@fbioyf.unr.edu.ar

P-gp is expressed in plasma membrane of different cells such as macrophages and is involved in the transport of a wide variety of drugs. BZL is the only drug available for the treatment of Chagas disease, although its efficacy is low and produces serious side effects. In this study we evaluated the effect of BZL on the expression of P-gp in RAW 264.7 cells. The cells were exposed for 48 h to BZL (0.5 mM) or vehicle (DMSO). P-gp protein levels were evaluated by western blotting in total homogenate. To estimate if P-gp is involved in BZL efflux, control and BZL-treated cells were incubated for 2 h with BZL (0.5 mM) in the absence or presence of the P-gp inhibitor Verapamil (V, 100 µM,) and BZL intracellular accumulation was measured by HPLC. **Results:** P-gp levels were increased by 200% (n=3) in BZL group vs. controls. Inhibition of drug efflux by V was more significant in BZL group. **Conclusion:** Induction of P-gp by BZL could reduce BZL intracellular concentration, and thus, the drug-parasite interaction. Co-administration of BZL with P-gp inhibitors could be useful to increase the efficacy of treatment.

BI-10.**SPIRONOLACTONE (SL) MODULATES THE EXPRESSION OF THE CANALICULAR TRANSPORTER P-GLYCOPROTEIN (P-gp) IN HepG2 CELL LINE**

Rigalli JP, Ruiz ML, Pellegrino JM, Mottino AD, Catania VA. IFISE-CONICET, Fac. de Cs. Bioq. y Farm. (UNR). Suipacha 570, 2000, Rosario, Argentina. E-mail: vcatania@fbioyf.unr.edu.ar

P-gp is involved in pumping lipophilic and cationic compounds out of polarized cells. We have previously shown that SL up-regulates the expression of P-gp in rat liver. Here, HepG2 cells, derived from a human hepatocarcinoma, were exposed for 48 h to SL (50 µM) or vehicle (DMSO). P-gp protein levels were evaluated by western blotting of total homogenates. To estimate P-gp activity, cells were incubated with its model substrate Rhodamine-123 (Rho-123, 5 µM, 2 h) with or without the P-gp inhibitor Verapamil (50 µM). After 30 min, the efflux of Rho-123 to the incubation medium was measured. **Results:** P-gp levels were increased by 400% (n=3) and the ratio extruded/intracellular Rho-123 was higher (3.5-fold) in SL-treated vs. control cells. Verapamil abolished the difference in this ratio between control and SL-treated cells. These data reveal that SL increases P-gp expression and, consequently, P-gp activity in HepG2 cells. **Conclusion:** Induction of human P-gp can influence the hepatic clearance of drugs that are P-gp substrates when they are co-administered with SL.

BI-11.**PREVENTIVE EFFECT OF SPIRONOLACTONE (SL) ON ETHYNYLESTRADIOL (EE)-INDUCED DOWNREGULATION OF Mrp2 IN RAT INTESTINE**

Ruiz ML, Arias A, Villanueva SSM, Rigalli JP, Luquita MG, Pellegrino JM, Mottino AD, Catania VA.

IFISE-CONICET, Fac. Cs. Bioq. y Farm., UNR, Suipacha 570 2000 Rosario, Argentina. E-mail: mruiz@fbioyf.unr.edu.ar

We previously observed that EE administration to rats down-regulates intestinal Mrp2. Here, we evaluate if treatment with SL, an inducer of hepatic Mrp2, is able to prevent the impairment in intestinal Mrp2 produced by EE. Adult male Wistar rats (n=3) were injected with EE (5 mg/kg/day, s.c.), or SL (200 µmoles/kg/day, 3 days, i.p.), or both (EE + SL; SL the last 3 days of the EE treatment). Control group received vehicle (propylene glycol s.c. and i.p.). Mrp2 protein expression was detected by western blotting in brush border membrane from jejunum. Mrp2 activity was estimated using isolated intestinal sacs (serosal-mucosal efflux), with dinitrophenyl-glutathione (DNP-SG) as a model substrate. **Results:** EE decreased (-80%) and SL increased (+400%) Mrp2 expression when administered individually. Co-treatment prevented the decrease in Mrp2 expression so that no differences were detected with respect to control rats. EE treatment decreased (-42%) and SL treatment increased (+56%) mucosal accumulation of DNP-SG at 30 min. The combined treatment showed no effect on DNP-SG efflux when compared to controls, correlating well with western blot studies. **Conclusion:** SL could be of potential therapeutic application to prevent downregulation of Mrp2 induced by EE.

BI-12.**QUANTIFICATION OF REDUCED AND TOTAL GLUTATHIONE IN PLASMA AND LIVER IN HEALTHY CATS**

Denzoin Vulcano L, Soraci A, Tapia MO.

Area de Toxicología, Dpto de Fisiopatología, FCV-UNCPBA. Campus Universitario. Paraje Arroyo Seco. Tandil, Argentina. E-mail: laura@vet.unicen.edu.ar

Glutathione is an important intracellular tripeptide with multiple functions. Glutathione has evolved to serve diverse functions in biological systems including detoxification of xenobiotics, transport of amino acids, stabilization of cell membranes and synthesis of proteins and DNA. Abnormal glutathione metabolism is thought to play an important role in various diseases of cats. The purpose of this work was to establish baseline data for future studies. A rapid, simple high performance liquid chromatography method for the quantification of reduced glutathione (GSH), oxidized glutathione (GSSH) and total glutathione (GSHt) in plasma and liver in healthy cats is described. The mean plasma concentrations of GSH, GSSH and GSHt were 4.51 ± 1 µM; 19.44 ± 3.79 µM (expressed as GSH equivalent) and 23.59 ± 3.89 µM, respectively. The mean concentrations of GSH, GSSH and GSHt in liver were 32.31±9.75; 14.46±7.94; 46.77±14.35; respectively expressed as nm/mg of proteins. More studies about the metabolism of GSH and its role in diverse pathologies in cats are needed.

BI-13.**EFFECTS OF TWO LEVELS OF DIETARY PROTEIN AND ENERGY:PROTEIN RELATION ON VARIABLES OF THE ENERGETIC METABOLISM IN MALE BROILER CHICKEN**

Sandoval GL², Terraes JC¹, Fernandez RJ¹, Esquivel GP², Obregón GRE².

Dptos. Cs. ²Básicas y ¹Producción, Fac. de Cs. Veterinarias, Univ. Nac. del Nordeste, Sgto Cabral 2139, 3400, Corrientes (Cap), Argentina. E-mail: bioquim@vet.unne.edu.ar

Two treatments with seven replications each one, were applied on 196 male chicken (six birds per m²), from a single commercial genetic lineage (Cobb Vantress, 2007, slow feathered gen). They consisted in two diets: broiler food (P) and fodder food (F). The cycle was divided in two stages: beginning (0 to 21 days) and fattening (21 days to end). The beginning food (I = 3000 Kcal/Kg) and the ending food (T= 3150 Kcal/Kg) had identical metabolic energy level, but with a lower protein level in F. The results of the analysis of repeated measures were as follows: except to total cholesterol (CT), the glucemia (Glc), body weight, canal proportion (Rend) and accumulated abdominal fat relative weight (AFat) were different between 21 and 49 days (p< 0,05). Glc decreased with the age, while Rend and AFat increased with the PC. The level of AFat increased with the higher energy:protein relation diet (0,88 and 1,24 for P and F respectively, p<0,05). This variation was not accompanied by the an increment of circulating CT. The tested nutritional formulations would have two important factors to consider for their election, the effect of the decrease of the clean weight (the extraction of abdominal fat) and the smaller relative cost of a diet which energy:protein relation is greater.

BI-14.**YIELD AND PROTEINEMIA OF MALE BROILER CHICKEN GROWN IN INDOOR CORRALS, FED WITH ISOENERGETIC FORMULATIONS OF DIFFERENT PROTEIN LEVELS**

Sindik M¹, Sandoval GL², Esquivel GP², Ceballos FA², Pino M¹.
Dptos. Cs. ²Básicas y ¹Producción, Fac. de Cs. Veterinarias, Univ. Nac. del Nordeste, Sgto Cabral 2139, 3400, Corrientes (Cap), Argentina. E-mail: bioquim@vet.unne.edu.ar

Two treatments with seven replications each one were applied on 196 male chicken (six birds per m²), from a single commercial genetic lineage (Cobb Vantress, 2007, slow feathered gen). They consisted in two diets: broiler food (P) and fodder food (F). The cycle was divided in: beginning (0 to 21 days) and fattening (21 days to end). The initiatory food (I = 3000 Kcal/Kg) and the ending food (T= 3150 Kcal/Kg) had identical metabolic energy, but with a lower protein level in F. The results of the analysis of repeated measures were as follows: alkaline phosphatase (ALP); body weight (PC); straight yielding (Rend); and relative weight of stomach, liver & gall bladder, and intestine & pancreas showed differences (p< 0,05) between two sample groups (21 and 49 days). The PC percentage corresponding to the digestive tract and annexed glands decreased with the age, while Rend increased with the PC. ALP was higher at 21 than at 49 day. Uni (2001) described that the alkaline phosphatase, from the intestinal mucous membrane, is positively correlated with the number of enterocytes and the longitude of the intestinal villi. These results showed that both formulae were able to sustain similar productive performances. The other detected differences showed ontogenic changes in yield and biochemical variables.

BI-15.**SERUM ALKALINE PHOSPHATASE OF FEMALE BROILER CHICKEN FED WITH TWO PROTEIN DIET LEVELS, ACCORDING TO AGE AND BODY GROWTH**

Sandoval GL², Terraes JC¹, Bettella A³, Pletsch C¹, Laffont GV².
Deptos. Cs. ²Básicas y ¹Producción, Fac. de Cs. Veterinarias, Univ. Nac. del Nordeste - EEA, El Colorado, Formosa, ³INTA. Sgto Cabral 2139, 3400, Corrientes (Cap), Argentina.
E-mail: bioquim@vet.unne.edu.ar

Females (H) Broiler chickens, from a commercial line (Cobb Vantress, 2007, slow fledge gen), were raised in corrals (6 birds/m²) at INTA-El Colorado, Formosa, Argentina. The cycle was divided in two stages: beginning (0 to 21 days) and fattening (21 to 56 days). Two treatments consisting in qualitative-quantitative different diets called broiler feed and fodder feed, were applied. No differences were found between diets. Proportional weight of leg & head and skeleton (Ske) –without legs & head- relatives to body weight, the serum alkaline phosphatase activity (ALP) and the relative weight of the digestive organs (stomach -Sto-, liver & gall bladder and intestine & pancreas -I&P-; p<0,01) lowed along growing. Positive correlations (Pearson) were found (p<0,005) between ALP vs nutritive efficiency (EfAlim) ratio, Ske, legs & head and I&P. Ske and I&P were not related between them, but they were related in equal sense with the other variables. EfAlim ran parallel to Sto. In general, the analyzed variables decreased with the age, correlating positively between them. The qualitative - quantitative differences between the diets did not affect the indicators at the end of the productive cycle. Considering a good practice the use of anyone of them according to availabilities and prices.

BI-16.**ESTIMATION OF HIDROXYL RADICAL GENERATION BY SALICYLATE HIDROXYLA TION METHOD IN PROSTATE, BREAST AND OVARY OF RATS EXPOSED TO 2,4-DICHLORO PHENOXYACETIC ACID (2,4-D)**

Pochettino A, Duffard R, Evangelista A.
LATOEX. Facultad de Cienciass. Bioquímicas y Farmacéuticas.
U.N.R. Suipacha 531. 2000 Rosario.
E-mail: aristidespochettino@gmail.com

Chlorophenoxy herbicides are widely used for the control of broadleaf weeds. We previously reported that 2, 4-D increase the oxidative stress and induction of death in brain areas and culture cells. The aim of the present work was to obtain direct evidence of oxygen radical activity in prostate, breast and ovary. We used a method based on the chemical trapping of hydroxyl in the form of the stable adducts 2,3 and 2,5- dehydroxibenzoic acid (DHBA) following salicylate (SA) administration. Virgin female 90 day-old Wistar rats were made pregnant, and the treated group was exposed to 2,4-D (70 mg/kg/day, sprayed on food) from gestation day 16 onward. On postnatal day 23, pups were weaned and the treated group continued to be fed with 2, 4-D until sacrifice by decapitation at 45, 60 or 90 days of age. Date showed the ratio of 2, 3- DHBA to SA was significantly increased in prostate at the three ages and in the ovary at 90 days old. No changes were observed in the breast. These results indicate that 2,4-D could increase the oxidative stress during the whole development of the prostate gland and in the adult ovary.

BI-17.
2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) EFFECTS ON THE RAT PROSTATE DEVELOPMENT

Pochettino A, Biolatto S, Duffard R, Evangelista A.
LATOEX. Facultad de Ciencias. Bioquímicas y Farmacéuticas
UNR. Suipacha 531. 2000 Rosario. Argentina.
E-mail: aristidespochettino@gmail.com

Hormone-dependent process such as growth, maintenance and development of the normal prostate gland could be altered by xenobiotics. The biological function of androgen in the prostate is mediated by androgen receptor (AR). The goal of the present work was analyze by Western blot and by histological and morphometrical studies, whether 2,4-D - which is an herbicide used to control the growth of broadleaf weeds- had effects on the developing prostate. Wistar rats were made pregnant and exposed to 2,4-D (70 mg/kg/day, sprayed on food) from gestation day 16 onward. On postnatal day 23, pups were weaned and the treated group continued to be fed with 2, 4-D until sacrifice by decapitation at 45, 60 or 90 days of age. We observed a significant decrease in the height of prostatic epithelial cells of the alveoli in the 2,4-D treated rats of 45 (37%) and 90 (40%) days old. An increase in luminal volume, a diminution in the number of cells per unit area (29%) with respect to control at 90 days of age was also determined. In addition, Western blot analysis indicated that the level of AR protein was increased (38%) at this age in the treated animals, In conclusion, the development of prostate was affected by 2,4-D herbicide.

BI-18.
DEVELOPMENT OF A MULTIRESIDUE ANALYTICAL-METHOD VALIDATION TO QUANTIFY BENZIMIDAZOLE DRUGS IN HEN'S EGG

Bistoletti M, Moreno L, Ceballos L, Alvarez L, Lanusse C.
Laboratorio de Farmacología, Facultad de Ciencias Veterinarias,
Universidad Nacional del Centro de la Provincia de Buenos Aires,
Tandil. CONICET. Argentina. E-mail: clanusse@vet.unicen.edu.ar

Albendazole (ABZ), fenbendazole (FBZ) and flubendazole (FLBZ) are antiparasitic drugs belonging to the benzimidazole group (BZD) widely used in veterinary medicine including in poultry. There are many published methods to quantify these molecules in diverse tissue/fluid of different mammalian species. However, the availability of recent method multiresidue published to determine these molecules in poultry is scarce. The goal of this research was to develop and validate a multiresidue method to quantify BZD in plasma and eggs of treated hens. Ten molecules were included in the validation: ABZ, FBZ, FLBZ and their respective metabolites. The quantification was realized by HPLC with UV detection (292 nm). A C18 stationary phase, mobile phase with gradient elution at 1.2 ml flow were used. The validation process was carried out following international criteria, including: selectivity, linearity, stability, precision, accuracy, recovery, limits of detection and quantification. All results obtained for each validation parameter in plasma and eggs fell within accepted range. The developed methodology allows the quantification of ten molecules simultaneously after a simple and low cost liquid-liquid chemical extraction.

BI-19.
THERMAL STABILITY OF IVERMECTIN RESIDUES DURING MILK PROCESSING

Iezzi S, Imperiale F, Lifschitz A, Lanusse C.
Laboratorio de Farmacología, Facultad de Ciencias Veterinarias,
UNCPBA. Tandil, Argentina. E-mail: fernanda@vet.unicen.edu.ar

Ivermectin (IVM) is broad-spectrum macrocyclic lactone (ML) antiparasitic drug extensively used in food-producing animals. The pattern of milk residue excretion for different ML compounds has been recently determined in our laboratory. The current trial addressed the evaluation of the stability of residual IVM concentrations in milk under conditions of heating treatment during milk processing. IVM concentrations were measured in milk using an HPLC-based methodology with fluorescence detection. IVM (0.1-20 ng/ml) was added to drug-free milk samples collected from untreated lactating cows. Milk samples with drug added were heated at 65°C for 30 minutes (pasteurization). IVM concentrations were measured prior and after the heating process. The results obtained indicated that no significant changes in the IVM residue profiles were observed after thermal treatment. The variation observed in heated milk residual concentration was within the range of the analytical method. The impact of these residual drug concentrations in milk-derived product on human safety and industrial processing are under evaluation.

BI-20.
DEVELOPMENT OF AN HPLC ASSAY TO DETERMINE FLUAZURON RESIDUES IN BOVINE TISSUES

Imperiale F, Farias C, Iezzi S, Sallovitz J, Lanusse C.
Lab. de Farmacología, Fac. de Ciencias Veterinarias. UNCPBA.
Tandil, Argentina. E-mail: fernanda@vet.unicen.edu.ar

Fluazuron (FZ) is a halogenated benzoylurea insecticide extensively used in cattle for tick control. Development of adequate analytical methodology is required to assess FZ residues in tissues from treated animals derived to human consumption. A simple reversed-phase HPLC analytical method with ultraviolet detection at 260 nm was developed, validated and applied for quantitative determination of FZ in bovine plasma and tissues. Linearity, precision, recovery and limit of quantification of the method were determined. Drug extraction from samples was effectively performed using liquid extraction and clean up by solid phase extraction. Regression analyses were linear over the concentration range examined (from 0.1 to 10 µg/ml) and correlation coefficients of calibration lines were >0.99. The developed HPLC method allowed the quantification of FZ up to 0.1 µg/ml (plasma) and 0.5 µg/g (tissues) with internationally accepted coefficient of variations (<15%). Recovery values were higher than 70%. The results obtained indicated that the developed chromatographic method is selective, accurate and easy to reproduce. The analytical method described here is a useful tool for the measurement of this insecticide molecule in different bovine tissues.

BI-21.
OXIDATIVE DAMAGE IN *MONIEZIA* SPP INCUBATED WITH DIFFERENT INCUBATION MEDIUMS

Cadenazzi G, García C, Álvarez L, Sansinanea A.
 Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina.
 E-mail: cadenazzi@vet.unicen.edu.ar

Anthelmintics drugs may induce oxidative damage in the target parasite, as an indirect consequence of its intrinsic mechanism of action. This feature could be used to evaluate the relative potency of different anthelmintics compounds. The aim of the present work was to evaluate the viability and basal oxidative damage in *Moniezia* spp after its incubation with different incubation mediums. The tapeworm material (1 g) was incubated at 37°C in 10 ml of buffer KRT or RPMI medium at pH 7.5 and 6.5 during 5, 15, 30, 60, 90 and 120 min. There were four replicate assays for each incubation time. Once the incubation time had elapsed, the parasite material was prepared for malonildialdeído (MDA) determination, which was used as an indicator of oxidative damage. Preliminary results indicate that in either, KRT buffer or in RPMI medium, MDA concentrations of 22 ± 0.8 nmol/mg protein were measured at the different incubation times evaluated. The *ex vivo* incubation of *Moniezia* spp under in our experimental conditions did not induce a significantly increase in the oxidative damage observed in tapeworms. Follow up studies on relative potency of anthelmintic drugs will be performed.

BI-22.
LIVER EXPRESSION OF CAT-1 IN RAT MODEL OF ACUTE HEPATOTOXICITY BY THIOACETAMIDE

Perdomo VG¹, Pretto L¹, Daniele SM², Nocito AL³, Palatnik JF⁴, Veggi LM^{1,2}.
¹IFISE (CONICET-UNR), ²Fac. C. Bioquím. y Farmacéuticas (UNR), ³Fac. Ciencias Médicas (UNR), ⁴IBR (CONICET-UNR).
 Suipacha 570, (2000) Rosario, Argentina.
 E-mail: veggi@ifise.gov.ar

The cationic amino acid transporter 1 (CAT-1) is expressed fairly ubiquitously in mammalian cells but its levels vary significantly in different cells and conditions. CAT-1 is required in the regenerating liver, being essential for liver cells to enter mitosis. The liver damage produced by thioacetamide (TA) in rats is a classic model of hepatotoxicity. Adult male wistar rats were treated with TA (i.p., 400 mg/kg, n=3) and saline solution (vehicle, i.p., n=3) being sacrificed 24 hs later. Serum samples were analyzed for liver markers of toxicity. Livers were extracted and used for histological and expression studies. We confirmed the hepatotoxicity in TA treated rats by Liver histopathological examination (perivascular injury) and serum toxicity markers (an increased activity of ALT, AST, ALP and LDH). We observed an induction of CAT-1 protein expression by western blot (relative expression RE, TA 2.01 ± 0.49 vs C 1.00 ± 0.28 , $p < 0.05$) and CAT-1 mRNA by quantitative real time PCR (RE, TA 27.44 ± 7.33 vs C 1.00 ± 0.55 , $p < 0.05$). We found that the expression of proliferating cell nuclear antigen, in the liver is significantly elevated in rats treated with TA by western blot (RE, TA 3.28 ± 0.50 vs C 1.00 ± 0.34 , $p < 0.05$). These experiments indicates that CAT-1 expression is increased by TA induced acute hepatotoxicity in liver rats and correlates with cell proliferation.

BI-23.
***ECHINOCOCCUS GRANULOSUS* KEY ENZYMES AS BIOCHEMICAL MARKERS OF *IN VITRO* PHARMACOLOGICAL DAMAGE**

Elissondo C, Denegri G, Cumino A.
 Laboratorio de Zoonosis Parasitarias, FCEyN, Universidad Nacional de Mar del Plata. CONICET. Funes 3350. Mar del Plata, Argentina. E-mail: mceliss@mdp.edu.ar

Enzymic markers could be used as endogenous indicators for identifying cellular changes and for providing quantitative data in pharmacological responses. The aim of this work was to find enzymes as biochemical markers of damage during *in vitro* drug treatment of *E. granulosus* protoscoleces. The levels of alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), cholinesterase (ChE), glutamic-pyruvic transaminase (GPT), glutamic-oxalic transaminase (GOT) and lactic acid dehydrogenase (LDH) were determined in medium supernatants of drug-treated parasite with benzimidazoles, avermectins and calcium modulators using kinetic spectrophotometric methods. It is the first study where the AP activity was determined in medium supernatant of *E. granulosus* cultures. AP and GOT showed considerable high levels in the control group. The rise of membrane-enzyme activities was related with later tegumental alterations. The enhancement of CHE and LDH levels were associated with the increase of mortality. We found at least a 10-fold increase of GGT and CHE activities in treated versus control groups. Therefore these enzymes would be applicable as possible markers in mass screenings for chemotherapeutic studies in *E. granulosus*.

BI-24.
IMMUNOLocalIZATION OF CERTAIN DETOXIFYING PROTEINS IN FASCIOLA HEPATICA. RELATIONSHIP WITH RESISTANCE MECHANISMS

Scarcella S¹, Solana H¹, Larsen M, Felipe A¹, Alzola R¹, Lanusse C².
¹Dpto. Cs. Biológicas and ²Dpto. Fisiopatología FCV-UNCPBA, Campus Universitario, 7000-Tandil, Buenos Aires.
 E-mail: silvanas@vet.unicen.edu.ar

Fasciolosis, is an emergent zoonotic disease. The drug of choice for its treatment is triclabendazole (TCBZ). Today, the extensive use of TCBZ has already generated resistance. The mechanisms of anthelmintic resistance include involvement of detoxification processes generated through the FMO enzymatic pathway or through drug extrusion pumps such as the P Glycoprotein (P Gp). The aim of the present work was to immune-locate the presence and distribution of P-Gp and FMO in TCBZ susceptible and resistant *Fasciola hepatica*. The immunohistochemical study confirmed the presence of P Gp in both strains, distributed in the enterocytes and a smaller quantity in the vitellogenic cells and specula. FMO location seems to be restricted to the vitellogenic cells. This distribution pattern was similar for both strains, without differences in their distribution and expression. It is inferred that the resistance may involve modifications at metabolic levels, without detectable morphologic changes, at least not observable by optical microscopy.

BI-25.**EFFECT OF LICKING ABOUT MILK EXCRETION OF MOXIDECTIN ADMINISTERED POUR-ON IN DAIRY CATTLE**

Imperiale F, Farias C, Sallovitz J, Lifschitz A, Virkel G, Lanusse C. Lab. Farmacología, Fac. Ciencias Veterinarias. UNCPBA. Tandil, Argentina. E-mail: fernanda@vet.unicen.edu.ar

Moxidectin (MXD) is a broad-spectrum endectocide antiparasitic drug extensively used in food-producing animals. Pour-on formulations of MXD are marketed internationally for use in dairy cattle. The current work was designed to measure plasma and milk concentration profiles of MXD using an HPLC-methodology after its pour-on administration to dairy cows. The influence of natural licking behavior of cattle on MXD plasma and milk concentrations was evaluated. Licking prevention during 5 days post-treatment determined changes on MXD plasma and milk kinetics. In licking-restricted cows, MXD plasma and milk availability were significantly lower than those in free licking animals. As shown earlier for other endectocide compounds, licking behavior may facilitate the oral ingestion of topically-administered drug in cattle. This would be consistent with the marked lower MXD concentration profiles measured in plasma and milk of licking prevented animals. The work reported here provides relevant information on the pattern of MXD excretion in milk after pour-on treatment, and contributes to understand the variability observed in the antiparasitic persistence of topically-administered endectocides in cattle. The implications of natural licking in topical treatments need to be seriously assessed to achieve accurate pharmacological studies aiming to a possible need of reviewing drug approval processes.

BI-26.**RESIDUE DEPLETION OF IVERMECTIN IN RABBIT TISSUES AFTER SUBCUTANEOUS ADMINISTRATION**

Daniele M, Errecalde F, Lambertini A, Mestorino N, Errecalde JO. Pharmacology Department, Faculty of Veterinary, Universidad Nacional de La Plata. (1900) La Plata, Argentina. E-mail: jerrecal@fcv.unlp.edu.ar

Ivermectin (IVM) is used against a wide spectrum of endo and ectoparasites. *Notoedres cati* var. *Cuniculi*, the agent of rabbit mange, is very sensitive to IVM. Pharmacokinetic and residual studies of IVM in rabbits, however, are rather scarce. The objective of the present paper was to study the tissue residue profile of IVM after SC administration of a 1% solution. Twelve young healthy male rabbits received 200 µg/kg of a 1% IVM formulation subcutaneously. Groups of three treated animals were sacrificed at 10, 20, 30 and 40 days after injection. Samples of liver, fat, kidney, muscle and injection tissue were obtained. IVM concentrations were determined by HPLC with fluorescence detection after SPE extraction.

IVM concentrations were determined after subcutaneous administration until 40 days. Muscle samples showed the lowest IVM concentrations throughout the study period. The highest IVM concentrations at all sampling times were measured in liver and fat tissues. Nevertheless IVM concentrations in all of the tissues analyzed were below the accepted maximum residue limits recommended by the European Union at 20 days post-treatment.

BI-27.**TYLOSIN CONCENTRATIONS IN HONEY AND BEES (*Apis mellifera*) AFTER ITS ADMINISTRATION TO HEALTHY BEEHIVES**

Huber B, Quintero M, Daniele M, Mestorino N, Errecalde JO. Cátedra de Farmacología. Facultad de Cs. Veterinarias. Universidad Nacional de La Plata. CC 296, 1900 La Plata, Argentina. E-mail: barhuber@gmail.com

Due to the limited information available about tylosin in honeybees, we decided to evaluate the evolution of concentrations and pharmacokinetic profile of the tylosin (Tyl) in honey and bees of high and low production hives during autumn. Twelve healthy beehives were inoculated with powdered sugar added with Tyl at a dose rate of 200 mg per week during three weeks. Honey and bees samples were obtained from the breeding chamber at different post-administration times. Tyl concentrations were determined by microbiological assay. The pharmacokinetic analysis was performed by WinNonlin Professional 5.2. We found significant statistical differences between pharmacokinetic parameters in honey and bees from different production levels. The high levels of tylosin residues in honey from low production hives (0.31 µg g⁻¹ 84 days after treatment) increased the risk of residues in this kind of exploitations in harvest time.

BII-28.**COMPARATIVE *IN VITRO* DERMAL ABSORPTION OF MOXIDECTIN AND DORAMECTIN THROUGH BOVINE SKIN**

Sallovitz J^{1,2}, Nejamkin P¹, Lifschitz A^{1,3}, Imperiale F^{1,3}, Virkel G^{1,3}, Lanusse C^{1,3}.

¹Lab. Farmacología, FCV, UNCPBA, Campus Universitario, 7000 Tandil, Argentina. ²CICPBA. ³CONICET.

E-mail: clanusse@vet.unicen.edu.ar

Endectocide drugs are used topically to treating ruminant parasitic diseases due to their easy administration, which also avoids residues in an edible administration site. However, available knowledge on their transdermal absorption in cattle is scarce. A practical *in vitro* method to evaluating new topical formulations before *in vivo* assays is not available. The transdermal absorption of moxidectin and doramectin topical formulations was evaluated using an *in vitro* model of bovine epidermis. Epidermal layers (500 µm) were placed on modified Franz diffusion cells. Receptor medium was buffer phosphate (0.1 M), albumin and ethanol (76:4:20). Sampling ranged from 0 to 48 h post-administration. Drug concentrations were quantified by HPLC. By 16.2±6.9 h (MXD) and 33.2±6.3 h (DRM) post-administration, permeation plateaus were reached. Permeation flows (MXD: 3.8±3.1, DRM: 10.1±8.1 ng·cm⁻²·h⁻¹) differed statistically (*P*<0.05). Permeation coefficients (MXD: 1.6x10⁻⁰⁶±1.3x10⁻⁰⁶ cm/h, DRM: 4.12x10⁻⁰⁶±3.44x10⁻⁰⁶ cm/h) were similar (*P*>0.05). These results agree with the faster plateau and shorter T_{lag} of MXD (2.4±0.6 h) compared to DRM (12.2±5.4 h), which could be due to different lipophilicity. Results are in agreement with *in vivo* kinetic data. The *in vitro* model presented here is useful to predicting and further understanding of endectocides' transdermal absorption process in cattle.

BII-29.
**EFFECTIVENESS OF ENHANCERS' COMBINATION ON
 TRANSDERMAL PERMEATION OF PROBENECID**

Lhez L, Allemandi DA, Pappano NB, Debattista NB.*
Universidad Nacional de San Luis. Lavalle 1155, San Luis.
*E-mail: llhez@unsl.edu.ar *Universidad Nacional de Córdoba.*

The pharmaceutical design is a very complex field due to the existence of multiple forms of administration. The oral route presents some disadvantages such as gastric irritation, exposition of the drug to extreme pH and degradation by hepatic first pass. A non-invasive alternative to avoid these effects are the transdermal formulations, which present the advantage to release the drug in constant and prolonged way. Nevertheless the transdermal drug administration itself is limited by the characteristics of the skin, which offers a remarkable resistance to the penetration of the active principles. Therefore, it is necessary to include in the formulations chemical compounds that, by different mechanisms, facilitate the penetration of the drug. In this study, using Franz diffusion cells, *in vitro* transdermal permeation of probenecid (uricosuric drug) in solid vaseline through dermatomized pig skin was assayed. Also, the isopropyl alcohol action and their combinations with terpene enhancers (L-menthol or D-limonene) were investigated. The obtained results allow to determine that isopropyl alcohol increases 5-fold the diffusion coefficient value ($D = 2.022 \times 10^{-7} \text{ cm}^2/\text{s}$) in relation to the formulation without enhancer ($D = 3.998 \times 10^{-8} \text{ cm}^2/\text{s}$). The addition of L-menthol or D-limonene to the formulation containing isopropyl alcohol increases 4-fold, approximately, its diffusion coefficient value. This fact shows the existence of a synergic effect between these terpenes and isopropyl alcohol.

BII-30.
CHRONOKINETIC STUDY OF INTRAMUSCULAR ADMINISTRATION OF CEFTAZIDIME IN DOGS

Monfrinotti A, Ambros L, Montoya L, Waxman S, Reuelto M.
Cátedra de Farmacología, Facultad de Ciencias Veterinarias,
Universidad de Buenos Aires. Chorroarín 280 (1427), Bs. As.
E-mail: amonfrinotti@fvet.uba.ar

The purpose of this study was to identify if time of day administration modified ceftazidime relevant pharmacokinetic parameters administered to dogs. Six healthy female mixed breed adult dogs weighing 14-22 kg were given a single 25 mg/kg dose of ceftazidime by the intramuscular route at 8.30 and 20.30 h after an 8 h fast, with a 2 week washout period. Blood samples were taken at predetermined times. Concentrations of ceftazidime were determined by microbiological assay. Data were analysed by noncompartmental techniques using PCNONLIN software. Results are reported as mean \pm standard deviation. For the 8.30 h administration, peak concentration, time to peak concentration and elimination half-life were $80.2 \pm 20.7 \mu\text{g/ml}$, $1 \pm 0.27 \text{ h}$ and $1.13 \pm 0.30 \text{ h}$, respectively. For the 20.30 h administration, peak concentration, time to peak concentration and elimination half-life were $104.30 \pm 24.98 \mu\text{g/ml}$, $0.75 \pm 0.44 \text{ h}$ and $1.1 \pm 0.33 \text{ h}$, respectively. No statistically significant differences were detected for all the pharmacokinetic parameters, however, high standard deviations may have accounted for this lack of difference. Our data suggest that time of administration may not modify the pharmacokinetics of intramuscular ceftazidime in dogs.

BII-31.
**ORAL ABSORPTION OF CEPHALEXIN ADMINISTERED
 IN DAY-TIME, NIGHT-TIME AND AFTER METOCLOPRAMIDE IN DOGS**

Prados AP, Reuelto M.
Cátedra de Farmacología, Facultad de Ciencias Veterinarias,
Universidad de Buenos Aires. Chorroarín 280 (1427), Bs. As.
E-mail: aprados@fvet.uba.ar

The purpose of this study was to compare if the time of administration or metoclopramide pretreatment modified the absorption of cephalexin after its oral administration to dogs. Six healthy adult Beagle dogs weighing 9-13 kg received a single dose (25 mg/kg) of cephalexin as a 5% oral suspension at 10.00 h (day-time) or 22.00 h (night-time) and also 5 min following the intravenous administration of metoclopramide (0.5 mg/kg) after 8 h fasting period. Blood samples were taken at predetermined times. Cephalexin plasma concentrations were quantified by a microbiological method with *Micrococcus luteus* 9341 ATCC as test microorganism. Mean cephalexin concentrations for each treatment were used to evaluate the absorption process by the Wagner-Nelson method. Results showed a first-order absorption process for the three treatments. Statistically significant differences were detected for the regression line slope of log-unabsorbed fraction of cephalexin versus time. Absorption rates were 1.06, 1.75 and 1.54/h for the 10:00 h-treatment (day-time), 22:00 h-treatment (night-time) and after metoclopramide pretreatment, respectively. Absorption times were 3.5 h (day-time), 2.5 h (night-time) and 1.5 h (metoclopramide pretreatment). Our results show that the moment of administration and also metoclopramide pretreatment affected cephalexin oral absorption in dogs.

BII-32.
**PLACENTAL TRANSFER OF LOCAL ANESTHETICS (LA):
 PREDICTIONS FROM A PHYSICO-CHEMICAL MODEL**

Miceli M, Serra H.
1^{ra} Cátedra de Farmacología, Facultad de Medicina, Universidad
de Buenos Aires. Paraguay 2155 piso 15. 1121 Buenos Aires, Argentina.
E-mail: haserra@fibertel.com.ar

Local anesthetics (LA) are commonly used drugs during labor. Despite their epidural or subarchnoidal application, maternal plasma levels are clinically relevant. In addition, LA produce many early (CNS depression, hypotension, jaundice) and long lasting (low rating in neurocognitive scores) neonatal side effects indicating certain placental transfer. Thus, a kinetic model of placental transfer could be useful to predict LA neonate's plasma levels. LA are cationic drugs, so their permeability and degree of compartmental trapping are pH dependent. Our model, running in MS Excel 2003 worksheet for Windows®, Microsoft Corp, allows calculate the LA compartmental trapping (R_b) and theoretical neonate concentrations for a given pH. If neonatal pH lies between 7 – 7.2 in an optimum labor conditions, the obtained R_b (1.4 – 2.3 according to each pH and LA) would indicate that neonate concentrations doubled the maternal concentrations. These data correlate well with the observed dose dependent side effects of LA.

BII-33.
PHARMACOLOGICAL STRATEGIES TO IMPROVE
IVERMECTIN EFFICACY AGAINST RESISTANT NEMA-
TODES

Lifschitz A^{1,2}, Entrocasso C³, Alvarez L^{1,2}, Lloberas M³, Ballent M^{1,2}, Manazza J³, Virkel G^{1,2}, Borda B³, Lanusse C^{1,2}.

¹Lab. Farmacología, FCV, UNCPBA, Tandil, Arg. ²CONICET. ³EEA INTA Balcarce, Argentina. E-mail: adrianl@vet.unicen.edu.ar

The involvement of the efflux-transport protein P-glycoprotein (Gp-P) on both the pharmacokinetic disposition and resistance mechanisms to macrocyclic lactones has been described. This work aimed to study the effects of loperamide (LPM), a Gp-P modulating agent, on ivermectin (IVM) pharmacokinetics and efficacy against resistant nematodes in sheep. Eighteen Corriedale lambs naturally infested with gastrointestinal nematodes were assigned into three experimental groups. Group A remained as untreated control. Other animals (Groups B and C) received ivermectin either alone or co-administered with LPM (0.2 mg/kg, 2 times every 12 h). Blood samples were collected between up to 14 days post-treatment and IVM plasma concentrations were determined by HPLC. The pattern of efficacy was estimated by the faecal egg count reduction test (FECRT) and adult nematode counts. LPM enhanced the IVM plasma availability (47%) in co-administered lambs. The FECRT values were increased from 79% to 96% in the presence of LPM. The general efficacy against nematodes was increased from 48% to 77% after the LPM co-administration. The clinical relevance of this pharmacokinetic/pharmacodynamic interaction is under study in our laboratory.

BII-34.
PHARMACOKINETICS OF CEFOPERAZONE IN NORMAL
AND MASTITIC GOATS

Lacunza J¹, Cordiviola C², Farina OH³, Rule R¹.

¹Commission of Scientific Research of the Province of Buenos Aires.

²Department of Introduction to Animal Production, Faculty of Agricultural and Forestry Science, ³Applied Pharmacology, Faculty of Medicine, La Plata University, Argentina.

E-mail: josefinalacunza@hotmail.com

Cefoperazone is a 3th generation semisynthetic cephalosporin antibiotic active against Gram-positive and Gram negative bacteria. In the present experience, six healthy lactating goats were used (Trial 1), these same animals were later induced with mastitis (Trial 2). In both Trials the animals received two doses of cefoperazone (Lab. Richet) by intramammary route (100 mg). Milk non-compartmental pharmacokinetic values in normal and mastitic glands were determined. Results. Elimination half-life (t_{1/2}) (Trial 1) 9.3 ± 1.9, (Trial 2) 11.9 ± 3.5 h; the area under the curve [AUC₍₀₋₁₀₈₎] (Trial 1) 7040.2 ± 3491.1, (Trial 2) 21044.0 ± 22319.9 µg.ml⁻¹.h and the mean residence times (MRT) (Trial 1) 9.5 ± 1.9 and (Trial 2) 10.3 ± 4.7 h. The results suggest that cefoperazone milk concentrations were high until 48 hours after second dose administration. Residues were detected in normal and mastitic milk during 108 hours postadministration of the antibiotic.

BII-35.
PHARMACOKINETIC OF CEPHALEXIN AFTER INTRA-
VENOUS AND INTRAMUSCULAR ADMINISTRATION TO
DOMESTIC CATS

Albarellos G, Denamiel G, Montoya L, Velo M, De Battista M, Landoni M.

FCV UBA Chorroarín 280, Cap. Fed. (1427); FCV UNLP. Calle 60 y 118, prov. Bs As. (1900). E-mail: albarell@fvvet.uba.ar

Introduction: Cephalexin (CFX) is a first generation cephalosporin widely used in domestic animals for the treatment of grampositive (Staphylococcus spp., Streptococcus spp.) and gramnegative (Escherichia coli, Proteus spp) infections. The aim of this study was to analyze the serum disposition of CFX after intravenous (IV) and intramuscular (IM) administration to cats and, to correlate plasma concentrations with the minimum inhibitory concentrations (MIC) for pathogen bacteria isolated from cats.

Materials and Methods: 5 and 6 adult cats received 10 mg/kg of CFX by IV and IM administration, respectively. Blood samples were withdrawn at pre-determined times over an 8 h period. CFX serum concentrations were determined by microbiological assay using Micrococcus luteus (ATCC 9341) as test micro-organism. Plasma disposition curves were analyzed by non linear methods. CFX MIC were determined for 11 Staphylococcus spp., 5 Streptococcus spp. and 8 E. coli by macrodilution broth method.

Results: Main pharmacokinetic parameters were: C_{max}: 44.15±14.64 µg/ml (IV) and 19.84±5.59 µg/ml (IM); AUC₍₀₋₈₎: 57.64±18.64 µg.h/mL (IV) and 40.37±6.12 µg.h/mL (IM); V_{d(ss)}: 0.41±0.14 L/kg; t_{1/2}: 1.66±0.24 h (IV) and 1.73±0.31 h (IM); MRT: 2.16±0.35 h (IV) and 2.28±0.36 h; Cl_B: 0.19±0.06 L/h.kg. Plasma CFX concentrations were above MIC=1 µg/ml for 6 and 8 h after IV and IM administrations, respectively.

BII-36.
EFFECT OF β-CYCLODEXTRIN ON THE TRANSDERMAL
DELIVERY OF QUERCETIN THROUGH PIG SKIN

Ortiz JE, Lhez L, Pappano NB, Debattista NB.

Universidad Nacional de San Luis. Lavalle 1155, San Luis.

E-mail: debattis@unsl.edu.ar

Quercetin (Q), a polyphenolic flavonoid, shows wide spectrum of pharmacological properties including anti-hypertensive and vasodilatador effects. Its use in pharmaceutical field is limited by its low aqueous solubility. β-cyclodextrin (β-CD), cyclic oligosaccharide, contains seven glucose monomers units in a ring creating a cone shape. It is able to host hydrophobic molecules in its interior, forming inclusion complexes. It greatly modifies the physical and chemical properties of the guest molecule, mostly in terms of water solubility. These complexes are able to penetrate body tissues and they can be used to release biologically active compounds under specific conditions. Formulations in carbopol gel were prepared and the effect of β-CD (0-10% w/w) on *in vitro* Q transdermal permeation through ear pig skin was studied. These studies were carried out by triplicate using Franz diffusion cells with 1.767 cm² area in automatic sampler Microette System. Te flux (J_m) values, from the slope of the linear graphs of Q cumulative quantities per area unit vs. time, were obtained and the diffusion coefficients values from J_m were calculated. The quercetin permeation increased over 3 fold when β-CD 3%, approximately, was employed. This formulation will be the most suitable for improving the transdermal penetration of this flavonoid.

**BII-37.
PHARMACOKINETICS OF CEPHALEXIN IN PREGNANT
AND LACTATING GOATS**

Ambros L¹, Veksler Hess J², Kreil V¹, Tarragona L¹, Prados AP¹, Hallu R¹, Rebuerto M¹.

¹Farmacología, ²Producción de Ovinos. FCV, UBA. Chorroarín 280 (1427), Buenos Aires. E-mail: ambros@fvvet.uba.ar

The objective of this study was to compare the pharmacokinetics of cephalexin administered by the intravenous (i.v.) route to pregnant and lactating goats. Six female pregnant goats received an i.v. dose of 10mg/kg of cephalexin lysine at 115-120 days of pregnancy and 25-28 days after the parturition. Blood samples were withdrawn at pre-determined times. Cephalexin concentrations were determined by microbiological assay using *Micrococcus luteus* ATCC 9341 as microorganism test. Plasma disposition curves were analyzed by non linear methods applying PcNonlin software. Lactating goats showed lower plasmatic cephalexin concentrations than pregnant goats. The elimination half-life was 0.31 ± 0.06 h and 0.37 ± 0.15 h for pregnant and lactating goats, respectively. $AUC_{0-\infty}$ was significantly higher in the pregnant goats ($40.70 \pm 6.36 \mu\text{g}\cdot\text{h}/\text{mL}$) than in lactating goats ($24.82 \pm 4.71 \mu\text{g}\cdot\text{h}/\text{mL}$). Volume of distribution and clearance were significantly shorter in pregnant goats than in lactating goats (0.11 ± 0.01 l/kg vs 0.22 ± 0.08 l/kg and 0.025 ± 0.03 l/kg-h vs 0.42 ± 0.09 l/kg-h, respectively). These results show that pharmacokinetics of cephalexin changes slightly in pregnant and lactating goats.

**BII-38.
PATTERN OF TRICLABENDAZOLE ACCUMULATION IN
LIVER FLUKES (*FASCIOLA HEPATICA*) COLLECTED
FROM TREATED SHEEP**

Ceballos L, Moreno L, Alvarez L, Lanusse C.

Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina. CONICET.

E-mail: ceballos@vet.unicen.edu.ar

The flucicidal compound triclabendazole (TCBZ) has a complex metabolic pattern that includes the systemic presence of active metabolites. The aim of this work was to evaluate the relative importance of oral ingestion or transtegumental diffusion in TCBZ/metabolites accumulation into *Fasciola hepatica*. Sheep infected with *F. hepatica* (n= 12) were treated with TCBZ (10 mg/kg) by the i.r. route. At 3, 24, 48 and 60 h post-treatment, animal was killed (n= 3) and plasma, bile and *F. hepatica* samples were obtained. TCBZ/metabolites concentrations were measured by HPLC. TCBZ sulphoxide (TCBZSO) and sulphone (TCBZSO₂) the only molecules found in plasma. These metabolites were also the main analytes recovered in *F. hepatica*, with low TCBZ concentrations detected at 24 h post-treatment. However, TCBZ, TCBZSO, TCBZSO₂ and hydroxi-TCBZ were found in bile of treated sheep at all sampling times. Plasma samples were collected (PK study) and analysed by HPLC. The similar concentration pattern observed for TCBZSO and TCBZSO₂ in plasma and *F. hepatica* may indicate that the oral ingestion is an important route of drug entry into the trematode when the assessment is done under *in vivo* conditions.

**BII-39.
SOLUBILITY AND pH-COMPATIBILITY OF SACCHARIN
SALTS OF FLUOROQUINOLONES**

Romañuk C, Manzo R, Olivera M*

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Ciudad Universitaria, 5000 Córdoba, Argentina. *E-mail: meoliver@fcq.unc.edu.ar

Fluoroquinolone Saccharinates (FQ-SACs, P-060105581) are new salts of FQs regarded as pharmaceutical alternatives. They have demonstrated an improvement in their organoleptic properties when evaluated in animals and humans. The aim of this work was to study aqueous solubility and pH compatibility of the saccharinate salts of the FQs norfloxacin, ciprofloxacin and enrofloxacin in order to establish their utility as pharmaceutical alternatives.

Solubility studies were comparatively conducted at 25°C in water and also at pH values embracing intestinal range (from 4 to 8) and compared to FQs. From 10 to 120% of the SAC, present in aqueous saturated solutions of FQ-SACs, was neutralized with NaOH. Solid phase in equilibrium was characterized by FTIR to determine the controlling solubility species.

FQ-SACs are 5-20 times more soluble in water than their respective FQ precursor and yield saturated solutions whose pH is higher than that of the respective hydrochlorides (4.3-4.6). Solubility of FQ-SACs decrease with pH increase, following the same pattern observed with the precursors. However, close to neutrality, were minimum solubility is observed, norfloxacin and ciprofloxacin salts are more soluble than expected. This behavior can be explained by ionic pair formation and may occur at intestinal pHs in which the FQs are absorbed. The increase observed in the solubility allows enlarging formulation possibilities and might have an impact in the *in vivo* amount of drug dissolved at intestinal level.

**BII-40.
REATMENT OF ORAL CANDIDOSIS: “IN VIVO” EVALUATION OF THE BIOPHARMACEUTICAL PERFORMANCE**

Llabot J, Manzo R, Allemandi D.

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Ciudad Universitaria, Córdoba, Argentina. E-mail: jmllabot@fcq.unc.edu.ar

Mucoadhesive tablets containing nystatin (10 mg), were evaluated *in vivo*. The assays were carried out with 12 healthy volunteer and the concentration of nystatin in saliva was determined at different times. Tablets remained attached to the buccal mucosa during 270 min. No evidence of ulceration or bleeding was observed. Typical appearance of intact buccal mucosa was seen before and after contact with the tablet. The tablets were well accepted by the volunteers. Concentration of nystatin in saliva was several times higher than MIC over a period of approximately 4.5 hours, which was in agreement with the behavior observed *in vitro*. These results permit to infer that the administration of this mucoadhesive tablets could be advantageous compared to conventional formulations and mucoadhesive extended release tablets might produce better therapeutic performance than conventional formulations in the treatment of oral candidosis.

BII-41.
IVERMECTIN AND NITROXYNIL CO-ADMINISTRATION
IN SHEEP: EFFECT OF BODY CONDITION ON THEIR
KINETIC DISPOSITION

Moreno L, Bistoletti M, Lifschitz A, Álvarez L, Lanusse C.
 Laboratorio de Farmacología, Facultad de Ciencias Veterinarias,
 Universidad Nacional del Centro de la Provincia de Buenos Aires,
 Tandil, Argentina. CONICET. E-mail: lmoreno@vet.unicen.edu.ar

Ivermectin (IVM) is an endectocide compound from the avermectin family. NitroxyNIL (NTX) is a trematocidal compound. Combination of different drug molecules is a modern and challenging approach in parasite control. The aim of the present work was to study the plasma disposition of both IVM and NTX after subcutaneous (SC) co-administration to sheep with different body conditions. Two groups (n= 8) of sheep with statistical different body weight (fat and thin) were treated with *Nitromectin*® (IVM 200 µg/kg and NTX 10 mg/kg) by the SC route. Blood samples were collected up to 60 days post-treatment and plasma concentrations of IVM and NTX were determined by HPLC. IVM and NTX were well absorbed after its co-administration. Higher IVM plasma concentrations were measured in "thin" sheep compared to the "fat" group. However, NTX plasma concentrations in the thin were lower than these measured in fat group. A large volume of distribution (IVM) and high plasma protein binding (NTX) help to explain the observed pharmacokinetic differences for these drugs in animals with different body condition.

BII-42.
COMPARATIVE ALBENDAZOLE SULPHOXIDE PHAR-
MACOKINETICS AFTER SINGLE ORAL ADMINIS-
TRATION OF THREE DIFFERENT FORMULATIONS IN
DOGS

Dib A¹, Palma S^{2,4}, Suárez G¹, Fariás C⁴, Cabrera A¹, Castro S^{2,4}, Allemandi D^{2,4}, Moreno L^{3,4}, Sánchez Bruni S^{3,4}, Lanusse C^{3,4}.
¹Universidad de la República, Uruguay, ²Universidad Nacional de Córdoba, Argentina, ³Universidad Nacional del Centro, Argentina.
⁴CONICET. E-mail: ssanchez@vet.unicen.edu.ar

New therapeutics alternatives using Benzimidazole compounds are being studied to improve posology and antiparasite efficacy. The aim of this work was to compare plasma pharmacokinetics (PK) profiles of three different oral Albendazole (ABZ)-based formulations in dogs. Nine animals were randomly divided into three groups and two phases (incomplete block design). **Phase I (n=9):** *Group I (formulation A)*: received 25 mg/kg of ABZ conventionally formulated. *Group II (formulation B)* received 25mg/kg of a modified ABZ formulation (poloxamer solid dispersion). *Group III (Formulation C)*, received Albendazole Sulfoxide (ABZSO) (equimolar dose). After 21 days of wash-out period the experiment was repeated as **Phase II**. Blood samples were taken over 24h and subsequently analyzed by HPLC. ABZSO and ABZSO₂ were the analytes recovered in plasma. Significant ($P < 0.001$) AUC values (+500%) and Cmax (+487%) for ABZSO, were obtained for the formulation C when statistically compared with A and B. However, no statistical differences on PK parameters were found between A and B formulations. In conclusion formulation C, showed the best trend as potential antiparasite activity.

BII-43.
EVALUATION OF PHARMACEUTICAL BIOEQUIVA-
LLENCE AND ANTHELMINTIC EFFICACY FOR DIFFE-
RENT ALBENDAZOLE GENERIC FORMULATIONS IN
PARASITIZED SHEEP

Suarez G¹, Alvarez L², Castells D³, Faggiolino P¹, Correa O¹, Lanusse C².
¹Universidad de la República, Montevideo, Uruguay. ²Laboratorio de Farmacología, FCV, UNCPBA, Tandil, Argentina y CONICET, Argentina. ³Secretariado Uruguayo de la Lana, Uruguay.
 E-mail: gsuarez@adinet.com.uy

Albendazole (ABZ) is a broad-spectrum anthelmintic drug widely used in human and veterinary medicine. The aim of this work was to evaluate the bioequivalence and anthelmintic efficacy of four different generic formulations of ABZ in sheep parasitized with resistant nematodes. Fifty parasitized lambs were divided into five groups (n=10): Untreated control and treated groups A (reference), B, C and D. Treated animals received different ABZ formulations (5 mg/kg). Plasma samples were collected over 3 days post-treatment and drug concentrations measured by HPLC. The efficacy of the different treatments was estimated by the faecal egg counts reduction test (FECRT). No bioequivalence was observed between the reference and either B or D generic formulations. The FECRT ranged between 36 and 59%. The study demonstrated a lack of bioequivalence for some of the generic ABZ formulations under study, which are commercially available for use in sheep.

BII-44.
INFLUENCE OF PREGNANCY IN THE PHARMACO-KI-
NETICS OF INTRAVENOUS CEFQUINOME IN GOAT
Litterio N¹, Lorenzutti M¹, Himelfarb M, Zarazaga MP¹, San Andrés
MP, Boggio JC¹.

¹Universidad Católica de Córdoba, Cdt. Farmacología. Obispo Trejo 323. 5000 Córdoba, Argentina. E-mail: nlitterio@ucc.edu.ar
²Universidad Complutense de Madrid. Dpto. de Toxicología y Farmacología.

Cefquinome (CFQ) is a fourth generation cephalosporin that was used in this study to assess its pharmacokinetics (PK) in pregnant goats, and their distribution to the fetus in the calving. Two groups of six Anglo Nubian goats were used; G1 with pregnant animals for four months, and G2 with non pregnant goat. All the animals were given CFQ (Cobactan® IV, 4.5%) at 2 mg/kg IV and were extracted blood between the 2 min to 48 hours postadministration. It was expected the birth in the G1 to administer CFQ at 2 mg/kg IV 30 minutes prior to a caesarean section, and take samples of maternal blood (pre-and postadministration), fetal blood (umbilical vein), amniotic fluid and placenta. Samples were analyzed by HPLC/uv. Pcnonlin 4.0 was used, by compartmental analysis. Results indicate that PK parameters of distribution and elimination in G2 were similar to happened in other species and significantly lower than that observed in G1 ($V_d = 0.67 \pm 0.3$ l/kg; $T_{1/2\beta} = 456 \pm 300$ min; $MRT = 675 \pm 450$ min). Probably this is due to the hemodynamic changes, such as venous stasis by the presence of one or more fetuses, and the increased body water in maternal tissues that occurs in late pregnancy. Moreover, were not detected CFQ in fetal blood or amniotic fluid. The nature hydrophilic the CFQ limit his passage to the fetus, leaving circulating in maternal blood, for longer than non-pregnant females. This will have a positive effect on the antimicrobial therapy with CFQ in a pregnant goat.

BII-45.
INTERACTION BETWEEN EUDRAGIT E100 AND DEXAMETHASONE PHOSPHATE

Guzman ML, Manzo RH, Olivera ME.

Departamento de Farmacia, Facultad de Ciencias Químicas, UNC. Haya de la Torre y Medina Allende. Ciudad Universitaria (5000). E-mail: meoliver@fcq.unc.edu.ar

The aim was to study the interaction between the components of a complex formed by a cationic methylmethacrylate polymer (Eudragit E100) and dexamethasone phosphate (DmP). Solid dispersions at various drug-to-polymer weight ratios were prepared by a wet granulation method. Similarly, DmP-loaded hydrogels with the same composition were prepared and characterized. Variable amounts of HCl were used to increase aqueous compatibility. The complexes were studied in solid state (PRXD, DSC, FTIR, solubility) as well as in dispersion (release in Franz cells, NaCl titration) to establish affinity and type of interactions. No residues of crystalline DmP are present in the solids. The phosphate groups of DmP are ionically linked to the dimethylamine groups of Eudragit E100, and showed high affinity. The dispersions are clear and allow obtaining concentrations of DmP far above its solubility with final pHs 2-6. They show slow and prolonged release (zero order kinetics) *in vitro* upon contact with physiologic simulated fluid. The interaction between Eudragit E100 and phosphate groups is stronger than that observed in systems whose acidic moieties were carboxylic acids. From the *in vitro* studies, the systems are interesting for the design of formulations for topic (ophthalmic, skin, oral mucosa) administration. In particular the 12.5-37.5% (DmP): 25-75% Cl⁻ compositions can be designated for further investigation.

BII-46.
INTERPOLYELECTROLITE-DRUG COMPLEX NANOPARTICLES AS BASIC DRUGS CARRIER

Palena M, Allemandi D, Manzo R, Jimenez-Kairuz A*.

Dpto. de Farmacia, Fac. de Cs. Químicas, UNC. Ciudad Universitaria, X5000HUA Córdoba. Argentina.

*E-mail: alvaro@fcq.unc.edu.ar

The aim of this project was to obtain polyelectrolyte/drug complex nanoparticles (NP). With this purpose two polymethacrylates, one anionic (eudragit L100 (EuL) and other cationic (eudragit E100 (EuE)) were selected to prepare ionic complexes with the model drugs (D), atenolol and propranolol. Aqueous dispersions of the obtained particles were subjected to physicochemical characterization and *in vitro* release studies. Aqueous dispersion of EuL was neutralized with D and different proportions of EuE were added to obtain (EuL-D₅₀-EuE_x) complexes (the subscript indicate the mole% of EuL-carboxylic groups neutralized with D or EuE, x=25, 50, 75, 100%). NP were characterized through ultracentrifugation, optical density, Z-potential, light scattering, microscopy and pH determinations. *In vitro* release was performed in Franz cells with synthetic membranes and water or saline solution as receptor media. The capacity of NP to be loaded with D increases from 57 to 80 w% with increase in the proportion of EuE but their physical stability decreases. D release from NP is slow when water is the receptor medium but it rises when saline solution is used. The rate and anomalous-kinetic remain unchanged with EuE and D proportions are modified. At present, permeability essay in everted rat intestine are being performed. This system of NP has potential properties to be used as D controlled release carriers.

BII-47.
SALT FORMATION DURING COGRINDING OF ENROFLOXACIN WITH SACCHARIN

Romañuk C, Manzo R, Olivera M*.

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Ciudad Universitaria, 5000 Córdoba, Argentina. *E-mail: meoliver@fcq.unc.edu.ar

Fluoroquinolone saccharinate salts (P-060105581) are pharmaceutical alternatives of fluoroquinolones presenting sweet taste to improve its palatability. The necessity of high amounts of hot water limits the convenience of the preparation method. The purpose of this work was to investigate the mechanism of salt formation during cogrinding of solid reactants. Enrofloxacin saccharinate salt was used as a model compound. Cogrinding in a ball mill of anhydrous solid reactants was studied. Coground samples were analyzed by FTIR, XRPD, DSC-TGA at regular intervals from 0-8 h and compared with enrofloxacin saccharinate obtained from solution method to establish amount and kind of interactions. The extent of salt formation during cogrinding was quantified by DSC and indicates the quick formation of the salt with a faster rate in the early stages of the process with not less than 45% of the salt formed after 30 min. The reaction was completed after 6 h. The solid obtained is a polymorph of that obtained from solution. This method yields enrofloxacin saccharinate in a faster and cheaper way since solvents or boiling are not needed and the synthesis is completed in a single step.

BII-48.
SOY PROTEIN POLYMERS AS VEHICLE OF FUNGI NEMATOPHAGOUS

Sagüés M¹, Saumell C¹, Purslow P², Fernández S², Fusé L¹, Iglesias L¹, Fiel C¹.

¹Área Parasitología y Enfermedades Parasitarias. Facultad Ciencias Veterinarias, Universidad Nacional del Centro, Buenos Aires. Tandil. Paraje arroyo seco s/n. Argentina. ²Universidad de Guelph. Depto de Patobiología. Canadá.

E-mail: federica@vet.unicen.edu.ar

Soy proteins polymers have a great potential as a controlled release system (CRS) for active compounds, such as nutrients, medicine and they also could be used therapeutically. The purpose of this trial was to evaluate the predatory capacity of *Duddingtonia flagrans* included in a soy protein polymers formulation; determine the fungi release from polymers and the suitable physic structure for bucoesophagic probe administration in ovine. The result showed that polymer formulation did not affect *D. flagrans* predatory activity and the fungi release was successful. Therefore the use of soy protein polymer as vehicle of nematophagous fungi into CRS for ruminant's nematode control could be the base for a potential biodegradable pharmaceutical formulation.

BII-49.**PHARMACOKINETIC VARIABILITY OF LOPINAVIR IN HIV INFECTED CHILDREN**

Bramuglia GF¹, Curras V¹, Hocht C¹, Hegoburu M¹, Niselman V², Mecikovsky D³, Bologna R³, Bellusci C, Mangano A⁴, Sen L⁴, Rubio MC¹.

¹Cátedra de Farmacología, ²Cátedra de Matemática, Facultad de Farmacia y Bioquímica, UBA; ³Servicio de Control Epidemiológico e Infectología, ⁴Laboratorio de Biología Celular y Retrovirus, Hospital de Pediatría Garrahan.

Lopinavir (LPV) is a protease inhibitor used in the treatment of HIV-1 infection. A large inter-patient variability in the disposition of this drug has been reported. The aim of this work was to study LPV plasma levels variability in HIV-1 infected children treated with lopinavir/ritonavir (LPV/r). 37 patients (ranged: 1.5-19 years old) treated with LPV/r (Kaletra, Abbott)-capsules or solution-were included. 70 LPV plasma levels were measured using HPLC-UV (33 peak and 37 through levels). A great inter-individual variability in LPV levels was observed (CV trough: 160.9%, peak: 127.4%). LPV levels showed no statistically significant differences after the administration of any of both formulations. Eight patients showed through levels < 1 µg/mL. We studied the relationship between C3435T MDR1 polymorphism in exon 26 ad LPV PK. Patients with T/T genotype showed higher LPV levels in comparison with C/T or C/C genotype. The high variability in LPV levels suggests that TDM of LPV may be advisable in children. No differences were observed in LPV levels using liquid or solid formulation. A relationship between LPV PK and MDR1 polymorphism in exon 26 was observed.

BII-50.**PHARMACOKINETICS AND T>MIC OF AMOXICILLIN-CLAVULANIC (AMX-CL) ACID IN MILK OF LACTATING DAIRY COWS WITH *S. aureus* SUBCLINICAL MASTITIS**

Lucas M, Moncada Cárdenas A, Marchetti L, Lambertini A, Mestorino N, Errecalde J.

Cátedra de Farmacología. Facultad de Cs Veterinarias. Universidad Nacional de La Plata. CC 296, 1900 La Plata.

E-mail: marianaflucas@gmail.com

Six Holstein lactating cows with subclinical mastitis caused by *S. aureus* were selected. Each quarter of all cows received 3 intramammary (IMM) infusions of AMX-CL (200-50 mg/6 mL) with a 12 h interval. Quarter milk samples were collected until 96 h after 3rd administration. Concentrations were measured by microbiological assay. PK analysis was by non-compartmental method. Experimental design was a factorial ANOVA applied to rank transformed data. The purpose was to evaluate the effects of the sanitary status of the quarters (SS): mastitis quarters (n=8) vs healthy ones (n=16) and the level of milk production (LP): quarters of high-producing cows (n=12) vs low-producing cows (n=12). LP had a significant effect on T_{1/2β} and MRT, which were higher in quarters of low producing-cows (*P*<0.05). The MIC₉₀ for AMX-CL 4:1 was 8 µg/mL and the T>MIC₉₀ was higher in quarters of low-producing cows. The LP modified the AMX-CL PK profile and it could be a determinant factor of bactericidal efficacy.

BII-51.**PLASMAIC DISPOSITION OF FLORFENICOL IN GOATS**
Himelfarb M¹, Lorenzutti M¹, Errecalde C², Prietto G², Litterio N¹, Boggio JC¹.

¹Universidad Católica de Córdoba., Cát. Farmacología Veterinaria. Obispo Trejo 323. 5000. Córdoba, Argentina.

E-mail: jcboggio@ucc.edu.ar ²Universidad Nacional de Rio Cuarto. Cát. Farmacología Veterinaria.

This study has the objective to determine the relation milk/plasma of the florfenicol given by intravenous (IV), intramuscular (IM), and intramammary (IMM) routes in lactating goats. It has been performed a cross-over experimental design to the three routes of administration using 6 lactating goats. A single 20 mg/kg dose of Florfenicol (Microflud®, Vetanco, Bs. As., Argentina) was administered by the IV and IM routes, and 600 mg per mammary half for the IMM route. The plasma and milk samples were collected at regular intervals. Florfenicol was quantified by HPLC. The data of plasmatic and milk concentration of florfenicol were analyzed on a non-compartmental model. The florfenicol demonstrated, by every routes, a very important diffusion, the concentration in milk, in relation to the plasma, is notable after the IV and IM administration, indicated by the high relation AUC_{milk}/AUC_{plasma}, of 7,4 and 2,35 for these routes respectively. By the IMM route an important passage from milk to plasma was observed. In every routes of administration it was obtained higher milk concentrations than the MIC (minimum inhibitory concentration) for *Staphylococcus aureus* (0,5 ug/ml), the main mastitis causing pathogen, until 72 hs (for the IV and IM) and 120 hs for the IMM route.

BII-52.**TYLOSIN PHARMACOKINETICS IN HEALTHY BEEHIVES (*Apis mellifera*) AFTER SUMMER ADMINISTRATION**

Huber B, Quintero M, Gräve E, Mestorino N, Errecalde JO.

Cátedra de Farmacología. Facultad de Cs. Veterinarias. Universidad Nacional de La Plata. CC 296, 1900 La Plata.

E-mail: barhuber@gmail.com

Tylosin (Tyl) exhibits better results than other macrolides in susceptibility *in vitro* tests with *Paenibacillus larvae subs. larvae*. A field experiment was carried out to determine the pharmacokinetic profile of Tyl among bees, young and older larvae and honey following oral dosing to adult bees in summer. Six healthy beehives were administered with powdered sugar added with tylosin at a target dose of 600 mg. Samples were collected from the breeding chamber at different times from all hives. Tyl concentrations were determined by microbiological assay. The pharmacokinetic analysis was performed by WinNonlin Professional 5.2. The elimination half life (T_{1/2β}) was longer in young larvae than old larvae-bees groups. Although it should be noted that T>MIC was 7 days in young larvae and 14.5 days in old larvae. Those findings suggest that the drug stays long enough in the target infection, explaining therapeutic efficacy. High levels of residues in honey and their persistence show that Tyl must be used cautiously in honey production.

BIII-53.**LIMONENE: DIFFERENT MECHANISMS OF ANTIPROLIFERATIVE ACTION ON A LYMPHOMA CELL LINE**

Manuele MG, Davicino R, Barreiro Arcos ML*, Ferraro G, Cremaschi G*, Anesini C.

Instituto de Química y Metabolismo del Fármaco (IQUIMEFA-CONICET) and *Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET). Junín 956, 2º piso. (1113) Buenos Aires, Argentina. E-mail: canesini@yahoo.com.ar

We demonstrated previously that limonene (L) increased total nitrates of a murine lymphoma cell line (BW5147). The aim of the study was to analyze the effect of limonene on cell proliferation (by tritiated thymidine uptake) and nitric oxide (NO) (by Griess assay) studying the participation of ERK and P-38 pathways. Results were expressed as Mean \pm SEM of three experiments made by triplicate: *P<0.05 (respect to basal) * P<0.05 (L alone and L+inhibitors)(ANOVA+Dunnnett). **Proliferation** (cpm): **Basal:** 2008 \pm 224; **L 40 μ g/ml:** 1169 \pm 50*; + L-NAME: 1369 \pm 40*; + Inh ERK: 1787 \pm 170*; + Inh P-38: 1814 \pm 22*; + Mevalonic (Mev): 1150 \pm 110; **L150 μ g/ml:** 1060 \pm 21; + L-NAME: 1308 \pm 100*; + Inh ERK: 1144 \pm 100; + Inh P-38: 1261 \pm 66*; + Mev: 1614 \pm 119*. **NO** (mM): **Basal:** 0.000011 \pm 0.000001; **L 40 μ g/ml:** 0.00077 \pm 0.00007*; + L-NAME: 0.00011 \pm 0.00001*; + Inh ERK: 0.00012 \pm 0.000010* + Mev: 0.00070 \pm 0.00007; **L 150 μ g/ml:** 0.0006 \pm 0.00006; + L-NAME : 0.00025 \pm 0.00002*; + Inh ERK: 0.0005 \pm 0.00009; + Mev: 0.00018 \pm 0.00001*. At low concentrations L activated P-38, and ERK via and increased NO by induction of iNOS and NOS activation. High concentrations inhibited ERK via and increased NO by NOS activation.

BIII-54.**EFFECT OF RUTA SSP. IN SMOOTH MUSCLE OF THE RAT**

Grigorjev C, Brizuela N.

Departamento de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. Santa Rosa 1085, 5000 Córdoba, Argentina. E-mail: cgrigorjev@hotmail.com

Ruta graveolens L. and *Ruta chalepensis L.* are plants used in folk medicine as antispasmodics, digestive and for intestinal gases. Animals used as experimental model were Wistar rats, adult females, clinically healthy and with a weight average of 250 g. We used strips of stomach and duodena. Each one of the segments mounted on two stirrups in a bath of organ isolated with Ringer-lactate solution, at 37°C, pH: 7.3-7.4, and bubbled with 95% O₂, 5% CO₂. One of the stirrups was connected vertically to the bottom of the bath and the other to a transducer of tension connected to a Beckman polygraph. We applied 500 mgs of basal tension. After the stabilization, the ethanolic extract of *Ruta ssp.* was added in increasing doses.

At 5 μ l/ml the tone lower 23% in small intestine and lower 27% in stomach. However at 10 μ l/ml the tone lower 32% and 35% respectively. In the other parameters the amplitude decrease only in the stomach at dose of 5 μ l/ml while in the small intestine no changes were observed. With 10 μ l/ml the amplitude change in both organs (60% in small intestine, and 75% in stomach).

In the frequency the change were different, in the small intestine not changes were observed however in the stomach the frequency down to 50%.

Rue showed decreased effects on isolated small intestine and stomach were is dose dependent, maybe we were demonstrated the effects digestive of *Ruta*.

BIII-55.**PANAX GINSENG EFFECTS ON ISOLATED ORGAN OF WINSTAR RAT**

Barberis M, Guerrini E, Huergo G, Romero Arijón A, Brizuela N. Cátedra de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. Córdoba, Argentina.

E-mail: nildabrizuela@hotmail.com

We studied the effects on tone, amplitude and frequency of contractions induced by Panax Ginseng extract in isolated smooth muscle. Animals used as experimental model were rats of the Wistar line, adult females, clinically healthy and with a weight average of 250 grams. We used strips of stomach, uterus and duodena. Each one of the segments mounted on two stirrups in a bath of organ isolated with Ringer-lactate solution, at 37°C, pH: 7.3-7.4, and bubbled with 95% O₂, 5% CO₂. One of the stirrups was connected vertically to the bottom of the bath and the other to a transducer of tension connected to a Beckman polygraph. We applied 500 mgs of basal tension. After the stabilization, the distillate extract of Panax Ginseng was added in increasing doses. Results: Uterus: to dose of 40 μ gr/ml the tone increase 81.4%, amplitude increases 5.7%, frequency decrease 23%. Duodena: to dose of 10 μ gr/ml amplitude decrease 60%, the rest of variables without significant changes. Stomach: to dose of 10 μ gr/ml tone increases 41%, amplitude decreases 50%, frequency without significant changes. Even though constrictors effects are observed on uterus and rat stomach, becomes necessary to realize a greater number of studies to found the relation between these effects and the used doses.

BIII-56.**EFFECT OF *Acacia visco* METHANOLIC EXTRACT IN ACUTE INFLAMMATION IN RAT**

Pedernera AM¹, Rotelli AE¹, Guardia T¹, Guardia Calderón CE², Pelzer LE¹

¹Farmacología, ²Bromatología. Fac. Qca. Bqca. y Fcia. Univ. Nac. San Luis. San Luis 5700. E-mail: tguardia@unsl.edu.ar

In previous studies we demonstrated chronic anti-inflammatory activity and antiulcerous gastric activity of methanolic extract from leaves (*AvMEI*) and bark (*AvMEb*) of *Acacia visco* (Biocell Vol 28(3), 2004). In this work we investigate the effect different doses of these extracts in the acute inflammation model carrageenan induced paw edema in rat (Winter *et al.*, 1962). Female Wistar rats were fasted for 24 h, water *ad libitum*, and were divided into nine groups of six animals each and were orally administered as follows: Group 1 (inflammation control) received vehicle; groups 2, 3, 4 and 5 received *AvMEI* 25, 50, 100 and 200 mg/kg respectively; groups 6, 7 and 8 received *AvMEb* 50, 100 and 300 mg/kg respectively and group 9 (reference) received indomethacin 10 mg/kg. One hour later, all groups were injected in the subplantar region of left hind paw with 0,1 ml/rat of carrageenan tipe IV 2% w/v in saline. Pedal edema was determined by volume difference between the left and right paws using a plethymometer Ugo Basile (Italy) at 1, 3, 5 and 7h. The percentage inhibition for each group was calculated with respect control group. Statistical analysis: ANOVA test. The tested extracts exhibited anti-inflammatory activity, showing *AvMEI* the highest anti-inflammatory effect. In conclusion, both leaves and bark methanolic extracts of *Acacia visco* showed significant inhibition of acute inflammation and it was dose dependent.

BIII-57.**TOPICAL ANTIINFLAMMATORY ACTIVITY OF MALVA SYLVESTRIS L. (MALVACEAE) ON CARRAGENIN-INDUCED EDEMA IN RATS**

Chiclana CF, Enrique A, Consolini AE.

Cátedra de Farmacología, Area Farmacia, Dpto Cs. Biológicas, Facultad de Ciencias Exactas, UNLP. Calle 47 y 115. (1900) La Plata. Argentina. E-mail: dinamia@biol.unlp.edu.ar

Malva (*Malva sylvestris* L., *Malvaceae*) is a medicinal plant frequently used in topical application as a demulcent, healing, anti-hemorrhoidal, for skin and mucus. Nevertheless, its topical anti-inflammatory effect has never been demonstrated by preclinical nor clinical tests. In this work, there were prepared malva extract creams at 5, 10 and 20% from a 50% decoction of malva commercially provided by herboristery. A subplantar edema was induced in rats by injecting carragenin 1%, and afterwards, either malva creams, 2% indometacine cream or placebo, were topically applied on the hind paw and covered until time of measurement. Edema was measured by pletismography at intervals of 60 min during 4 hours, while the respective creams were re-applied during each interval. Results were expressed as the relative increment in paw volume $\Delta V = (V_t - V_i) / V_i$ % (at t time vs. initial), and they were compared by a two-way ANOVA ($F = 2.901$, $p = 0.0247$, df: 4 for treatments) and a-posteriori tests. A significative inhibition of the edema (from $38.7 \pm 8.7\%$ to $23.5 \pm 4.7\%$) was obtained with the 5% malva cream regarding the placebo, and the effect of malva was higher than that of the 2% indometacin cream. The results give support to the topical antiinflammatory effect of malva on the skin, and to the indication of this plant in local inflammation.

UNLP X-408-2005/08.

BIII-58.**EFFECTS OF GENISTEINE ON ISCHEMIA-REPERFUSION IN RAT HEARTS WITHOUT AND WITH HIGH K^+ - LOW Ca^{2+} - CARDIOPLEGIA**

Consolini AE¹, Ragone MI¹, Bonazzola P².

¹Cát de Farmacología, Fac. Cs. Exactas, UNLP (47 y 115 La Plata), and ²ININCA, Fac. Medicina, UBA-CONICET (M.T. Alvear 2270, Buenos Aires). E-mail: dinamia@biol.unlp.edu.ar

Genistein (Gen) is a phytoestrogen from soya beans, which is thought to decrease the incidence of cardiovascular disease. On cardiomyocytes, it was reported that Gen can reduce $I_{Ca,L}$ but also to increase cell shortening, to rise the contraction rate and increase the sarcoplasmic reticulum (SR) load. Then, it was evaluated whether Gen can protect hearts from contractile failure associated to ischemia-reperfusion (I-R) and/or modify the protection induced by a 25 mM K^+ -0.5 mM Ca^{2+} -cardioplegia (CPG). Isolated rat hearts were perfused with Krebs-C (C) and pretreated with C or CPG before exposition to 45 min I- 45 min R with C. The intraventricular pressure (P) and total heat release (H_t) were simultaneously measured in a flow-calorimeter. Adding 20 μ M Gen to C before I reduced P to 70% but not H_t in R. A diastolic contracture was induced during I (ΔPr : 24.8 ± 10) and start of R (27 ± 9 mm Hg), which then reverted. I- ΔPr was higher than in C-hearts ($p = 0.033$, 2-way ANOVA). Gen also improved P during R (to $123 \pm 39\%$ of pre-I vs. $64 \pm 10\%$ in C-hearts, $p < 0.05$) and increased H_t (18 ± 3 mW.g⁻¹, $145 \pm 36\%$ of pre-I). Contrarily, 20 μ M Gen in CPG before I did not change ΔPr , P ($76 \pm 6\%$ of pre-I vs. $79 \pm 14\%$) nor H_t (19.5 ± 5 vs. 17.3 ± 3.5 mW.g⁻¹) in R ($n = 4$, NS vs. CPG-hearts). Then, Gen could protect hearts by an effect non-additive to that of CPG, possibly by increasing the SR Ca^{2+} load during R.

X-408 UNLP-2005/08, PIP 6024/05.

BIII-59.**ANTIOXIDANT AND APOPTOTIC PROPERTIES OF HEDEOMA MULTIFLORUM BENTH, (TOMILLO SE-RRANO)**

Dadé M¹, Fioravanti D², Schinella G^{2,3}, Tournier H^{2,3}.

¹CUFAR. ²Cátedra de Farmacología Básica. Facultad de Ciencias Médicas. UNLP 60 y 120, La Plata, Argentina. ³CIC- Pcia Buenos Aires. E-mail: martindade26@hotmail.com.ar

In the present work we assessed the antioxidant activity (AA) and the cytotoxicity (CT) of an extract (TS) of *H. multiflorum* Benth (Lamiaceae), an Argentinean plant used popularly as digestive. Herbal bags were commercial products from a market of Villa de las Rosas, Córdoba, Argentina. AA was assessed using different experimental models. TS reduced the Fe^{3+} in the FRAP assay (658 ascorbic acid eq / mg dry extract) and shown a high scavenging capacity in the ABTS ^{•+} and DPPH tests (1.60 μ mol and 0.77 μ mol Trolox equiv/ mg dry extract respectively). When tested in rat brain homogenates at a concentration of 100 μ g/ml, TS inhibited significantly (58%) the lipid peroxidation measured as TBARS production. Total phenol and flavonoid content of TS (1.05 μ mol eq. caffeic acid and 0.7 μ mol eq. rutin/mg dry extract respectively) were determined. CT of TS was assessed on PMN cells by the MTT assay. TS (100 μ g/ml) decreased the cell viability by 34% after 3 h incubation. Using different flow cytometry methods we measured: a) the exposure of membrane phosphatidyl serine by the binding of Annexin V-FITC ($26.1 \pm 5.1\%$) b) the mitochondrial membrane integrity by DIOC-6 (3) retention ($29.9 \pm 8.2\%$) and c) the development of hypodiploid nuclei by propidium iodide ($18.2 \pm 3.2\%$). We conclude that the *H. multiflorum* extract is an important source of compounds with antioxidant activity and shows apoptotic activity on human neutrophils.

BIII-60.**EVALUATION OF GASTROPROTECTIVE EFFECT OF SOLIDAGO CHILENSIS EXTRACTS**

Bucciarelli A, Skliar MI.

Laboratorio de Farmacognosia, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. (8000) Bahía Blanca, Argentina. E-mail: mksliar@uns.edu.ar

Solidago chilensis Meyen is a native species from South America, widely used in the popular medicine of different countries. The aim of the present study was to assess the gastroprotective activity of different fractions (ethyl acetate 250 mg/kg, butanolic 500 mg/kg and aqueous 500 mg/kg) obtained from the capitula of the plant in mice subjected to an experimental model of ethanol-induced gastric lesions. The control group received vehicle, the reference control group was treated with omeprazole and the remaining groups were given the corresponding extract. All the animals received absolute ethanol as necrotizing agent.

Gastric lesions in groups receiving pretreatment with extracts and omeprazole were reduced to varying degrees as compared with control group ($P < 0.05$). Ethyl acetate was the most active fraction, which showed a gastroprotection of 99.5%. Phytochemical assays revealed the presence of flavonoids. These data suggest that the activity could be due, at least partly, to their presence. The precise mechanism of action of the extract is unknown. Further studies are needed to elucidate the active principles and mechanisms involved in this activity.

BIII-61.**ANTIOXIDANT ACTIVITY OF ARGENTINEAN MEDICINAL PLANT EXTRACTS**

Haag G¹, del Valle ME¹, Debenedetti S¹, Tournier H², Schinella G²
¹Cátedra de Farmacognosia. Facultad de Ciencias Exactas. UNLP.
²Cátedra de Farmacología Básica. Facultad de Ciencias Médicas.
 UNLP- CIC - La Plata, Argentina. E-mail: schinell@uv.es

Naturally occurring antioxidant compounds can reduce the harmful activities of free radicals on cells and tissues. The present work assessed the antioxidant capacity (AC) of dichloromethane (DCM) and methanolic (M) extracts obtained from 19 native plants from Argentina: *Gentianella parviflora*, *Gaillardia megapotámica radiata*, *Gaillardia cabreræ*, *Gaillardia megapotámica scabiosoides*, *Bahunia candicans*, *Pellaea flavens*, *Baccharis crispa*, *Achyrocline satureoides*, *Equisetum giganteum*, *Gentianella achalensis*, *Lippia turbianata*, *Pterocaulon polystachium*, *Terminalia australis*, *Terminalia triflora*, *Picrosia longiflora*, *Macfadyna ungui-cati*, *Adesmia bicolor*, *Lippia germinata*, *Proto usnea* using as experimental model the scavenging of DPPH and ABTS •⁺ stable free radicals. Total phenol content of extracts was determined. All extracts were able to bleach the radicals in the range of 2-1820 eq Trolox/mg dry extract. There was a very good correlation between the phenol content and the AC (P<0.01). The M extract of *T. australis* exhibited the highest AC (862 and 1822 eq Trolox/mg dry extract for DPPH and ABTS respectively). DCM extract of *P. usnea* showed a high trapping activity on ABTS. (767 eq Trolox/mg extract). Our results permit to suggest that the extracts from *T. australis* and *P. usnea* are important sources for the isolation of compounds with a great total antioxidant activity and potential use as pharmacological tools.

BIII-62.**STRUCTURAL BASIS OF THE ANTI-INFLAMMATORY ACTIVITY OF QUERCETIN: INHIBITION OF THE 5-HYDROXYTRYPTAMINE TYPE 2 RECEPTOR**

Rotelli AE¹, Aguilar CF², Pelzer LE¹.

¹Farmacología, ²Biología Molecular Estructural. Fac. QByF. UNSan Luis. Chacabuco y Pedernera (5700) San Luis.
 E-mail: arotelli@unsl.edu.ar

The anti-inflammatory activity of quercetin was evaluated through serotonin induced rat paw edema. The experiments showed that quercetin had an important effect on acute inflammatory processes. Docking of serotonin and quercetin into the homology model of the 5-hydroxytryptamine type 2 receptor allowed to analyze the structural basis of the anti-inflammatory activity. Paw edema induced by serotonin: Wistar rats (200-250g), divided into groups of six animals received by ip: saline (control); quercetin 80 mg/kg. One hour later, all animals were injected in left paw with serotonin 0,01%. Edema was measured at 30, 60 and 120 minutes using a plethysmometer. The three-dimensional model of serotonin receptor was constructed with MODELLER 9.4 using as template the crystallographic structure of the human β_2 Adrenergic G Protein-coupled receptor (PDB dataset 2RH1). The docking of serotonin and quercetin were done using AUTODOCK 4 (Scripps Res Inst. La Jolla, Cal.). Quercetin showed antiinflammatory activity against the inflammation induced by serotonin, with inhibition percentage of inflammation of 35%, 38% and 44% at 30, 60 and 120 minutes, respectively. Results showed that serotonin and quercetin bind in the same region of the active site with a similar binding energy but quercetin has a much bigger inhibition constant. Therefore, it seems possible that quercetin may act as a natural inhibitor of the receptor 5-HT₂ blocking the acute inflammation generated by serotonin.

BIII-63.**DEHYDROLEUCODINE AMELIORATES CAPSAI CIN AUGMENTED ACETIC ACID-INDUCED COLITIS**

Wendel G¹, María A¹, Giordano O², Pelzer L¹.

¹Farmacología and ²Química Orgánica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. Chacabuco y Pedernera. (5700) San Luis. Argentina.
 E-mail: gwendel@unsl.edu.ar

Dehydroleucodine (DhL), a sesquiterpene lactone of the guaianolide type, isolated from *Artemisia douglasiana* Besser, shows pharmacological cytoprotective effect in experimental colitis. In the present study, the role of capsaicin-sensitive neurons in the cytoprotection of DhL on experimental colitis was evaluated. Rats were treated with capsaicin 20, 30 and 50 mg/kg, on three consecutive days, a regimen shown to ablate primary afferent neurons. Colitis was induced two weeks later by 2 ml 10% acetic acid. Colon rats received saline, acetic acid, or capsaicin alone. Another group of rats received DhL 1 hr prior to damage induction. Rats were sacrificed 24 hr after damage induction, the colon isolated and damage was quantified by the scoring system of Wallace *et al.* All acetic acid-treated rats experienced diarrhea manifested as watery, loose stools. Acetic acid induced extensive colonic damage (8.2 ± 0.58). No damage was observed in the colon of rats treated only with saline or capsaicin. DhL pretreatment significantly decreased the macroscopic damage (1.21 ± 0.34, p<0.001). Capsaicin pretreatment resulted in significant reduction of the cytoprotective action of DhL (3.25 ± 0.45, p<0.05). Our results suggest that the protective activity of DhL in experimental colitis is mediated, at least in part, through the afferent sensory neurons.

BIII-64.**DESIGN OF VALERIANA OFFICINALIS TABLETS USING A COPROCESSING DRY PLANT EXTRACT**

Gallo L^{1,2}, Bucciarelli A², Castro S¹, Piña J³, Bucala V³, Skliar M², Palma S^{1,2}, Allemandi D¹.

¹Dpto. de Farmacia, Univ. Nac. de Córdoba. Ciudad Univ.(5000). Córdoba. ²Dpto. de Biología, Bioqca. y Fcia. ³Dpto de Ing. Qca. ^{2,3}Univ. Nac. del Sur. (8000) Bahía Blanca, Bs. As.
 E-mail: loreana.gallo@uns.edu.ar

Many people suffer sleep problems, and consumed benzodiazepines to resolve this pathology, about this it have been reported that *V. officinalis* L. (Valerianacea) possesses hypnotic activity and few adverse effects.

A solid pharmaceutical dosage formulation using a novel dry plant extract (NDPE) of *V. officinalis* is proposed. The botanical evaluation of the plant material is presented, it permits a correct identification of the species and guarantees a good phytomedicine quality. Physical and mechanical properties of DPE alone and with excipients were studied. Tablets containing a DPE and common excipients were evaluated. *V. officinalis* DPE possesses suitable rheological properties and compressibility, permitting its use in direct compression. Formulations like: *V. officinalis* DPE (300mg), Avicel PH101 (146mg), Emcompress (146mg), magnesium stearate (8mg) and *V. officinalis* DPE (300mg), Avicel PH101 (146mg), Lactose CD (146mg) and magnesium stearate (8mg) showed the best pharmaceutical performance.

BIII-65.**INTOXICATION BY ALKALOIDS FROM *Ipomoea carnea* spp. *fistulosa* IN GUINEA PIGS: HISTOPATHOLOGICAL LESIONS ON PANCREAS AND KIDNEY**

Cholich L, Gimeno E, Teibler G, García Denegri E, Acosta O.
 Cátedra de Farmacología. Facultad de Ciencias Veterinarias,
 UNNE, 3540, Corrientes; Argentina. Tel.: 03783-425753.
 E-mail: lucianacholich@hotmail.com

Intoxication by *Ipomoea carnea* in ruminant is characterized by cytoplasmic vacuolation in cells of organs. Calystegines (0, 05%) and swainsonine (0, 02%) were isolated in this plant and produce inhibition of α and β galactosidases, α glucosidase and lysosomal α -manosidase respectively. The aim of this study was demonstrate the toxic effects of the alkaloids present in dry leaves of the plant administered to guinea pigs. Histological evaluation and lectin histochemistry was applied to identify stored specific sugars in cells. In the cytoplasm of exocrine pancreas and renal tubular cells was showed the presence of vacuoles and were identified lectins such as Con-A (*Concanavalina ensiformis*), sWGA (*Succinil triticum vulgaris*), WGA (*Triticum vulgaris*) and LCA (*Lens culinary*). This result is coincident with the lectin histochemistry staining pattern of the vacuoles described for ruminant and also indicates that they contain N-glycosidically bound oligossacarides.

BIII-66.**EFFECT ON GASTRIC MOTILITY OF *JODINA RHOMBI-FOLIA* AND *PLANTAGO LANCEOLATA***

Toso R, Toribio M, Boeris M.
 Centro de Investigación y Desarrollo de Fármacos, FCV, UNLPam.
 Calle 5 y 116, General Pico, La Pampa.
 E-mail: retoso@vet.unlpam.edu.ar

Hydro-alcoholic extracts of *Jodina rhombifolia* (*Jodina*) and *Plantago lanceolata* (*Plantago*), collected in Province of La Pampa, Argentina, were administered to mice to determine effects on gastric motility. The time from administration *per os* of barium sulphate until gastric evacuation was observed using radiography. Neostigmine (0.08 mg/kg) and Hyoscine-N-butylbromide (0.05 mg/kg) were administered as reference drugs. Mice in the control group began emptying immediately after administration. Evacuation was delayed 4 h by *Plantago* and 30 minutes by *Jodina*. Gastric evacuation was in 2 h with Hyoscine and Neostigmine completed evacuation in 15 minutes. Combined administration of *Plantago* and Hyoscine did not show differences with mice treated with only Hyoscine, but inhibited the effect of the mice treated with Neostigmine. In conclusion, *Plantago* has an anticolinergic effect on gastric motility antagonizing the effect of Neostigmine, while *Jodina* neither showed effect on gastric motility nor pharmacological interactions with reference drugs.

BIII-67.**PHARMACOLOGICAL ACTIVITY OF ETHANOLIC EXTRACT OF *URTICA URENS***

Marrassini C², Miño J¹, Acevedo C¹, Ferraro G², Gorzalczany S¹.
 Cátedras de ¹Farmacología y ²Farmacognosia. IQUIMEFA (UBA-
 CONICET), Facultad de Farmacia y Bioquímica, UBA. Junín 956
 (1113). Buenos Aires, Argentina. E-mail: sgorza@ffyb.ub.ar

Urtica urens L. (Uu) belongs to the Urticaceae family and it is known with the common names of "ortiga", "ortiga crespá", "ortiga chica". This is a native herb widely distributed in South America and also in Europe, Africa, Asia and Australia. In South America, is widely used in popular medicine as diuretic, antirheumatic and for muscular pain. For this study, the dried aerial parts were ground to a fine powder and were extracted by maceration with 80% ethanol at room temperature for 24 hours. The extract of Uu was evaluated for antinociceptive activity using writhing, formalin and hot-plate tests in mice. A dose-related antinociceptive response was obtained in the writhing test at doses between 10 - 500 mg/kg i.p. (percentage of inhibition 59 to 96%). The extract also inhibited the second phase of formalin test (77%) at dose of 100 mg/kg i.p. Furthermore, no significant effect was obtained in the hot-plate test. The anti-inflammatory activity was analyzed with the carrageenan-induced paw edema in rats at doses of 100 and 300 mg/kg i.p. A significant antiedematogenic effect was obtained at doses of 300 mg/kg (42% of inhibition), no effect was seen in the ear edema induced by 12-*O*-tetradecanoylphorbol-13 acetate (TPA) in mice. These results indicate that Uu has antinociceptive and antiinflammatory activities that could be support the folk medicinal use of the plant.

BIII-68.**IN VITRO ANTIFUNGAL ACTIVITY OF EXTRACTS FROM *LARREA DIVARICATA* CAV. (JARILLA) AND *BLEPHAROCALYX SALICIFOLIUS* (HBK) BERG (ANACAHUITA)**

Dallia S, Montrull H, Brizuela N.
 Departamento de Salud y Educación. Universidad Nacional de La
 Rioja. Avda. Menem y Favaloro. 5300 La Rioja. Argentina.
 E-mail: nilda.brizuela@gmail.com

Larrea divaricata and *Blepharocalyx salicifolius* are plants widely used by the folk medicine in Argentina. Aims: *To get the different extracts, *Larrea divaricata* Cav. (Jarilla) and *Blepharocalyx salicifolius* (HBK) Berg (anacahuita). *To use dermatophytes causing *Tinea Pedis* typified (CEREMIC), to isolate and to assess the fungistatic activity. *To evaluate the fungitoxic activity of *Larrea divaricata* Cav. and *Blepharocalyx salicifolius* extracts against dermatophytes causing *Tinea Pedis*: *Epidermophyton floccocum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. This activity was compared with the action of Ketoconazole and Amphotericin B. Antifungal activity of *Larrea divaricata* and *Blepharocalyx salicifolius* was investigated using dichloromethane, methanol, hydroalcoholic (Ethanol 70% v/v 30% water) and aqueous extracts. Our data suggest that *Larrea divaricata* and *Blepharocalyx salicifolius* extracts contain compounds with fungitoxic potency against dermatophytes causing *Tinea Pedis*: *Epidermophyton floccocum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

BIII-69.**IN VITRO ANTHELMINTIC EFFICACY OF A PLANT WITH POTENTIAL ANTIPARASITIC EFFECT AGAINST GASTROINTESTINAL NEMATODE PARASITES OF CATTLE AND GOATS**

Moreno F^{1,3,4}, Saumell C⁴, Gordon I¹, Wright A², Benvenuti M^{1,3}, Fiel C⁴.

¹CSIRO CSE, Australia; ²AIMS, Australia; ³INTA EEA Cerro Azul, Mnes; ⁴Fac Cs. Veterinarias U.N.C.P.B.A. Tandil, Argentina.
E-mail: Fabiana.Moreno@csiro.au

The purpose of this study was to test *in vitro* the possible direct anthelmintic effects of plant extracts, rich in secondary metabolites (PSM), upon the migration of infective larvae (L3) of *Haemonchus placei*, *Cooperia punctata* (cattle) and *H. contortus*, *Trichostrongylus colubriformis* (goats) in order to identify plants that can then be tested *in vivo*. The effect of PSM extracted from several plant species on the motility of nematodes, were evaluated using a larval migration inhibition (LMI) assay. The effect of plant extracts on parasite migration was analysed by ANOVA. Almost all plant extracts showed inhibitory effect against *H. placei* y *C. punctata*. Extracts from *Allocauarina torulosa*, *Neolitsea dealbata*, *Acacia holosericea*, *Acacia salicina*, *Callitris endlicheri* and *Casuarina cunninghamiana* were the most effective against these parasites ($p \leq 0.05$). In general plant extracts showed low inhibitory effect against *H. contortus* and *T.colubriformis*. However plant extracts from *C. endlicheri*, *C. cunninghamiana* and *A. holosericeae*, *A. nilotica* and *A. farnesiana* caused a significant reduction ($p \leq 0.05$) in *H. contortus* and *T. colubriformis* larval migration. We conclude that the impact of plant extracts with PSM on larval migration suggests a possible role for these plants in ruminant diets as a means to reduce dependence of anthelmintic drenches.

BIII-70.**ANTISPASMODIC EFFECT OF "BURRITO" (ALOYSIA POLYSTACHYA) AND ITS Ca²⁺ NON-COMPETITIVE ANTAGONISM ON ISOLATED RAT INTESTINE**

Berardi A, Ragone MI, Consolini AE.

Cátedra de Farmacología, Dpto Cs. Biológicas, Fac. Cs. Exactas, UNLP. 47 y 115 (1900) La Plata, Argentina.

E-mail: dinamia@biol.unlp.edu.ar

Plants from the genus *Aloysia* (Verbenaceae) like "cedrón", "palo Amarillo" (Pam) and "burrito" (*Aloysia polystachya*, Griseb., Mold.) are used in Argentinean folk medicine as eupeptics. We studied the antispasmodic effect of "burrito". A 20% aqueous extract (AEB) was prepared and lyophilized (yield: 11.2%). The effects of AEB was tested on isolated rat duodenum and ileum submerged in Tyrode solution (Ca²⁺ 1.8 mM, pH 8.2) at 37°C, while the longitudinal force was measured by WPI isometric transducers, and A/D acquired. The AEB non-competitively inhibited the dose-response curves (DRC) of acetylcholine (Ach), with an IC₅₀ of 1.96±0.32 mg lyoph/ml (n=5), which was similar to that of "cedrón" (1.34±0.5 mg/ml). The maximal inhibition was 37 ± 4% of the E_{max}^{Ach} at 3 mg/ml AEB, which was comparable to that of "cedrón" (23% at 6 mg/ml) but higher than that from "Pam" (67% at 1 mg/ml). The mechanism was assayed on DRC of Ca²⁺ under a depolarizing 80 mM K⁺-0 mM Ca²⁺-Tyrode. AEB also non-competitively inhibited the Ca²⁺-DRC, with an IC₅₀ of 3.94±0.37 mg lyoph/ml (n=7) up to 64.8±9% of E_{max}^{Ca} at 6 mg lyoph/ml. The present results suggest that: **a)** the spasmolytic effect of AEB was similar to that of "cedrón" but higher than that of "Pam", **b)** it is not due to a competitive antagonism on muscarinic receptor nor on Ca²⁺ influx, but to an interference with another intracellular pathway, as well as "cedrón". UNLP X-408-2005/08.

BIII-71.**HEPATOPROTECTIVE ACTIVITY OF ARTEMISIA DOUGLASIANA BESSER. STUDY OF ACUTE TOXICITY**

Gil L, García Aseff S, Wendel G, Pelzer L.

Farmacología. Fac Qca, Bioqca y Fcia, U.N.S.L. Chacabuco y Pedernera, San Luis 5700. E-mail: aseff@unsl.edu.ar

Artemisia douglasiana Besser (*Ad*), known as "matico", have been used in folk medicine for gastrointestinal disorders. The aim of this work was to study the hepatoprotective activity, using the model of experimental liver damage induced by acetaminophen (640 mg/kg) in rats and the acute toxicity in mice. Infusion (20%) was prepared. Hepatoprotective activity: serum aspartate (AST) and alanine aminotransferase (ALT) were determined. The *Ad* infusion produced reduction of AST ($p < 0.05$) in males, but significant differences were not observed in ALT in both sex. Acute toxicity: mice were fasted for 4 hours and given i.p. increasing doses (5-2000 mg/kg) of lyophilized water infusion. It was administered to five (one group served as control) groups of 6 mice each (3 male and 3 female). Animals were observed for 14 consecutive days to register body weight, mortality or other toxic symptoms. The *Ad* infusion, at the dose of 2000 mg/kg killed all mice at 24 h; however, it showed any visible symptoms of toxicity at dose as 5-300 mg/kg: there were no signs on symptoms of restlessness, respiratory distress, diarrhea, convulsions, coma and did not induce change on the spontaneous activity. Relative wet weights of organs were not statistically different. *Ad* infusion showed hepatoprotective activity in the acute liver injury induced by acetaminophen. In the acute toxicity test only the highest dose of *Ad* infusion presented signs of toxicity.

BIII-72.**PHARMACOLOGICAL ACTIVITY OF THE PURIFICATE FRACTION FROM DECOCTION OF THE CHILIOTRICHUM DIFFUSUM (ASTERACEAE) FLOWERS**

Alcalde SM¹, Córdoba OL², Gorzalczy S³, Höcht C³, Flores ML⁴, Taira CA³

¹Farmacología I, ²Química Biológica II and ⁴Farmacognosia, CRIDECIT - Facultad de Ciencias Naturales, UNPSJB. Km 4, Comodoro Rivadavia, 9000, Chubut; ³Farmacología e INFIBIOC, FFyB, UBA, Junín 956, 1113, Buenos Aires, Argentina.

E-mail: ctaira@ffyb.uba.ar

In this work we studied pharmacological effects of the purification fraction from decoction of *Chiliotrichum diffusum* (Asteraceae). Flowers were collected in Santa Cruz, Argentina and air-dried after its collection. Powdered flowers were extracted by decoction and purification with solvents. Carragenin antiinflammatory test was carried out in rats and antinociceptive tests (hot plate and writhing tests) were carried out in mice. For cardiovascular studies, arterial pressure was calculated from the intraarterial registers in anesthetized rats. Aqueous fraction of the purification decoction showed flavonol glycosides: quercetagenin-7-O-glucoside, quercetin-3-O-glucoside, quercetin-3-robinobioside-7-O-rha and the other derivatives from kaempferol and quercetin. The fraction (300 mg/kg ip) showed anti-inflammatory activity when it was studied by the carragenin test (inhibition 64%), and antinociceptive activity by the writhing test (500 mg/kg, inhibition 82.3%). Effect was not seen by hot plate test. The fraction had a dose-dependent depressor effect (0.3-30 mg/kg, iv). In conclusion, this fraction from *C. diffusum* has antinociceptive, anti-inflammatory and vascular depressor activities.

BIV-73.**PROTECTION OF CHIMERIC SUBCELLULAR VACCINES AGAINST *BRUCELLA OVIS* IN RAMS**

Estein S¹, Fiorentino A², Paolicchi F², Claussé M¹, Manazza J², Cassataro J², Giambartolomei G³, Coria L³, Zylberman V⁴, Fossati C³, Goldbaum F⁴

¹Lab. de Inmunología, F.C.V., U.N.C.P.B.A., Pinto 399, Tandil, Bs. As., Argentina. E-mail: silmares@vet.unicen.edu.ar; ²Lab. de Bacteriología, INTA-Balcarce; ³IDEHU-CONICET, FFyB, U.B.; ⁴Fundación Instituto Leloir.

Chimera BLS-OMP31, as a recombinant protein (BLS-Omp31) or DNA vaccine (*pCIbbs-Omp31*), has been identified as a protective antigen against *B. ovis* in mice. In this work, groups of 10 rams were vaccinated three times with: a) Chimera in oil based adjuvant (AFI), b) Chimera in saponin (QUIL A), c) DNA with electroporation, d) DNA without electroporation and e) Prime-boost (PB) (3 times with electroporated DNA, and a fourth with protein). Control group was immunized with hot saline extract (HS) of *B. ovis*. Unvaccinated group was included. Rams were challenged with virulent *B. ovis* 7 months after last immunization and slaughtered 6 months thereafter, taking bacteriological samples from 12 organs. Chimera in AFI and PB strategy induced the highest IgG specific antibodies followed by chimera in QUIL A. Electroporation enhanced humoral immune response in DNA vaccinated rams. However PB stimulated the best levels of specific gamma IFN. The highest rates of protection were obtained with PB (75%) and chimera with AFI (63%). In the other groups the level of protection remained between 10-20%, including HS vaccine. Percentages of infection in unvaccinated rams and DNA without electroporation were 100%. Altogether these results indicate that chimera should be considered as potential vaccine in ovine brucellosis.

BIV-74.**EFFECTS OF DICLOFENAC AND PARACETAMOL ON INTERLEUKIN 1 AND NITRIC OXIDE PRODUCTION IN OSTEOARTHRITIC HUMAN ARTICULAR CHONDROCYTES**

Ricarte Bratti JP, Montrull HL, Brizuela N, Demurtas S, Meirovich CI.

Dpto. de Farmacología. FCM. Universidad Nacional de Córdoba. Santa Rosa 1085. Córdoba, Argentina. E-mail: hmontrull@fibertel.com.ar

Osteoarthritis (OA) is characterized by slowly progressive loss of articular cartilage, in which the breakdown leads to matrix fibrillation and full-thickness loss of the joint surface. As an irreversible step in OA occurs when collagen is degraded. Moreover, OA-affected chondrocytes show an up-regulation of various inflammatory mediators including cytokines, matrix-degrading enzymes, nitric oxide. To determine the effects of two drugs Diclofenac (DICLO) and Paracetamol (PARA) on interleukin-1 β (IL-1 β), and nitric oxide (NO) production by OA-affected chondrocytes. Human chondrocytes were enzymatically isolated from osteoarthritic knee cartilage and then maintained in culture in suspension for 48 h in the absence or in the presence of 10 μ g/mL de DICLO y PARA. The age of patients ranged from 45 to 79 years. NO-[2]/NO-[3] concentrations were determined using the Griess assay, enzyme-linked immunosorbent assay (ELISA) was used to quantify IL-1 β DICLO and PARA had no significant effect on NO production. DICLO was associated with significant decrease of IL-1 : 16.4 \pm 9.2 vs. 0.8 \pm 0.3 pg/ml ($p < 0.05$). PARA had no significant effect on production of IL-1.

BIV-75.**ANTIGEN PRESENTATION CELLS (APC) AND CD8 LYMPHOCYTE RECRUITMENT ARE INDUCED BY IMIQUIMOD SKIN APPLICATION**

Pretti R, Diaz Aquino V, Martire K, Roque G, Marin GH, Mansilla E.

Lab. Ing. Tisular, CUCAIBA. 1931 Ensenada, Bs. As. E-mail: edmansil@netverk.com.ar

Although new chemotherapy has been developed in last 50 years, cancer is still a major cause of mortality. Though, stimulating immune system remains indisputable. In order to induce APC immune recruitment, imidazoline components were tested as local treatment. Twenty Californian Rabbits were randomly divided in control (C) and treatment (T) groups. One specimen of each group was sacrificed for basal pathology organ studies. All rabbits were submitted to 7x7 cm square skin shave. Half gram Imiquimod-5% (Imimore®) crème was applied diary to T group and placebo crème to C rabbits. Each application was covered with Tegaderm® (3M) film. Blood samples for laboratory tests and skin punch biopsies were obtained weekly. After 3 weeks, necropsies were done to measured drug organ consequences. Epidermis with horny layer increased in thickness and decrease of thorny stratum in T group biopsies compared with C one's. T group dermis showed edema and mesenchymal and mononuclear cells increment when compared with Control's. (73% \pm 24 vs. 27% \pm 18% $p < 0.04$ and 65% \pm 18% vs. 31% \pm 28 respectively). Presence of these both types and also dendritic cells were seen inside blood vessels and local lymph nodes. Flow cytometry showed 40% \pm 14 CD8T lymphocyte increment in Tgroup. No differences were detected in hemoglobin, liver enzymes, proteins, creatinine or urea levels between both groups. Liver, renal, spleen or brain, did not develop changes from basal necropsy group Changes induced by imiquimod may becomes a promising drug in treatment of skin atypical lesions.

BIV-76.**BONE MARROW ADRYAMICIN INDUCED ERYTHROPOIETIC INJURY AND RENAL NEPHROTOXICITY**

Stoyanoff T, Stemberg E, Todaro J, Cardoso L, Juaristi J, Aguirre M, Brandan N.

Cátedra de Bioquímica. Fac.Medicina. UNNE. Moreno 1240 (3400) Corrientes. E-mail: nbrandan@med.unne.edu.ar

Adryamicin (ADR) is a chemotherapeutic agent that induces genotoxicity in hematopoietic system. However, its effects on bone marrow (BM) erythropoiesis related to nephrotoxicity are not well known. This study evaluates the effects of ADR on the erythropoietic response and its relationship with renal injury. CF-1 mice were injected with ADR (15 mg/kg ip) for a time course study of 120 days. BM mitosis and apoptosis, BM microenvironment, EPO-R expression, differential erythroid precursors and erythroid colonies were determined. Renal HIF expression, functional and structural assays were performed in parallel. BM erythropoiesis was deeply affected: erythroid cells, CFU-e and BFU-e colonies decreased by day 3. Apoptosis was maximal on day 7. EPO-R levels decrease was coincident with BM microenvironment disruption. Erythropoietic BM recovery was observed from day 30. HIF was detected since day 15 in accordance with hypoxic nephrotoxicity. These results suggest that BM recovery to ADR-induced erythropoietic injury might be associated with BM microenvironmental regulations rather than with renal hypoxia.

BIV-77.**DOXORUBICIN EFFECTS ON BONE MARROW MICRO-ENVIRONMENT, APOPTOSIS AND BAX EXPRESSION**

Stemberg E, Stoyanoff T, Todaro J, Cardoso L, Reyes J, Aguirre M, Brandan N.

Cátedra de Bioquímica. Facultad de Medicina. UNNE. Moreno 1240. (3400) Corrientes. E-mail: nbrandan@med.unne.edu.ar

Doxorubicin (DOX) is a chemotherapeutic with wide clinical applications. Dose dependent-life-threatening toxicity is a crucial limiting factor for its therapeutic use. It induces apoptosis, inhibits topoisomerase II, and produces reactive oxygen species. However, it is not well known the features of BM hematopoiesis upon DOX treatment. The aim of this study was to evaluate the effects of a single dose of DOX (15 mg/kg) on bone marrow (BM) hematopoiesis in mice (CF-1) for 45 days. BM cellularity and viability, mitotic and apoptotic indexes, BM microenvironmental organization (scanning electron microscopy) and Bax expression (immunoblotting) were determined. BM cellularity decreased by day 3 ($p < 0.01$ vs control) until the end of the experience. BM viability was transiently affected by day 5. Erythroid and myeloid compartments were the most affected subsets. Fluorescent microscopy revealed increments of the apoptotic indexes from day 3 to 7. A drastic disruption of the BM microenvironment and Bax (a pro-apoptotic protein) over-expression were significant on the 3rd day. These results indicate that DOX causes apoptosis in BM with substantial deleterious effects on hematopoiesis.

BIV-78.**APPLICATION OF A 32P-PATCH IN THE TREATMENT OF A MURINE MELANOMA**

Salgueiro MJ¹, Arnoldi S¹, Barreiro Arcos ML², Medina V¹, Nicolini J³, Ughetti R³, Cremaschi G^{1,2}, Zubillaga M¹

Laboratorio de Radioisótopos, FFyB, UBA. ²CEFYO-CONICET-UBA, ³Laboratorios Bacon SAIC. Junín 956 Piso Bajo-1113 CABA. E-mail: jsalgueiro@ffybu.uba.ar

We designed a 32P-patch that in previous works had demonstrated its safety and therapeutic efficacy in a skin cancer model in Sencar mice. The objective in this work was to evaluate the therapeutic effects of the 32P-patch in the treatment of a murine melanoma. 20 male C57BL6 mice were divided in 2 groups: treated (T) and control (C). Superficial tumors were induced in T and C by injecting B16F10 melanoma at about 10⁵ cells/mouse subcutaneously. Tumors developed 10-15 days after transplantation and the 32P-patch was applied on palpable tumors of T group. Tumor growth was followed during 21 days in both groups by measuring tumor size with a caliper. Finally, all animals were sacrificed and skin samples were collected for histological study and stained with H&E. The skin at application site of 32P-patch appeared hairless and erythema developed, but reversed to normal after a few days in T group. Tumor growth control was achieved in T group compared with C group. Preliminary histological analysis revealed vascularized melanoma tumors in C group and both complete and partial regression in T group. Conclusion: the 32P-patch may be considered as a promising approach for the treatment of melanoma tumors.

BIV-79.**FORMOCRESOL-INDUCED CELL DEATH IN THE MURINE PERITONEAL MACROPHAGES HAS BOTH APOPTOTIC AND NECROTIC FEATURES**

Cardoso ML, Alvarez M, Aguirre M, Todaro J, Brandan NC.

Cátedra de Bioquímica. Facultad de Medicina. UNNE. Moreno 1240. (3400) Corrientes. Argentina. E-mail: nbrandan@med.unne.edu.ar

The potential toxicity of Formocresol (FC), a compound widely used in pediatric dental care, is a cause of concern worldwide. The aim of the present study was to quantify the rate of macrophage viability and apoptosis/necrosis following FC exposure. Additionally, heat shock protein (Hsp60), pro-apoptotic proteins (Fas and Bax), as well as the pro-survival protein Bcl-x expressions were analysed. Peritoneal murine macrophages (pMø) were cultured in 1:100 FC 2 to 24 h. Viability (Trypan-blue), cell morphology (Scanning Electron Microscope), apoptosis and necrosis (light and fluorescent microscopy) were studied at different scheduled times. Furthermore, the expression of proteins related to stress, survival and cell death was measured by western blotting. FC exposed macrophages exhibited maximal apoptosis from 2 h to 6 h, coincident with Bax over-expression ($P < 0.001$). Additionally, Bcl-x_L showed maximal expression between 12 and 24 h suggesting its survival effect in pMø. An increase in the necrotic rate from 4 h to 12 h in accordance to Hsp60 and Fas over-expression and the lowest pMø viability was also observed ($P < 0.001$). Taken together these results suggest that FC induces in pMø an initial period of apoptosis (2-6 h) controlled by the Bax/ Bcl-x_L offset followed by a period of necrosis (4-24 h) regulated by Fas and HSP 60. We also believe that Bcl-x_L over-expression did not interfere in the pMø necrotic progression program.

BIV-80. **α -MSH CAN MODULATE LPS+IFN- γ SIGNALING TRANSDUCTION PATHWAYS INVOLVED IN INFLAMMATION**

González P¹, Caruso C², Sanchez M³, Cabanillas A⁴, Lasaga M², Simonelli T¹

¹IFEC CONICET, Dpto Farmacol. FCQ, UNC. ²Instituto Investigaciones en Reproducción. Fac. Medicina, UBA. ³CEPROCOR Córdoba. ⁴CIBICI-CONICET Dpto Bioq,Clinica. FCQ, UNC. 5000 Córdoba, Argentina.

E-mail: vgonzalez@fcq.unc.edu.ar

There is evidence that the melanocortin alpha-melanocyte stimulating hormone (α -MSH) has immunomodulatory and anti-inflammatory actions within the brain. The aim of the present study was to establish the effect of α -MSH on LPS+IFN- γ -induced signalling pathways in hypothalamic neurones or astrocytes in culture. We found that α -MSH suppresses LPS+IFN- γ -induced nuclear translocation of the transcription factor NF-kB. Besides, treatment with LPS+IFN- γ stimulates phosphorylation of cAMP-responsive element-binding protein (CREB), but this effect was not modified by α -MSH. In neurones, LPS+IFN- γ elicited a slight increase in CREB activation. Treatment with α -MSH induced CREB phosphorylation and the combination of LPS+IFN- γ and α -MSH induced an additive increase in CREB activation. Our results demonstrated that α -MSH can differently modulate signalling transduction pathways induced by LPS+IFN- γ .

BIV-81.**TH1/TH2 IMBALANCE INDUCED BY CHRONIC STRESS EXPOSITION IS RELATED TO DIFFERENT VULNERABILITY TO STRESS EFFECTS IN BALB/C AND C57BL/6 MICE**

Palumbo ML, Canzobre MC, Ríos H, Wald M, Genaro AM. CEFYBO-CONICET-UBA and Instituto de Biología Celular y Neurociencias, Fac. Medicina, UBA. Paraguay 2155, Piso 15, Bs As, Argentina. E-mail: molecula_21@yahoo.com.ar

Stress has been related to cognitive deficit. The hippocampus, a limbic area involved in learning and memory, is particularly sensitive to the stress effects. Cytokines have been shown to affect some behaviours, including effects on sleep, appetite, sexual behavioural, memory and motor activity. Moreover, IL-2, INF- γ (TH1-cytokines) and IL-6 (TH2-cytokines) has been implicated in psychiatric disorders. The aim of the present work was to analyze the correlation between the cytokine production and behavioural alteration induced by stress in Th1-biased C57BL/6 and Th2-biased BALB/c mice. We found that BALB/c but not C57BL/6 mice exposed to chronic stress had a poor learning performance respect to control mice. Histological studies showed that the number of neurons and the thickness in the area CA1 y CA3 of hippocampus were lower in stressed BALB/c but not in stressed C57BL/6 mice respect to control. Moreover, an increase in ROS production was observed in BALB/c but not in C57BL/6 mice under stress. Finally, we found an increase of INF- γ and IL-2 in stressed C57BL/6 mice and a decrease of INF- γ and an increase of IL-6 in stressed BALB/c mice. These results indicate that BALB/c mice are more vulnerable to stress effects than C57BL/6 mice associated to a differential regulation of TH1/ TH2 cytokine balance.

BIV-82.**TRANSFORMING GROWTH FACTOR β STIMULATES BOVINE LEUKEMIA VIRUS EXPRESSION IN NATURALLY INFECTED CELLS**

Gutiérrez S, Juliarena M, Ceriani C, Esteban E. Laboratorio de Virología, Fac de Cs Veterinarias, UNCPBA. Pinto 399, Tandil, Argentina. CONICET. E-mail: segutier@vet.unicen.edu.ar

Fetal calf serum (FCS) and platelet extracts (PE) have been shown to stimulate Bovine Leukemia Virus (BLV) expression in a naturally infected cell line (NBC-10) as well as in primary cultures of bovine peripheral blood mononuclear cells (PBMC). In order to identify the platelet factor responsible for this stimulation we have tested the activity of several recombinant or highly purified platelet factors on the expression of BLV in NBC-10 cells. The 3 mammalian isoforms of transforming growth factor- β (TGF- β stimulated the synthesis of the major core BLV protein (BLVp24) in a dose dependent manner. Anti TGF- β antibodies neutralized the effect of FCS and PE on the synthesis of BLVp24 in NBC-10 cells. Recombinant TGF- β also stimulated the synthesis of BLVp24 in 24 hr cultures of BLV infected PBMC. It is concluded that TGF- β is the main factor involved in the *in vitro* activation of BLV expression induced by FCS and PE in NBC-10 cells. The bioassay employing NBC-10 cells as indicator system proved to be useful for the identification of substances regulating the expression of BLV.

BIV-83.**BLOOD CELLS PACKETS, FABRICIUS BURSA AND SPLEEN IN BROILER CHICKENS WITH TWO DIETS**

Sandoval GL², Revidatti FA¹, Terraes JC¹, De Biasio MB², Romero CR².

Dptos. ²Cs. Básicas y ¹Producción, Fac. de Cs. Veterinarias, Univ. Nac. del Nordeste, Sgto Cabral 2139, 3400, Corrientes (Cap), Argentina. E-mail: bioquim@vet.unne.edu.ar

Males (M) and females (H) Broiler chickens (considered as separate blocks) were raised indoor (six birds per m²). Two cycles of production of 49 and of 56 days (for M and H respectively) were carried out, each divided in two stages: beginning (0 to 21 days) and fattening (21 days to end). Two treatments with seven (M) and eight (H) replicas each one were applied. They consisted in qualitative - quantitative different diets called broiler feed (P) and fodder feed (F). The samplings took place at the end of each stage. Analysis of repeated measures were applied with a significance level of 5% (Statistix program for Windows). At slaughter, the corporal weight (PC), the relative proportion of spleen and Fabricius bursa were not different between blocks, nor between treatments. Differences were found between blocks' haematocrites ($p < 0.05$). The total leukocyte fraction did not demonstrate significant variations, only insinuated a tendency in favor of H at slaughter ($p = 0.09$). These two variables showed higher values in H, probably due to the different circumstances of slaughter. Both formulations gave similar results, consequently the election of one of them, will depend on the evaluation of other indicators such as the economic ones.

BIV-84.**ATTENUATED SALMONELLA AS ADJUVANT IN CANCER VACCINES**

Vendrell A, Di Sciullo P, Gravisaco MJ, Colman E, Rizzo N, Mongini C, Waldner C.

CEFYO, CONICET-UBA. Paraguay 2155, P16 (CP 1121). Buenos Aires. E-mail: avendrell@fvet.uba.ar

The role of innate immunity in stimulating adaptive immune responses is the basis of the action of adjuvants which are useful in clinical vaccines by promoting a nonspecific pro-inflammatory response to elicit specific host defense.

Based on previous studies that demonstrated the ability of *Salmonella* to elicit an inflammatory Th1 response when it is inoculated by mucosal route, we propose to study the use of an attenuated *Salmonella* strain as an adjuvant in cancer vaccines to stimulate the innate and modulate the adaptive immune response against tumors. Two times at seven days intervals BALB/c mice were immunized via the orogastric route with 2×10^9 UFC of CVD915 or PBS (control) using a gavage tube needle, and simultaneously, 1×10^6 irradiated LBC tumor cells (LBCi) or PBS were inoculated by intraperitoneal (i.p) injection. Seven days after the second immunization, all groups of animals were challenged i.p with wild-type LBC cells. Mice immunized with bacteria simultaneously with LBCi display a significant increase in the medium survival time respect to PBS-treated mice ($p < 0.05$, Log rank test).

Our findings suggest that immunization with *Salmonella* in combination with irradiated tumor cells could be a useful strategy for prophylactic vaccination that merits further studies to prove its potential in cancer vaccines.

BIV-85.**A COMMUNITY OF MULTISPECIES AND MULTISTRAIN ACID LACTIC BACTERIA AND YEAST FERMENTING WHEY IS A POTENTIAL PROBIOTIC IN CALVES**

Indart H, Landi R, Cerone S, Esteban E, Zavaleta R, Inza G, Mogni S, Igarza L.

Departamento de Fisiopatología, Facultad de Ciencias Veterinarias y LactiTec SRL, Universidad nacional del Centro. Campus Universitario. (7000) Tandil. Argentina.

E-mail: mindart@vet.unicen.edu.ar

The aim of this work was firstly to achieve a simple and costless propagation of potentially probiotic agents using plain whey as culture medium. Second, to mitigate diseases of calves when they rise under stressful conditions or environmental hostilities. After a systematic selection of agents by their growing capacity in whey, the constituted microbial community was considered as a unit. The community is composed by agents from separated dominium like Bacteria and Eukaria. Selected lactic acid bacteria and yeast are multispecies and also multistrain assuring high biodiversity. The consistency found in continuous cultures fermenting whey suggests a symbiotic relationship and high adaptability.

Experiments were designed assessing alimentary security or any beneficial effect on calves. The community not only lack adverse effects when supplied as food additive but surprisingly showed remarkably health benefits. The prevention of infection and highly significant increasing of phagocytic activity in peripheral blood leukocytes seen in calves, strongly suggests an efficient connection between the community and the immune system. Studies are under way examining quorum sensing between community members, host microbiota and host immune system.

BIV-86.**CHARACTERIZATION OF *Staphylococcus aureus* STRAINS IN A PUBLIC HOSPITALARY UNIT**

Sparo M¹, Ranno G¹, Delpech G¹, Sanchez Bruni S^{2,3}.

¹Laboratorio de Microbiología, Hospital Ramón Santamarina-Tandil. ²Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, UNCPBA. (B7000APA) Tandil. ³CONICET.

E-mail: monicasparo@speedy.com.ar

Methicillin resistant *Staphylococcus aureus* (Sa) strains are the more frequent hospitalary pathogen worldwide (H-MRSA). But recent infections have been reported in patients from the community without risk factors (Ca-MRSA). The goal of this study was to investigate the epidemiology and antimicrobial resistance of Sa strains isolated from patients during the period 2006-2008. Patients' inclusion criteria were used under the CDC (USA) policy. For the Sa strains were performed PLP2a and also *in vitro* antimicrobial sensitivity. 340 different patients showed Sa infections. The 36% of them were MRSA, and from the latter, 25%, Ca-MRSA. H-MRSA strains were associated with elderly patients (51% >60 old years). However, Ca-MRSA mainly invaded paediatric and young patients (57% < 20 years old). In whole MRSA were detected PLP2a. Cefoxitin and oxacilin were predictors of resistance to beta-lactams. H-MRSA strains showed resistance associated to gentamicin (94%), erythromycin (96%), clindamycin (96%), cloramphenicol (100%), rifampicin (79%), TMS (27%) and ciprofloxacin (87%). Ca-MRSA showed resistance to eritromycin (7%), gentamycin (17%), clindamycin (7%) and rifampicin (18%); being it sensitive to TMS, ciprofloxacin and cloramphenicol. Whole MRSA were sensitive to vancomycin, teicoplanin and minociclin. It is necessary a rapid differentiation between H/Ca-MRSA strains for a rational therapeutic scheme.

BIV-87.**EUDRAGIT E100 POTENTIATES ACTIVITY OF OFLOXACIN AGAINST FQ-RESISTANT *PSEUDOMONAS AERUGINOSA***

Romero V, Manzo R, Alovero F.

Departamento de Farmacia. Fac. de Ciencias Químicas. UNC. Ciudad Universitaria 5016. Córdoba. Argentina.

E-mail: fallover@fcq.unc.edu.ar

Fluoroquinolones (FQs) resistance is a clinically significant issue. Efforts to improve the efficacy of existing FQs could be an alternative to restrict the development of FQ-resistant bacteria prolonging their future utility. Eudragit E100 (Eu) is a cationic polyelectrolyte able to react with the acidic group of OFLOXACIN yielding Eu-OFLO_x complexes where OFLO neutralizes 20-50% of the basic groups of Eu. The addition of a second counterion (Cl⁻) turns the complexes water soluble giving clear dispersions (Eu-Cl₅₀-OFLO_x) with positive electrokinetic potential (ξ) and pHs 6.4-6.5. The activity against FQ-R *P.aeruginosa* was enhanced exhibiting MICs two or four-fold lower than free OFLO and improved bactericidal effect. Eu-Cl₅₀ without OFLO exhibits an initial antibacterial effect reversible along the time while Eu-Cl₅₀-OFLO_x exhibits enhanced effect leading complete bacterial eradication at concentrations or time assays where free OFLO does not show it. This behaviour could be attributed to the improved drug-bacterial cell interaction, topic in which the high ξ of Eu-Cl₅₀-OFLO_x could play a preponderant role.

BIV-88.**"In vitro" ACTIVITY OF A NOVEL ANTIMICROBIAL PEPTIDE CECT7121 AGAINST HUMAN CLOSTRIDIUM STRAINS**

Sparo M^{1,2}, Confalonieri A³, Ceci M², Ranno G¹, Urbizu L^{3,4}, Rivulgo M, Sánchez Bruni S^{3,4}

¹Laboratorio de Microbiología Hospital R. Santamarina, Tandil, ²Centro de Estudios Bioquímicos. ³Laboratorio de Farmacología, FCV-UNCPBA, (B7000APA) Tandil, Argentina. ⁴CONICET.

E-mail: ssanchez@vet.unicen.edu.ar

The emergence of anaerobes Gram (+) multi-resistant bacteria, involves a serious therapeutic concern in clinical practice. Antimicrobial peptide (AP) CECT7121 is a purified bacteriocin isolated from an environmental strain of *Enterococcus faecalis* CECT7121 with 5 kDa of MW and high lipophilicity. The goal of this work was to investigate the "*in vitro*" bactericidal action of this compound against different strains of *Clostridium perfringens* and *Cl. difficile* isolated from hospitalized care unit patients who failure to the standardized treatment. The strains assayed were six of hospital acquired methicillin-resistant *Clostridium perfringens* HRS37, HRS64 (cellulite aspiration fluid) HRS71, HRS73, HRS148 (necrotising fasciitis biopsy) and *Clostridium difficile* HRS21 (feces). Sensitivity was assessed by agar diffusion method and the bactericidal activity of AP CECT7121 characterised by killing curves method. A killing effect on bacterial populations was observed within 180 min for the *C. perfringens* strains. The CFU Log₁₀ mL⁻¹ viable counts for *C. difficile* were decreased threefold after 90 min incubation, showing also a bactericidal effect against this strain. Developing of AP-CECT7121 may be a potential tool for the treatment of Human multi-resistant bacterial infectious diseases.

BIV-89.
NOVEL THERAPEUTIC STRATEGY BASED ON ENROFLOXACIN TO IMPROVE THE EMBYO TRANSFER IN MARES

González C¹, Moreno L^{1,2}, Fumuso E¹, Rivulgo M¹, García J¹, Fernández H¹, Sparo M¹, Sánchez Bruni SF^{1,2}.

¹Facultad de Ciencias Veterinarias, UNCPBA. ²CONICET. ³Centro de Estudios Bioquímicos, Tandil (B7000APA). Argentina.
 E-mail: ssanchez@vet.unicen.edu.ar

Enrofloxacin (EFX) is often empirically used for preventing uterine infections in mares to improve the efficiency of the Commercial Embryo Transfer Farms. The research goal of this work was to correlate the uterine distribution of EFX in healthy mares. Values of Minimum Inhibitory Concentrations (MIC), for Gram (-) and Gram (+) were 0.5 and 2 µg/mL, respectively. Uterine pharmacokinetic studies were performed in 2 groups (n=5) of healthy mares in estrous, after the intravenous (IV) administration of EFX (Baytril®, Bayer) at 2.5 and 5 mg/kg. Endometrial tissue samples were taken over 48 h post-treatment and analyzed by HPLC. EFX and its active metabolite Ciprofloxacin (CFX) were recovered from endometrial tissue. The endometrial concentrations of EFX (5 mg/kg) decayed with values of 2 µg/g at 36h post-treatment above the MIC for Gram (+) and 0.96 µg/g at 48 h post-treatment for Gram (-), exceeding it twice the MIC reported for Gram (-). However, the endometrial concentrations of EFX (2.5 mg/kg) and its metabolite were below the MIC for Gram (+) and Gram (-) at 12 h post-treatment, respectively. The rational use of EFX as preventive therapeutic tool may be recommended as follows: one usual dose (5mg/kg) pre-breeding and 2 doses q 36-48h post-breeding to prevent the infection.

BIV-90.
MODULATION OF THE EFFLUX TRANSPORTER BCRP (ABCG2) ACTIVITY BY THE ANTI-HIV DRUG EFAVIRENZ IN RATS

Peroni RN^{1,2}, Rubio MC^{1,2}, Bramuglia GF².

¹Instituto de Investigaciones Farmacológicas (CONICET-UBA);
²Cátedra de Farmacología (Fac. Fcia. y Bioq.-UBA)

The safety and effectiveness of highly active antiretroviral therapy (HAART) is challenged by viral resistance to antiretrovirals and the frequent occurrence of drug interactions which may limit the access of these drugs to the target sites. Particularly, drug distribution and elimination may be modified by active efflux transporters such as BCRP. Previous studies demonstrated significant inhibition of BCRP by the non-nucleoside reverse transcriptase inhibitor efavirenz *in vitro* (Weiss *et al.*, 2007). The aim of this study was therefore to investigate the influence of the chronic treatment with efavirenz on the expression of BCRP in adult male Sprague-Dawley rats. BCRP expression was assessed by Western Blot after oral administration by gavage of 25 mg/kg efavirenz or the corresponding vehicle (corn oil), once daily during five days. The treatment with efavirenz was selected on the basis of a 98.8% reduction of the HIV-1 cDNA load in the spleen of Sprague-Dawley rats (Goffinet *et al.*, 2007). An increase in the BCRP protein abundance was observed in monocytes, lymphocytes as well as in the blood brain barrier of efavirenz-treated compared with vehicle-treated animals. Our study demonstrated that efavirenz could modulate BCRP expression which in turn could influence the bioavailability and targeted delivery of this drug to target or sanctuary HIV-sites.

BIV-91.
INCREASED LEUKOTRIENE CONCENTRATION IN SUBMANDIBULAR GLANDS FROM RATS WITH EXPERIMENTAL PERIODONTITIS

Busch L, Miozza V, Sterin-Borda L, Borda E.

Cátedra de Farmacología, Facultad de Odontología, UBA. Marcelo T de Alvear 2142. Capital. E-mail: lucybusch@yahoo.es

Mucins are heavily glycosylated, high molecular weight glycoproteins produced by epithelia of the respiratory, gastrointestinal and reproductive tracts and by salivary glands. Increased production of mucus commonly occurs in diseases involving inflammation. We observed that 22 days after inducing experimental periodontitis in the rat, by placing a sterile silk ligature around the two lower first molars, basal mucin secretion by submandibular gland was increased. The increment was inhibited by blocking β-adrenergic receptors and leukotrienes (LTs) production. In this work we evaluated LTs concentration and its relation with sympathetic system and mucin release in submandibular glands from rats with experimental periodontitis. Results showed that LTs were increased in glands from rats with periodontitis and the increment was inhibited by the inhibition of LTs production with NDGA and by blocking the β₁-adrenergic receptor with atenolol. Isoproterenol induced a dose-dependent increase of LTs concentration in rats with ligature and control. On the other hand, LTs induced mucin release in a dose dependent manner. The increment of basal mucin release in rats with periodontitis was decreased in the presence of the LTs receptor antagonist FPL 55712. It is concluded that experimental periodontitis induces an increment of sympathetic activity in submandibular gland that results in an increase of mucin secretion and LTs production. These two substances are known to participate in the oral defense mechanism.

BIV-92.
DETERMINATION OF MINIMUM EFFECTIVE CONCENTRATION OF FLUBENDAZOLE IN *IN VITRO* CULTURES OF *ECHINOCOCCUS GRANULOSUS* PROTOSCOLECES

Elissondo C, Denegri G.

Laboratorio de Zoonosis Parasitarias, FCEyN, Universidad Nacional de Mar del Plata. CONICET. Funes 3350. Mar del Plata, Argentina. E-mail: mceliss@mdp.edu.ar

In a previous work, we reported the *in vitro* effect of flubendazole (FLBZ) on *E. granulosus* protoscoleces (PSC). The aim of this work was to define the minimum concentration of flubendazole required to kill PSC *in vitro*. PSC were incubated with FLBZ at the following final concentrations: 0.1, 0.05, 0.01, 0.005, 0.003, 0.002 and 0.001 µg/ml. PSC incubated with culture medium containing DMSO were used as controls. Vitality was assessed every 6 days using the methylene blue exclusion technique and samples were taken for electron microscopy. During the first 20 days of culture, FLBZ at concentration of 0.001 µg/ml showed the same behavior that the control group. Later, the percentage of vitality diminished lightly, but no structural and ultrastructural alterations were observed. Higher concentrations produced a greater fall in vitality showing a dose-dependent effect at the lower assayed concentrations. Morphological changes included contraction of the soma region, formation of blebs, rostellar disorganization, loss of hooks and destruction of microtriches. In the current study we found FLBZ to be effective against *E. granulosus* PSC even at 0.002 µg/ml.

BIV-93.**ASSESSMENT OF PREDATORY CAPACITY OF *DUDDINGTONIA FLAGRANS* IN FAECES EXPOSED TO SUN AND SHADE**

Sagüés F¹, Saumell C¹, Fusé L¹, Iglesias L¹, Fiel C¹.

¹Área Parasitología y Enfermedades parasitarias. Facultad Ciencias Veterinarias, Universidad Nacional del Centro, Buenos Aires. Tandil. Paraje arroyo seco s/n. Argentina.

E-mail: federica@vet.unicen.edu.ar

The potential use of *Duddingtonia flagrans* as a possible biological agent for controlling nematode parasites in livestock production is widely accepted. Sun radiation could inhibit the predation action against infective larvae (L3) in faeces. The purpose of this study was to test predatory activity of *D. flagrans* on fecal matter exposed to sun radiation and shade in naturally infected horses. During 2 weeks inside temperature of faeces was registered twice a day (8:30 and 13:30 hs). The efficacy percentage with *D. flagrans* chlamydozoospores on fecal matter larvae reduction was 77% in faeces exposed to sun (mean temperature: 30.5°C, range: 13-51°C) while faeces exposed to shade larvae reduction was 100% (mean temperature: 21°C, range: 9-27°C). Therefore, *D. flagrans* showed efficient predatory activity at extreme temperature.

BIV-94.**PARASITOSIS PREVALENCE IN THE POPULATION OF CATAMARCA ASSISTED BY REMEDIAR HELATH PROGRAM**

Montrull H, Demurtas S, Murua D, Brizuela N.

Departamento de Salud Universidad Nacional de La Rioja. Argentina. E-mail: hmontrull@fibertel.com.ar

The Remediare health program assures people access to essential treatments through the Primary Health Care Centre. Its efficiency can be affected by the lack of diagnosis, laboratories, follow up and neglect the treatment. To understand the efficacy of in the use of Remediare medication provided for Parasitosis, the Remediare database was analyzed during 2003-2006 years. The objective was to evaluate the pharmacological treatments in the Sole Form of the R medical prescription. Metronidazole in the ovule or tablets pharmaceutical forms and Mebendazole (tablets or solution) were tabulated. 26782 R forms were collected. The frequency in medical prescription of both anti-parasitic drugs was similar. The database did not allow for analysis of the prescription-diagnostic relationship. In 2005, a higher number of prescriptions for both medications were detected. 60-80% of the prescriptions were for women of all ages, being the incidence higher during the productive age.

BV-95.**EFFECT OF VITAMIN E ADMINISTRATION ON ACETYLCHOLINE-INDUCED AORTIC RING RELAXATION IN PANCREATECTOMIZED RATS**

Linares LM, Wallinger ML, Reyes Toso ML, Rosón M, Planells F, Reyes Toso CF.

Departamento de Fisiología. Facultad de Medicina. UBA. Paraguay 2155 Piso 7. Buenos Aires. Argentina.

E-mail: creyesto@fmed.uba.ar

In previous studies we showed that a decreased acetylcholine-induced relaxation (Ach-IR) followed subtotal pancreatectomy (Ppx) in rats. The effect was amplified by pre-incubation in a high glucose solution -HG- (44mM/l), a situation that results in oxidative stress mainly through superoxide anion (O₂⁻) accumulation. Based on these results, we hereby investigated the effect of vitamin E (VE) treatment on Ach-IR of rats turned intolerant to carbohydrates. Subgroups of sham- and Ppx rats were chronically treated with VE (C-VE and PPx-VE) (300 mg/kg per os daily during 8 weeks). Dose-response curves for Ach-IR of aortic rings (after previous exposure to phenylephrine) were conducted in a HG. PPx decreased significantly Ach-IR of aortic rings when compared with those obtained from sham-operated rats (P< 0.001). This effect was partially prevented in PPx-VE rats (P< 0.01). When rings obtained from this subgroup were incubated with Tiron (a scavenger of O₂⁻), or the enzyme superoxide dismutase (that removes O₂⁻), Ach-IR was completely restored (P< 0.001). These results suggest that hyperglycemia leads to an excessive generation of O₂⁻ and consequently peroxynitrite anions, and that this may in turn trigger an impairment of endothelium-dependent relaxation. It also supports further evidence on the ability of VE to restore altered relaxation in PPx rats, probably through the scavenging property of O₂⁻ accumulation.

BV- 96.**VITAMIN E ADMINISTRATION PARTIALLY RESTORES AORTIC RELAXATION IN FRUCTOSE FED RATS**

Wallinger ML, Linares LM, Reyes Toso ML, Viglione PN, Ricci CR, Reyes Toso CF.

Departamento de Fisiología. Facultad de Medicina. UBA. Paraguay 2155 Piso 7. Buenos Aires. Argentina.

E-mail: creyesto@fmed.uba.ar

The fructose-fed (FF) rat (60% fructose) is used as an animal model of insulin resistance and is considered to parallel multiple metabolic syndrome observed in humans. A decreased acetylcholine-induced relaxation (Ach-IR) is observed in these animals. This effect was amplified by pre-incubation in a high glucose solution -HG- (44mM/l), a situation that results in oxidative stress mainly through superoxide anion (O₂⁻) accumulation. We hereby investigate the effect of vitamin E (VE) treatment on plasma thiobarbituric acid reactive substances (TBARS) and Ach-IR of rats turned intolerant to carbohydrates. Fasting blood glucose and oral glucose tolerance tests (OGTT) were performed. Subgroups of control and FF rats were chronically treated with VE (C-VE and FF-VE) (50 mg/day during 16 weeks). TBARS in plasma were higher in FF than in control rats (P< 0.01) and this effect was suppressed by VE treatment. Dose-response curves for Ach-IR of aortic rings were conducted in a HG. Fructose administration decreased significantly Ach-IR of aortic rings when compared with those obtained from control rats (P< 0.001). This effect was partially prevented in FF-VE rats (P< 0.05). When rings from this subgroup were incubated with Tiron (a scavenger of O₂⁻), or superoxide dismutase, Ach-IR was restored (P< 0.001). These results support further evidence on the ability of VE to restore altered relaxation in FF rats, probably through the scavenging property of O₂⁻ accumulation.

BV-97.**AQP2 EXPRESSION IN RESPONSE TO BILATERAL URETERAL OBSTRUCTION**

Villar SR, Torres AM.

Area Farmacología. Facultad de Cs. Bioquímicas y Farmacéuticas. UNR. CONICET. E-mail: villar_silvina@hotmail.com

It was demonstrated that bilateral ureteral obstruction (B) is associated with postobstructive diuresis 1 day after obstruction releasing, being normalized after 7 days. The aim of this work was to study the time course effect of B on aquaporin 2 (AQP2) protein abundance (Prot.) in apical membranes and mRNA in rat kidney. Ureters were obstructed for 24 h in all experiments, and released for 1 (B1, n=4), 2 (B2, n=4) and 7 (B7, n=4) days. A parallel group of Sham rats (S, n=4) was employed. AQP2 (%) expression was determined by Immunoblotting and RT-PCR techniques in renal cortex (Cx) and medulla (Med). Data were analysed with ANOVA plus Newman-Keuls $P < 0.05$: [a]vsS, [b]vsB1, [c]vsB2, [d]vsB7.

AQP2	Prot. (Cx)	Prot. (Med.)	mRNA (Cx)	mRNA (Med)
S	100±5	100±2	100±5	100±6
B1	71±7 ^{a,c,d}	72±6 ^{a,c,d}	60±3 ^{a,c,d}	49±3 ^a
B2	129±8 ^{a,b}	120 ± 14 ^{b,d}	84±8 ^{b,d}	56±2 ^a
B7	149±7 ^{a,b}	152 ± 10 ^{a,b,c}	154±4 ^{a,b,c}	57±2 ^a

The decreased expression of AQP2 in B1, might contribute to the obstructive polyuria. In B2 and B7 groups, the increased AQP2 expression in apical membranes might be sufficient to normalize water reabsorption. The no parallel changes in protein and mRNA levels suggest that the cellular responses to obstruction might involve different regulatory processes.

BV-98.**OAT1 AND OAT3 EXPRESSION IN ACUTE RENAL FAILURE (ARF)**

Di Giusto G, Villar SR, Torres AM.

Area Farmacología. Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR. CONICET. E-mail: giseladg@hotmail.com

The renal expression of the organic anion transporters (Oat1 and Oat3) was evaluated in three experimental models of ARF using Male Wistar rats. Ischemic ARF (I/R, n=4) was induced by occlusion of both renal pedicles during 60 min, followed by 60 min of reperfusion. Nephrotoxic ARF (HgCl₂, n=4) was produced by administering a single dose of HgCl₂ (5 mg/kg b.wt., s.c.) 18 h before. Post-renal ARF (BUO, n=4) was obtained by bilateral obstruction of ureters for 24 h plus a releasing period of 24 h. A parallel group of sham operated rats was employed. Oat1 and Oat3 expression was evaluated by immunohistochemistry and by immunoblotting (homogenates (H) and basolateral membranes (M)). Global renal function was assessed by urea plasma levels.

(*) $P < 0.05$.	Sham	I/R	HgCl ₂	BUO
Uremia (g/L)	0.46 ± 0.02	0.58 ± 0.01*	1.25 ± 0.09*	2.91 ± 0.3*
Oat1 H (%)	100 ± 5	70 ± 6*	145 ± 8*	156 ± 8*
Oat1 M (%)	100 ± 2	87 ± 3*	70 ± 4*	24 ± 9*
Oat3 H (%)	100 ± 5	78 ± 5*	68 ± 7*	48 ± 11*
Oat3 M (%)	100 ± 3	81 ± 6*	76 ± 4*	35 ± 3*

Immunohistochemistry confirmed these results. A good correlation was observed between uremia and Oat1 M ($r = 0.99$) and Oat3 M ($r = 0.97$). These data suggest that nitrogenous waste products might be involved in the regulation of both carriers expression.

BV-99.**CARDIOVASCULAR VARIABLES AFTER DEXMEDETOMIDINE AND DEXMEDETOMIDINE-DEXTROPROPOXIPHENE ADMINISTRATION IN DOGS**

Tarragona L, Ceballos M, Rebuelto M, Otero P.

Cátedras de Anestesiología y Farmacología, Facultad de Ciencias Veterinarias, UBA. Chorroarin 280 (1427), Bs. As.

E-mail: ltarragona@fvet.uba.ar

The purpose of this study was to compare the cardiovascular variables modifications after the administration of dexmedetomidine (DM) and the combination dexmedetomidine plus dextropropoxiphene (DP) by the intravenous route to dogs. Twelve healthy beagle adult dogs weighing 10 – 14 kg were randomly administered DM 2 µg/kg (n=6) or DM 2 µg/kg plus DP 2 mg/kg (n=6). Cardiovascular variables (heart rate, systolic, diastolic and mean blood arterial pressure) were measured by non invasive methods at 0 (baseline) and at predetermined times up to 90 min after treatment administration. Area under the curve (AUC) calculated for all, above and below the baseline cardiovascular variables data versus time curve for each animal, and data from each sample point measured after DM administration was compared against DM+DP administration by non paired statistical t tests. After DM administration, emesis was observed on 4 of 6 dogs. No significant differences were found for AUCs, significant differences were found only for the 45 min mean arterial blood pressure sample point. Our results suggest that the addition of DP to the anaesthetic protocol does not affect the DM cardiovascular stability in dogs.

BV-100.**HYPOXIA INDUCIBLE FACTOR 1 (HIF-1) EXPRESSION IN NORMOXIC AND HYPOXIC MURINE TISSUES: EFFECTS OF AGING**

Todaro J, Aguirre M, Cardoso L, Juaristi J, Brandan N.

Cátedra de Bioquímica. Facultad de Medicina. UNNE. Moreno 1240. (3400) Corrientes. E-mail: nbrandan@med.unne.edu.ar

Hypoxia-inducible factor 1 α (HIF-1 α) is the oxygen sensitive subunit of a transcriptional master regulator. The aim of this study was to assess the relationship operating between HIF-1 expression in normoxic and hypoxic murine organs (spleen, liver, heart and kidney) and aging. Young and old CF-1 female mice were used in this study (two months and 16 months age, respectively). Both groups (n=8) were divided in two subgroups, one was maintained in normoxia and the other was injected with a single ip dose of Cl₂Co 45 mg/Kg (acute hypoxia inducer agent). Nuclear proteins were fractionated onto 7,5% SDS-PAGE respectively. HIF-1 α was studied in nuclear fractions (immunoblottings) assuming the expression of this factor in post hypoxic renal extracts (6 h at 0,4 atm in hypoxic chamber) as positive control. Experimental data show that HIF-1 is physiologically expressed in all tissues of young animals, mainly in cardiac tissue. Hypoxia triggers the up-regulation of HIF-1 α in all tissues with the same pattern. However, senescent mice exhibit a constitutive diminution of HIF-1 expression and respond poorly to hypoxic stress. These findings might contribute to the knowledge about molecular mechanisms in response to hypoxic stress in elderly.

BV-101.

ROLE OF MITOCHONDRIAL Ca²⁺-UNIORTER AND MTP DURING ISCHEMIA-REPERFUSION IN RAT HEARTS EXPOSED TO HIGH K⁺- LOW Ca²⁺- CARDIOPLEGIA

Ragone MI, Consolini AE.

Cát. Farmacología, Dto Cs. Biológicas, Fac. Cs Exactas, UNLP, 47 y 115, La Plata. E-mail: dinamia@biol.unlp.edu.ar

Cardioplegic solutions protect heart from injury during ischemia-reperfusion (I-R) in a surgery. Although mitochondria (Mit) could be deleterious during I-R, we showed that in a reversible I-R on rat hearts Mit can contribute to the contractile recovery (CR), especially after pre-treatment with a 25 mM K⁺-0.5 mM Ca²⁺-Krebs (CPG). CR was sensitive to the blockade of the Mit-NaXCa-exchanger (mNCX) and to drugs which act on Mit-K_{ATP} channels (mK_{ATP}). Now, we evaluated whether the Mit Ca²⁺ uptake during I affect CR, by blocking the Mit-Ca²⁺-uniporter (m-CaU) with Ro-360. Also, we tested whether the Mit-transition pore (MTP) was opened during I-R, by using cyclosporine-A (Cys-A). Isolated rat hearts were perfused with Krebs-C (C) and CPG before exposing to 45 min I- 45 min R with C, while intraventricular pressure (P) and total heat release (H_t) were simultaneously measured. CPG+1 μM Ro-360 before I induced a diastolic contracture (ΔPr: 10.3±4.9 mm Hg, n=6) at the start of R, which reverted during it. It also reduced CR (to 39.9±16% of pre-I P, p<0.05 vs. CPG-hearts) and increased H_t (to 17.3±3.1 mW.g⁻¹, 161.9±23.3% of pre-I). On the other hand, 0.2 μM Cys-A added to CPG before I and during R did not change ΔPr, P (67.3±8% of pre-I) nor H_t (119±40% of pre-I), n=4, NS vs. CPG-hearts. Results suggest that the Mit-Ca²⁺ uptake by the m-CaU is determinant for the post-I CR in CPG-hearts. Nevertheless, the MPT is not opened during I nor R, in agreement with the CPG protection.

X-408 UNLP-2005/08, PIP 6024/05.

BV-102.

INCREASED EFFICIENCY TO ANGIOTENSIN II INDUCED BY SIMULTANEOUS INHIBITION OF EPOXYGENASE AND OMEGA HYDROXYLASE

Sierra L, Scacchi F, Peral de Bruno M, Jerez S.

Laboratorio de Fisiología y Farmacología Vascular. Departamento de Fisiología Vascular. (INSIBIO-CONICET). UNT. Tucumán. E-mail: sierraliliana@arnet.com.ar

This study was designed to analyze the mechanisms of contractile response increase to Angiotensin II (Ang II) in presence of 17-Octadecynoic acid (17-ODYA). Thoracic aorta from rabbits was excised. Rings were cut and mounted in an organ bath to register isometric contractions in arteries with endothelium. Arteries were incubated or not (control) with 17-ODYA, 17-ODYA+Indomethacin, NS398, SQ 29548, 17ODYA + NS398 o SQ 29548, Miconazol, CAY 10434 y Miconazol+CAY10434 and stimulated with NA and one CDRC (cumulative dose response curve) to Ach. After washing, one CDRC to Ang II was performed. 17-ODYA increased maximal response (Emax) to Ang II and diminished Ach-relaxation. Arteries treated with 17-ODYA plus indomethacin, NS398 or SQ29548 induced a shift to the right to AngII-CDRC. However, only indomethacin and NS398 blocked Emax-increase. Miconazol increased affinity but not Emax. CAY10434 did not modify Ang II response. Furthermore, Miconazol+CAY10434 induced AngII- Emax increase similar to 17-ODYA. Simultaneously inhibition of epoxygenase and hidroxylyase induced release of COX-dependent metabolite which increased efficiency and potency to Ang II. This metabolite modulates Ang II potency through TXA2/PGH2 receptors. EETs but not 20-HETE would modify Ang II affinity in physiological conditions.

BV-103.

SIMPLE, VERSATILE EQUIPMENT FOR RECORDING BIOLOGICAL SIGNALS

López Fernández L¹, Ruiz Mostacero J¹, Cuezco L², Castillo G³, Orce G^{1,3}.

¹Facultad de Medicina, UNT; ²Sociedad Argentina de Bioingeniería; ³INSIBIO (UNT- CONICET). Avda. Roca 1900, S.M. de Tucumán. E-mail: orcegap@yahoo.com

In order to be measured and recorded, biological variables are often converted into voltage values by means of transducers. We have developed a low cost recording system with self-adjustable scale, which processes data generated by strain gauge transducers. Acquisition and digitalization are carried out by means of the sound card of a standard PC, and the data are visualized in real time and simultaneously stored in the memory of the machine. Sampling frequency is user selectable up to a maximum of 100 samples/sec, with 16-bit resolution. The hardware consists of separate units, each containing two independent operational amplifier channels. The gain is set by software, and tools are included in the software which allow for the inclusion of markers during acquisition, and the calibration of each channel separately. Data can be exported to Excel for further off-line processing.

BV-104.

THE ENDOCANNABINOID ANANDAMIDE INHIBITS KININ B₁ RECEPTOR SENSITIZATION THROUGH CB₁ RECEPTOR STIMULATION IN HUMAN UMBILICAL VEIN (HUV)

Gago J, del-Rey G, Diana-Menéndez S, Mendes-Garrido F, Ireizo J, Errasti A, Pelorosso F, Rothlin R.

³Cátedra de Farmacología. Fac. de Medicina. UBA. Paraguay 2155, piso 9, 1121 Bs As. E-mail: [farmaco3@fmed.uba.ar](mailto:farmac3@fmed.uba.ar)

Endocannabinoids and kinins are relevant signaling systems in inflammation and pain. While kinin B₁ receptor up-regulation appears to be a key step in the development of such pathophysiological processes, endocannabinoids are thought to reduce their signs and symptoms. Considering the potential antinociceptive and anti-inflammatory actions of endocannabinoids we evaluate the possible inhibition by anandamide (AEA) of kinin B₁ receptor sensitization in isolated HUV, a well characterized human model of kinin B₁ receptor up-regulation. AEA effects on kinin B₁ receptor up-regulation were evaluated by prolonged exposure of HUV rings to the endocannabinoid before the construction of contractile concentration-response curves to selective kinin B₁ receptor agonists. AEA and its metabolically stable analogue, R-(+)-methanandamide, produced a selective and dose-dependent inhibition of kinin B₁ receptor-sensitized responses. The inhibitory effect of AEA failed to be modified by either LY2183240, a selective AEA uptake inhibitor, or by AM630, selective cannabinoid CB₂ receptor antagonist. However, the cannabinoid CB₁ receptor antagonist, AM251, abolished AEA effects on kinin B₁ receptor sensitization. The present results provide novel pharmacological evidence indicating that endocannabinoid AEA inhibits kinin B₁ receptor sensitization, possibly by inhibition of its up-regulation. This finding may be relevant for the understanding of endocannabinoid mechanisms of action in inflammatory and painful processes and for the rational development of new pharmacotherapeutic approaches.

BV-105.**BIOPHARMACEUTICAL PROPERTIES OF A POLYELECTROLYTE- ENALAPRIL COMPLEX**

Ramírez Rigo MV, Quinteros DA, Arduzzo MS, Olivera ME, Allemandi DA, Manzo RH.

Dep. de Farmacia. Fac. de Cs. Químicas. Univ. Nacional de Córdoba. Ciudad Universitaria (5000) Córdoba.

E-mail: vrrigo@fcq.unc.edu.ar

In a R+D project on drug delivery systems, enalapril (Ena) was selected as a model of peptide-mimetic drug to be associated to a polyelectrolyte carrier (PE). This drug is absorbed by PEPT1 and 2 transporters and has low oral bioavailability (36- 44%). It has basic and acid groups able to interact with PE.

The aim of this work is to study the biopharmaceutical properties of an aqueous dispersion of (PE-Ena) complex. Drug release evaluation was performed using different receptor media. The complex solution of pH~5.7 released Ena slowly when the medium is water. Release rate is increased as water is replaced by physiological solutions. In both cases the data fits the linear kinetic model. The ionic interaction modulates the release rate.

Evaluation of intestinal permeability was performed in everted rat intestine. It was observed an enhancer effect of the PE because it increase 1.7 times the speed of Ena permeation. The mechanism is not clear and it is necessary complementary experiments to determine it.

The complex solutions can be consider like a drug carrier system of Ena that increases the drug permeation and could improve the bioavailability of the drug.

BV-106.**INVERSE AGONISM OF CARVEDILOL AND NEBIVOLOL IN ISOLATED AURICLE OF FRUCTOSE FED RATS**

Di Verniero C, Silberman E, Mayer M, Taira C, Höcht C.

Cátedra de Farmacología, INFIBIOC, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Junín 956, 1113 CA de Buenos Aires, Argentina. E-mail: chocht@ffyb.uba.ar

In previous works we reported that inverse agonist activity of metoprolol was reduced in SH rats. The aim of this work was to study the inverse agonism of carvedilol and nebivolol in isolated auricle of fructose fed rats. Male rats were divided into two groups: F, fructose (10% W/V, to drink for 6 weeks); C, control. Inverse agonist activities were studied in isolated auricle by the corresponding cumulative concentration-response curves of carvedilol and nebivolol (10^{-8} - 10^{-4} M). Maximal chronotropic responses of carvedilol were similar in F and C rats. Carvedilol showed a minor inverse agonist activity in F rats (F: pEC₅₀: 4.80 ± 0.04 , n=6, p<0.05 vs. C rats; C group: pEC₅₀: 5.22 ± 0.10 , n=6). Nebivolol only showed inverse agonist activity in C rats (E_{max}: $53 \pm 10\%$; n=6; pEC₅₀: 4.22 ± 0.16 ; n=6). In conclusion, inverse agonism activities of carvedilol and nebivolol are reduced in the fructose fed rats experimental model of hypertension. Differences between both drugs are also seen.

BV-107.**ALLOPREGNANOLONE MODIFIES 3 β -HSD ACTIVITY AND EXPRESSION IN HYPOTHALAMUS AND OVARY OF RATS IN AN ESTRUS CYCLE DEPENDENT MANNER**

Giuliani F, Vega Orozco AS, Casas S, Nanfaro F, Bazocchini V, Yunes R, Cabrera R.

LINCE-IMBECU-CONICET. Area de Farmacología, Facultad de Ciencias Médicas, UN de Cuyo., Ciudad Universitaria, (CP 5500) Mendoza, Argentina. E-mail: fgiuliani@fcm.uncu.edu.ar

3- β -hydroxysteroid dehydrogenase (3- β -HSD) converts pregnenolone to progesterone. Previously we demonstrated that Allopregnanolone (Allo) icv inhibits the LH release, ovulation and luteal apoptosis. The aim was to study if Allo icv modifies enzymatic activity and gene expression of hypothalamic and ovarian 3- β -HSD during proestrous (P) and diestrous (D). Female Holtzman adults rats were injected in third ventricle with Allo 6 μ M or vehicle (KRBG) and sacrificed 30 min later. 3- β -HSD activity was measured by spectrophotometric method. The gene expression was studied by RT-PCR. The results were analyzed by T-test, p<0.05 was considered significant. In P, Allo decreased 3- β -HSD activity in hypothalamus (p<0.001) and ovary (p<0.01), but no significant changes were observed in the gene expression. In D, Allo increased the enzymatic activity (p<0.01) and the gene expression (p<0.05) in ovary and hypothalamus respectively. We conclude that Allo modulates the activity and the gene expression of 3- β -HSD in hypothalamus and ovary in an estral cycle dependent manner. Moreover we show that the neurosteroids are important modulators of the molecular mechanisms involved in the reproductive physiology of the female rat.

BV-108.**MAMMARY BIOPSIES IN PREPUBERTAL HEIFERS. HISTOLOGICAL STUDIES OF THE DEVELOPMENTAL GLAND. EFFECT OF ANTHELMINTIC TREATMENT**

Licoff N, Diab S, Perri A, Mejía M, Formía N, Becú-Villalobos D, Lacau-Mengido I.

Lab. Regulación Hipofisaria, IBYME-CONICET, Obligado 2490, Buenos Aires. E-mail: ilacau@dna.uba.ar

As IGF-1 is involved in prepubertal mammary growth, and we demonstrated that IGF-1 is diminished in parasitized heifers, we wished to determine if parasite infection could also affect mammary gland development. Twenty new born female Holstein calves were randomly assigned to treated (strategic treatments with ivermectin, fenbendazole or levamisole to minimize parasite burden) or untreated group. Three heifers per group were used to set up a mammary biopsy technique and then to compare development of the mammary parenchyma by histology. Four biopsy samples were taken (using a Tru-Core® Biopsy Needle, Medical Device Technologies, Inc.) from each heifer at 20, 30, 40 and 60 week of age, and then kept in formalin solution for histology. The mammary biopsy technique was reliable and efficient. Biopsy sites healed rapidly and without infections. Biopsies showed mammary parenchyma embedded in fat pad, conforming ductal developing structures of epithelial cells. As the animal grew up more organized ductal structures were observed. Heifers in the treated group had higher ratio of epithelial cells/total area at 20 weeks of age. We conclude that minimal parasite burden during development could increase early ductal mammarygenesis in association with increased IGF-1 levels. Molecular studies will be performed in further studies.

BV-109.**DIFFERENTIAL REGULATION OF STEROIDOGENIC GENES EXPRESSION BY GLUCOCORTICIDS AND IGF-I IN CULTURES OF IMMORTALIZED GRANULOSA CELLS**

Agostini M, Chedrese P.

Fac. Cs. Vet. UNICEN. Paraje Arroyo Seco S/N. Tandil Argentina. Department of Biol. U. of SK College of Art and Sciences. Saskatoon SK Canadá. E-mail: agostini@vet.unicen.edu.ar

Ovarian steroidogenesis is hormonally regulated at the level of transport of cholesterol into the mitochondria and the conversion of cholesterol into pregnenolone. Experimental and clinical information suggest that glucocorticoids regulate ovarian steroidogenesis and that insulin like growth factor I (IGF-I) regulates expression of StAR and P450scc genes. JC-410 cells were genetically modified to express the promoter region of the StAR and P450scc genes, driving the luciferase reporter gene (1423-StAR-LUC and 2320-P450scc-LUC, respectively). The expression of LUC was determined by a luminometric assay. The effect of the protein kinase A activator, cholera toxin (CT), was used as positive control. Dexametasonone (Dx) at 10 and 30 μ M inhibits activity of 2320-P450scc-LUC, but stimulates 1423-StAR-LUC. On the other hand, IGF-I stimulates activity of both promoters. In addition, IGF-I reverts the inhibitory effect of Dx on 2320-P450scc-LUC. In conclusion, these results indicate that IGF-I and glucocorticoids participate in a local mechanism of differential expression of the steroidogenic genes. By inhibiting expression of the P450scc gene, glucocorticoids limit the conversion of cholesterol into pregnenolone, while IGF-I facilitates the synthesis of steroid in the ovary by stimulating expression of the StAR gene.

BV-110.**CONSUMPTION OF ORAL HYPOGLYCEMIC DRUGS IN THE HOSPITAL PROVINCIAL DEL CENTENARIO. ROSARIO.**

Marzi M¹, Odetto C², Bertero J², Nuñez M³, Quaglia N².

¹Dpto de Matem. y Estad. ²Area Farmac. Fac Cs Bio y Farmac. UNR. ³ Cátedra Farmac. Fac Medicina. UBA. Suipacha 531. Rosario. Santa Fe. Argentina. E-mail: nquaglia@fbioyf.unr.edu.ar

Diabetes is an illness with high morbimortality. Drugs consumption researches are necessary to approach to appropriate drugs utilization. Objective: to evaluate the consumption of oral hypoglycemic drugs (OHD) in the first 45 days of 2007 and 2008, in the Hospital Provincial del Centenario from Rosario. Methods: metformin (Mt) and glybenclamide (Gb) consumption was calculated from the number of defined daily dose (DDD) and the number of prescribed daily dose per treatment (PDTs). It was considered that each treatment has 30 days of duration. Data was collected from all prescriptions received at hospital's pharmacy office in the studied period. Results: it was collected 899 prescriptions from 554 outpatients. The median age=56. OHD consumption (DDD), 2007: Mt: 3816, Gb: 5503; 2008: Mt: 2432, Gb: 3207. PDTs (means \pm SEM, 2007-2008): Mt: 16,0 \pm 0,4, Gb: 26,1 \pm 0,6. Conclusion: the consumption of OHD was lower than the previous year which would be justified, partially, due to restrictions imposed to drugs dispensation at the end of 2007. To Mt, the PDTs means was about half of the expected, thus, it must be projected studies which aim to revise if the described situation is correlated with the correct control of diabetes.

BV-111.**THE ANALYSIS OF THE LIFE STRATEGIES OF THE POPULATION AT RISK FOR CATAMARCA ASSISTED BY THE PROGRAM REMEDIAR**

Montrull H, Meirovich YF, Paez MA, Murua D, Brizuela N.

Departamento de Salud Universidad Nacional de La Rioja. Argentina. E-mail: hmontrull@fibertel.com.ar

The Remediari program assures people access to the essential drug treatments through the Primary Health Care centre. However, its efficiency can be affected by lack of control and not reliable or falling treatments. The cardiovascular diseases are the leading cause of death in Argentina, and if associated to Diabetes it makes for a worse prognosis. In the context of this program the pharmacological and non-pharmacological treatments were submitted for evaluation. Using the database of the Remediari medication provided to the CAPS and the R prescription Sole Form. The prescriptions for Hypertension and Diabetes were tabulated separately and together. From year 2003-2006, patients were grouped for each year by age and sex, chosen-age group range was of 10 years. It was observed that female patients predominated (80%). Patient showed limited and variable adhesion to treatment over time, which was remarkable in diabetics: out of the 9061 diagnosed patients, only 10% accomplished the daily dosing defined for hypoglycemic agents.

BV-112.**INFLUENCE OF CHRONIC MILD STRESS ON DIABETES PROGRESSION AND IMMUNE RESPONSE IN STREPTOZOTOCIN-INDUCED TYPE I DIABETES MODEL**

Rubinstein R, Wald MR, Genaro AM.

CEFYO-CONICET-UBA. Buenos Aires, Argentina. E-mail: roxirubin@yahoo.com.ar

Experimental literature suggests a relation between diabetes and immunosuppression. However, there are diabetic patients with normal evolution after an infection challenge. Among the factors that can regulate this susceptibility are the stressful life events. Here, we studied the effect of chronic mild stress exposition (CMS) on the development and evolution of STZ induced type I diabetes in Balb/c mice and its correlation with the immune response. Results indicate that CMS exposition after diabetes induction lead to significantly higher levels of glucose than those observed in non exposed animals. Concerning the immune response, in animals with CMS exposition after diabetes induction, T and B lymphocyte proliferative response to selective mitogens was lower than animals exposed to diabetes or CMS alone. Participation of hyperactivation of hypothalamo- pituitary-adrenal (HPA) axis was estimated by determination of serum level of corticosterone, an hyperglycemic hormone. No correlation was observed between increased corticosterone levels, glycemia and decrease of immune response. However a positive correlation was observed between hyper-glycemia and inhibition of immune response. These findings indicate a detrimental association of stress with the prognosis of diabetes through a glycemia increase and a reduction of immune response.

BVI-113.
EXPRESSION PROFILE OF DOPAMINE AGONISTS IN AN ANIMAL MODEL OF PARKINSONISM

Larramendy C, Saborido MD, Taravini IRE, Murer GM, Gershanik OS.

Instituto de Investigaciones Farmacológicas, CONICET-UBA. Junin 956, 5to piso, Buenos Aires, Argentina.

E-mail: larramendy@ffyb.uba.ar

L-dopa and dopamine agonists (DA) are treatments available for Parkinson's disease. L-dopa is the most effective to alleviate motor disability but induces dyskinesias. DA have been proposed to have neuroprotective effects and less induction of dyskinesias. Rats were injected with 6-OHDA in the left striatum and a partial and homogeneous striatal lesion was characterized. After surgery, animals were treated with L-dopa, or pramipexole at doses that produced similar therapeutic benefit. A control group received vehicle alone. The therapeutic benefit was determined by the reversal of akinesia of the contralateral forepaw, induced by the lesion, and evaluated with the cylinder test. Three independent groups of normal and lesioned rats will be analyzed by microarray technology in order to compare the genetic profile of the groups: normal/vehicle, lesioned/vehicle, lesioned/levodopa, and lesioned/pramipexole. Within the profiles thus expressed we hope to identify transcripts related to the therapeutic effects, as well as those possibly related to neuroprotection, plasticity and dyskinesias. We hope to be able to speculate on the possible underlying mechanisms and their relationship with the pharmacodynamic and pharmacokinetic properties of the drugs under study.

BVI-114.
D1 AND D2 DOPAMINE RECEPTOR ANTAGONISTS EFFECTS ON LEVODOPA-INDUCED DYSKINESIAS

Saborido M¹, Taravini I¹, Larramendy C¹, Murer G², Gershanik O¹

¹Laboratorio de Parkinsonismo Experimental, ININFA CONICET-UBA. ²Laboratorio de Fisiología de Circuitos Neuronales, Dto. de Fisiología y Biofísica, Facultad de Medicina, UBA.

E-mail: msaborido@ffyb.uba.ar

A common and troublesome complication of L-dopa therapy in Parkinson's disease is the development of L-dopa-induced dyskinesias (LID). To evaluate if it is possible to separate the generation of LID from the motor response induced by L-dopa, rats with severe lesion of the nigrostriatal pathway were treated during 12 days with L-dopa. Once LID were established, rats were treated with increasing doses of SCH-23390 (D1 antagonist) or raclopride (D2 antagonist) in order to obtain a dose that ameliorated LID without interfering with the motor response. We found that SCH-23390 at a dose >0.005mg/kg ameliorates the severity of LID. However, at these doses also the motor response evaluated by means of contralateral rotations were also reduced. On the other hand, we were not able to find a dose of raclopride that reduced the LID score induced by L-dopa. This was probably because of the very low doses used here. These data in part supports the idea that once dyskinesias are established it is not possible to separate its generation from the motor response induced by a L-dopa.

BVI-115.
PROTEIN SYNTHESIS INHIBITION EFFECT ON DYSKINESIA INDUCTION

Saborido M¹, Larramendy C¹, Taravini I¹, Murer G², Gershanik O¹

¹Laboratorio de Parkinsonismo Experimental, ININFA CONICET-UBA. ²Laboratorio de Fisiología de Circuitos Neuronales, Dto. de Fisiología y Biofísica, Facultad de Medicina, UBA.

E-mail: msaborido@ffyb.uba.ar

Dyskinesias are one of the major limiting side effects encountered in the treatment of Parkinson's disease. To demonstrate that protein synthesis is required for the priming effect of dopamine agonists on the generation of dyskinesias, 22-gauge stainless steel cannulas were implanted hemilaterally in the striatum of rats. Anisomycin (160µg/1.6µl) or vehicle were infused 15 min before apomorphine (0.025mg/kg) or vehicle once every 48 h, for a total of three times (priming). Forty eight hours later, all groups received 0.05mg/kg apomorphine. After apomorphine administration contralateral rotations and dyskinesias were tested. Twenty minutes after apomorphine akinesia of the contralateral forepaw was also tested by means of the cylinder test. Our results suggest that the administration of anisomycin during priming with apomorphine diminishes dyskinesia severity induced by a new challenge with apomorphine. These preliminary data indicate that protein synthesis could be necessary for the plastic changes that occur in response to dopamine agonists and that give origin to dyskinesia in an animal model of parkinsonism.

BVI-116.
ROLE OF THE VENTROMEDIAL MEDULLA IN THE ANALGESIC MODULATION OF THE STRIATUM

Barceló AC¹, Fillipini B, Pazo JH.

¹Facultad de Odontología, Cátedra de Fisiología and Facultad de Medicina, Departamento de Fisiología, Laboratorio de Neurofisiología. UBA, Paraguay 2155, Buenos Aires, Argentina. *E-mail: acbarce@odon.uba.ar*

Electrophysiological and pharmacologic studies done in our laboratory provided data suggesting that the striatum plays a key role in the endogenous analgesia mechanism. In rats, striatum stimulation reduced or suppressed the jaw opening reflex (JOR) induced by tooth pulp stimulation. This action is mediated by D2 dopamine receptors. The pathway followed by the analgesic affect is partially known and involves the GP and the SNr nuclei. However, connections among these nuclei and the sensory trigeminal nuclei are not known. In order to elucidate the network involved in the striatum pain modulatory mechanism upon the trigeminal nuclei, we carried out a study in anesthetized rats with urethane. The uni or bilateral neurotoxic lesion (0.3 µl per side, kainic solution 0.25nM/ 0.1 µl) of the periaqueductal gray matter (PAG), implicated in pain modulation and with projections to the trigeminal nuclei, did not suppress the striatal inhibition of the JOR. However, the neurotoxic bilateral lesion (0.3 µl per side, kainic acid 0.25nM/ 0.1 µl) of the rostroventromedial medulla (RVM), another area related to pain modulation and connected to the sensory trigeminal nuclei, suppressed the striatal inhibition of the JOR. (ANOVA F2,15=1413, *p* =0.281). The unilateral lesion of the RVM left unaffected the JOR inhibition (ANOVA F2,20=13.767, *p*<0.001). The present results suggest that the analgesic effect of the striatum on the trigeminal nucleus is mediated by the RVM.

BVI-117.**ACTION OF GABA_A RECEPTORS OF THE SUBTHALAMIC NUCLEUS ON THE RELEASE OF DOPAMINE IN THE STRIATUM**

Pazo JH, Fillipini B, Hocht C¹.

Fac. de Medicina, Dpto. de Fisiología, Lab. de Neurofisiología, and ¹Fac. de Farmacia y Bioquímica, Cát. de Farmacología, UBA. Paraguay 2155, Buenos Aires, Argentina. E-mail: jpazo@fmed.uba.ar

Previous studies from this laboratory have demonstrated that high frequency stimulation of the subthalamic nucleus (STN) increases the release of dopamine in the ipsilateral striatum, which is mediated by the substantia nigra compacta. However, the therapeutic effect of high frequency stimulation of STN was attributed to inhibition of STN neuronal activity. To further clarify the mechanisms involved, we have analyzed the effect of intrasubthalamic microinjection of gabaergic agonist and antagonist on the release of DA in the striatum. The experiments were performed in rats anesthetized with urethane. The microinjection of bicuculine (25 µg / 0.2 µl) into the STN increased the release of DA in the striatum (104 ± 0.38 vs 196.84 ± 15, expressed as % of control values, P < 0.007). The DOPAC concentration was not modified. The microinjection of bicuculine into the adjacent zona incerta did not change the concentration of both DA and DOPAC in the striatum. The microinjection of muscimol (0.2 µg / 0.2 µl) in the STN decreased the release of DA (103.04 ± 2.49% vs 43.72 ± 3%, P < 0.001), but not of DOPAC. The observation of similar results obtained by microinjections of bicuculine and high frequency electric stimulation of the STN, indicate that the latter is due to activation of the neurons of the nucleus.

BVI-118.**REPEATED EARLY MATERNAL SEPARATION AND COLD STRESS**

Rodríguez CB, Salatino AE, Odeon M, Acosta GB.

ININFA. Junín 956. 5° floor, C1113AAD, Buenos Aires. E-mail: gacosta@ffyb.uba.ar

Early life events have profound consequences in growth and development. The aim of this study was to investigate the consequences of repeated early maternal separation and exposed to cold stress on adult brain on GABAergic function and determine whether the desensitization to maternal separation was an age-specific. Rats' pups were separated from their mother plus cold exposure (4°C) for 1 h at postnatal day (PD) 5, 7 and 13 during 20 days. These animals were allowed a 30 days recovery period until adulthood. The rats were killed by decapitation and collecting trunk blood. Frontal cortex (FC) and hippocampus (HIC) were dissected. We studied GABA uptake, corticosterone levels and GAT-1 expression by western blot. Repeated stress decreased GABA uptake on FC and HIC significantly at PD5 as also the levels of corticosterone. While GAT-1 expression increased at PD13 on FC. These preliminary results support the notion that early life environmental manipulations have an influence on hypothalamic-pituitary-adrenal (HPA) axis and alter the expression of plasticity related neuronal proteins.

BVI-119.**α-MSH INHIBITS THE EFFECT OF IL-1β ON MEMORY CONSOLIDATION**

Gonzalez P¹, Lasaga M², Scimonelli T¹.

¹IFEC CONICET. Depto Farmacol. FCQ. UNC. Ciudad Universitaria, 5000 Córdoba, Argentina. ²Inst. de Invest. en Reproducción. Facultad de Medicina. UBA.

E-mail: vgonzalez@fcq.unc.edu.ar

It has been described that the immune activation causes deficits in learning and memory. Interleukin-1beta (IL-1β), a potent pro-inflammatory cytokine, has deleterious effects on memory consolidation. The melanocortin α-MSH exerts potent anti-inflammatory actions by antagonizing the effect of pro-inflammatory cytokines. Five subtypes of melanocortin receptors (MC1R-MC5R) have been identified, being the MC3R and MC4R predominant in the central nervous system. The present experiments show that the bilateral injection of IL-1β (5 ng) in dorsal hippocampus immediately after training decreases freezing during the contextual fear conditioning test. Treatment with α-MSH (0.05 µg) blocked this effect. Administration of the MC4 receptor antagonist HS014 (0.5 µg) reverses the effect of α-MSH. However, the treatment with γ-MSH, an MC3 agonist, did not affect the IL-1β-induced impairment of memory consolidation. Our results also showed that IL-1β can modulate the persistence of memory. These results suggest that α-MSH, through MC4R may inhibit the effect of IL-1β on memory consolidation.

BVI-120.**ACTIVITY-DEPENDENT REGULACION OF GABA_A RECEPTOR FUNCTION**

Gutiérrez ML, Gravielle MC.

Instituto de Investigaciones Farmacológicas-CONICET-UBA. Junín 956, C1113AAD. Buenos Aires, Argentina.

E-mail: mlgutierrez@ffyb.uba.ar

Alterations in the GABA_A receptor function are associated with tolerance to the sedative-hypnotic effects of benzodiazepines. Chronic activation of GABA_A receptors by GABA has been shown to induce down-regulation of receptor number and uncoupling of GABA/benzodiazepine site interactions with a half-time of 24 h. In a previous work we reported that a single brief exposure of cultured neocortical neurons to GABA for 5-10 min (t_{1/2}=3 min) initiates a process that results in uncoupling hours later (t_{1/2}=1 h) in the absence of a change in receptor number, providing a paradigm to selectively study the mechanism of uncoupling. Uncoupling is contingent upon receptor activation and is accompanied by down-regulation of selective receptor subunits. In order to determine whether uncoupling is produced by positive allosteric modulators we studied here the effect of brief applications of benzodiazepines. Results from these experiments indicated that diazepam treatment induced a small but significant uncoupling that is blocked by flumazenil. To further analyze the mechanism of uncoupling we first investigated whether uncoupling is mediated by the activation of a protein kinase cascade. Our results showed that the protein kinase inhibitors studied blocked the GABA-induced uncoupling suggesting a role of phosphorylation on the mechanism of uncoupling. This activity-dependent modulation of GABA_A receptor function may be relevant to physiological and pharmacological conditions where synaptic receptors are exposed to GABA agonists for several minutes.

BVI-121.
CHANGES IN HIPPOCAMPAL Arc PROTEIN EXPRESSION AND SYNAPTIC PLASTICITY BY THE PRESENTATION OF CONTEXTUAL CUES LINKED TO DRUG EXPERIENCE

Monti C, Almiron R, Ramirez O.
 IFEC-CONICET. Dpto. de Farmacología, Facultad de Ciencias Químicas, UNC. Córdoba, Argentina.
 E-mail: cmonti@fcq.unc.edu.ar

Hippocampal synaptic plasticity has been related to learning memory and adaptive processes developed during chronic administration of drug abuse. In this study, we investigated if the environmental context associated with drug experience was able to evoke the same behavioural alteration observed after chronic diazepam administration. We also studied the hippocampal synaptic plasticity and anatomical expression of Arc protein during withdrawal and retrieval as a marker of neuronal activity. We demonstrated that re-exposition to the initial context was associated with the expression of the anxiety sign, characteristic of benzodiazepine withdrawal, evoked on days 15 and 25. An increased hippocampal synaptic plasticity, on dentate gyrus, was observed in animals dependent on diazepam and during retrieval until day 15. However this correlation disappeared 25 days after the first exposure to the context. Moreover an over expression of Arc protein in dorsal dentate gyrus and CA1 during the first day, in the dependent animals and also during their re-exposition on day 15 was observed. In conclusion the behaviour evoked by the environmental context associated with the experience of the drug on day 25, but not linked to an increased hippocampal synaptic plasticity or over expression of Arc protein, may indicate that this behaviour on day 25 is not dependent on the hippocampus but may be dependent on other cortical areas of the brain.

BVI-122.
MODULATION OF FEAR CONDITIONING BY MK-801 IN ETHANOL WITHDRAWN RATS

Bertotto ME, Boiero C, Molina VA, Martijena ID.
 IFEC-CONICET, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5016 Córdoba, Argentina. E-mail: mebertotto@fcq.unc.edu.ar

We have recently shown that discontinuation from chronic ethanol administration results in a clear enhancement of contextual fear conditioning. It is known that NMDA receptors are a major site for ETOH action and that they play a critical role in memory processes and in associative aversive learning. Therefore, in the present study we investigated the effect of MK-801, a NMDA receptor antagonist, on fear conditioning in ETOH withdrawn rats.

Wistar rats made dependent via an ethanol (6% v/v) containing liquid diet for 14 days. Three days after the last ethanol administration animals were injected with MK-801 (0.1 mg/kg i.p.) 30 min before a contextual fear conditioning paradigm (3 electrical foot-shocks 0.4mA, duration 3s; ITI: 30s). Twenty-four hours later animals were exposed to the context without shock delivery and the freezing response was evaluated.

The present study shows that: 1). The formation of a new fear memory is facilitated in animals that have previously experienced ETOH withdrawal. 2). Administration of MK-801, at a dose that did not affect the acquisition of contextual fear conditioning in control animals, normalized the freezing response in ETOH withdrawn rats. 3). 0.3 mg/kg seems to be an effective dose of MK-801 to block the fear conditioning acquisition in control rats.

The enhanced sensitivity to the facilitatory effect of MK-801 in ETOH withdrawn animals may be mediated by adaptive changes in NMDA receptors provoked by ETOH dependence.

BVI-123.
ROLE OF ADHESION MOLECULES IN THE SYNAPTIC REMODELLING INDUCED BY AN EXPERIMENTAL MODEL OF DEPRESSION AND THE ANTIDEPRESSANT FLUOXETINE

Podestá MF, Lorenzo Lopez JR, Sonnemberg M, López M, Brusco A, Wikinski S, Reinés A.
 Instituto de Investigaciones Farmacológicas (ININFA, UBA-CONICET), Junín 956, Buenos Aires, Argentina.
 E-mail: areines@ffyb.uba.ar

Hippocampal plasticity seems to be involved in the pathophysiology of depression and in the therapeutic actions of antidepressants. NCAM and N-cadherin adhesion molecules participate in building and maintaining synaptic structure but also play a role in neuroplasticity. The aim of this work was to examine the synapse morphology and adhesion molecule expression in the hippocampus of animals exposed to the learned helplessness (LH) paradigm. The effects of the antidepressant fluoxetine (FLX) were also studied. At the CA3 synapses of LH animals, synaptic cleft was wider and total number of vesicles was lower than controls, whereas length and thickness of postsynaptic density were, respectively, reduced and increased. While immunostainings for NCAM decreased and for PSA-NCAM, a less-adhesive form, increased in the CA3 region of LH rats, results were exactly the opposite in the CA1. In LH group, total NCAM and N-cadherin expression failed to differ from controls, possibly due to a counteracting effect between CA3 and CA1 contributions. Interestingly, FLX treatment strongly reduced total NCAM expression, accompanied by a slight N-cadherin diminution. Our results indicate that LH exposure induces a decrease in CA3 connectivity and that FLX might exert its action by reducing NCAM expression and so that inducing synaptic remodelling.

BVI-124.
MIDAZOLAM PREVENTS BOTH THE INCREASE ON p35 LEVELS IN BASOLATERAL AMYGDALA AND THE ENHANCED ANXIETY PROVOKED BY STRESS

Bignante EA, Paglini G, Molina VA.
 Dpto. de Farmacología (IFEC). Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Ciudad Universitaria, 5016 Córdoba, Argentina. E-mail: anahibignante@fcq.unc.edu.ar

Amygdala is crucially involved in the processing of fear and anxiety. Recently we have reported that stress increase the expression of p35 selectively in the Basolateral Amygdala (BLA). This increase is consistent with an enhanced anxiety-like behaviour in stressed animals. In the present work, we try to elucidate if the normalization of this anxiogenic behavior with prior midazolam (MDZ) administration also prevents the increase on p35 expression induced by restraint.

Adults Wistar rats (290-320 grs) received a dose of 0.5 mg/kg of MDZ i.p. or vehicle (VEH) and later on subjected to a 30 min restraint session. One day later, animals were behaviourally tested on the elevated plus maze (EPM). Another group of animals received a dose of 0.5 mg/kg of MDZ i.p. or vehicle (VEH) followed by the stressful experience. Thirty minutes after stress rats were sacrificed for the immunohistochemistry.

Restraint provoked a significant and selective increase of p35 levels in BLA and an enhancement of the anxiety-like behavior. Both effects were prevented by prior MDZ administration.

BVI-125.**BONE MARROW ADRYAMICIN INDUCED ERYTHROPOIETIC INJURY AND RENAL NEPHROTOXICITY**

Stoyanoff T, Stemberg E, Todaro J, Cardoso L, Juaristi J, Aguirre M, Brandan N.

Cátedra de Bioquímica. Fac. Medicina. UNNE. Moreno 1240. (3400) Corrientes. E-mail: nbrandan@med.unne.edu.ar

Adryamicin (ADR) is a chemotherapeutic agent that induces genotoxicity in hematopoietic system. However, its effects on bone marrow (BM) erythropoiesis related to nephrotoxicity are not well known. This study evaluates the effects of ADR on the erythropoietic response and its relationship with renal injury. CF-1 mice were injected with ADR (15 mg/kg ip) for a time course study of 120 days. BM mitosis and apoptosis, BM microenvironment, EPO-R expression, differential erythroid precursors and erythroid colonies were determined. Renal HIF expression, functional and structural assays were performed in parallel. BM erythropoiesis was deeply affected: erythroid cells, CFU-e and BFU-e colonies decreased by day 3. Apoptosis was maximal on day 7. EPO-R levels decrease was coincident with BM microenvironment disruption. Erythropoietic BM recovery was observed from day 30. HIF was detected since day 15 in accordance with hypoxic nephrotoxicity. These results suggest that BM recovery to ADR-induced erythropoietic injury might be associated with BM micro-environmental regulations rather than with renal hypoxia.

BVI-126.**NEUROPROTECTIVE EFFECT OF 17 β -ESTRADIOL IN A RAT MODEL OF NEONATAL X RADIATION**

Caceres LG¹, Aón L², Saraceno E², Capani F², Guelman LR¹.

¹Facultad de Medicina, UBA-CEfyBO-CONICET, ²Facultad de Medicina, UBA. Depto. Bioquímica Humana. Paraguay 2155, Buenos Aires, Argentina. E-mail: lugcaceres@gmail.com

Developing Central Nervous System (CNS) is vulnerable to radiation-induced reactive oxygen species (ROS). The consequent oxidative stress has been shown to produce changes at behavioral, biochemical and histological levels in cerebellum (CE) and hippocampus (HIP).

The aim of the present work was to test if 17 β -estradiol, a potential neuroprotector, was able to counteract these changes.

Neonatal male Wistar rats were X-irradiated (5 Gy) in their cephalic ends up to 48hs of postnatal life and a group of this animals was treated with 17 β -estradiol (5 μ g/g). Open field (OF) test, ROS levels, as well as a histological assessment, were performed at 30 post-natal days.

Administration of 17 β -estradiol improved the short-term habituation and decreased the time spent in the centre in the OF. ROS levels returned to control in HIP and the cytoarchitecture of CE was reconstituted.

These results suggest that 17 β -estradiol was able to counteract the effects of X-rays at behavioral, biochemical and histological levels, probably acting through an antioxidant mechanism.

BVI-127.**ANXIOGENIC EFFECT OF A NEW BIOACTIVE DERIVED OF QUINOLONES**

López Rivilli MJ¹, Bignante EA¹, Molina VA¹, Yranzo GP.

¹Dpto. de Farmacología (IFEC), ²Dpto. de Química Orgánica (INFIQC), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Ciudad Universitaria, 5016 Córdoba, Argentina. E-mail: marisalr@fcq.unc.edu.ar

The pharmacological properties of benzodiazepines are due to their GABA_A receptor modulating property, consequently benzodiazepine binding site (BBS) is an interesting target for the development of novel drugs with potential effect on anxiety. Several types of compounds, such as the *pyrazolo-quinolinones* are known to bind to the BBS with high affinity, and show a continuous pharmacological activity as agonists, inverse agonist or antagonists.

A serie of novel 2-aryl-2H-pyrazolo[4,3-c]quinolin-3-ones derivatives were synthesized. Multistep synthesis was carried out starting from bromoanilines and diethyl ethoxymethylenemalonate via Gould-Jacobs reaction, and the further treatment with the appropriate analog of phenylhydrazine gave the *pyrazolo-quinolinone* nucleus.

We evaluated the effect of one of these drugs on the anxiety-like behavior of Wistar adult male rats (250-300 g) in the Elevated Plus Maze (EPM), an experimental paradigm to monitor anxiety. Animals were injected and tested thirty minutes later in the EPM. We evaluated the following doses: 0.25, 0.5, 1 y 3 mg/kg, a significant anxiogenic effect was observed with 3 mg/kg, since there was a significant reduction in the percentage of time spent on open arms of the EPM. These results suggest a possible role of this drug as an inverse agonist of the BBS.

BVI-128.**UTILIZATION OF PSYCHOTROPICS IN PAVÓN ARRIBA DURING 2007**

Quaglia N, Paciaroni J, Elías MM.

Area Farmacol. Facultad de Cs Bioq y Farm. UNR. Suipacha 531. 2000 Rosario. E-mail: nquaglia@bioyf.unr.edu.ar

Central nervous system drugs are widely used in Argentina. **Objective:** To study the consumption of antidepressant (ATDs) and benzodiazepines drugs (BZDs) during 2007 in Pavon Arriba, a town located in the south of Santa Fe with about 2000 inhabitants. Moreover, to value if the utilized drugs fit with National Therapeutic Formulary (NFT). **Methods:** ATDs and BZDs consumption was calculated from the number of defined daily dose (DDD) and DDD-1000 inhabitants-day (DHD). Data was collected from the total of prescriptions received in the whole pharmacy's offices from the town in the studied period. Drugs utilization 90% method (DU90%) was employed to value the adjustment of drugs to NFT. **Results:** ATD: total consumption (DHDs): 8.13. In (DDD): 5868; in the segment DU90% was found: fluoxetine: 1545, paroxetine: 1480, citalopram: 848, escitalopram: 652, sertraline: 630, amitriptyline: 313. The 50% of these are included in the NFT. BZD: total consumption (DHDs): 82.90. In (DDD): 59870, in the segment DU90% were found clonazepam (Cln): 28185, lorazepam (L): 13174, alprazolam: 12980. The NFT includes diazepam, L and Cln. The last one used only for panic crisis treatments when it is not indicated to comitial crisis use. **Conclusion:** It is observed a relatively low consumption of ATDs and high of BZDs. Generally, it is scarce the adjustment to FTN. It would be suitable to revise the utilization of these drugs in order to promote a higher rationalization in its use.

BVI-129.**PRENATAL STRESS INDUCES OXIDATIVE AND BEHAVIOURAL ALTERATIONS IN OFFSPRING'S CEREBELLUM AND HIPPOCAMPUS RAT**

Maur DG, Palumbo ML, Bourdet B, Genaro AM, Zorrilla Zubilete MA.

Cátedra de Farmacología, Facultad de Medicina, UBA, CEFYBO-CONICET. Paraguay 2155, Buenos Aires, Argentina.

E-mail: mariazorrillaz@gmail.com

Stress during early development stages induces both early and later alterations. The objective of this study was to evaluate the role of the cellular oxidative mechanisms in the behavioural alterations in adulthood induced by prenatal stress during of neurodevelopment.

Methods: pregnant Wistar rats were individually restrained three times a day, 45 minutes each. Was analysed the offspring's hippocampus and cerebellum for Nitric Oxide Synthase (NOS) activity, NOS expression by nNOS by RT-PCR and Western Blot. **Results:** The offspring of stressed rats showed an increased activity of total NOS (pmol/gtissue/30min) at PN7, PN15 and PN90. We also detected an increased production of Reactive Oxygen Species (ROS) stimulated by NMDA in PN15 cerebellum. Finally, we observed an increased protein profile of neuronal NOS (nNOS) at PN15, PN30, PN45 and PN90 together with an alteration in the expression of NOS RNA. **Conclusion:** Stress during early development stages induces alterations in cerebellar and hippocampal oxidative mechanisms. Taking this results together with behavioural abnormalities we hypothesise that the oxidative alterations might be primary events during development, which manifest in adulthood as significant changes in animal behaviour.

BVI-130.**NEUROTENSIN DECREASES HIGH AFFINITY [³H]-OUABAIN BINDING TO CEREBRAL CORTEX MEMBRANES**

Rosin C, López Ordieres MG, Rodríguez de Lores Arnaiz G.

Inst Biol Cel y Neuroc "Prof. E. De Robertis", Fac Med, and Cátedra de Farmacol, Fac Farm y Bioq, UBA. Paraguay 2155, 1121-Buenos Aires, Argentina. E-mail: grodrig@ffyb.uba.ar

Previous work showed that neurotensin inhibits synaptosomal membrane Na⁺, K⁺-ATPase activity, an effect blocked by SR 48692 (SANOFI-AVENTIS, US INC), antagonist for high affinity neurotensin receptor (NTS1). Neurotensin effect on high affinity [³H]ouabain binding was studied, to observe a dose-dependent decrease, with the following values (% vs control): 87 ± 10; 66 ± 5 and 22 ± 10 (n=3) at 10⁻⁶ M, 10⁻⁵ M and 10⁻⁴ M peptide concentration, respectively. SR 48692 at 10⁻⁶ M, 10⁻⁵ M and 10⁻⁴ M decreased [³H]ouabain binding (in %) to 96 ± 19; 85 ± 16 and 34 ± 17 (n = 3-6), respectively. Neurotensin at 10⁻⁶ M and 10⁻⁵ M concentration respectively decreased 15% and 30% binding to membranes after injection of SR 48692 (100 µg/kg, icv, 30 min). It is concluded that neurotensin is able to modulate [³H]ouabain binding, an effect which hardly involves NTS1 receptor.

BVI-131.**NON RADIOACTIVE *IN SITU* HYBRIDIZATION OF CRFR1 mRNA IN AN ANIMAL MODEL OF DEPRESSION**

Fernández Macedo GV, Sifonios L, Cladouchos ML, Wikinski S.

ININFA. Junín 956 piso 5°. Buenos Aires, Argentina.

E-mail: georginafm@ffyb.uba.ar

Corticotrophin releasing factor (CRF) has been shown altered in depressive patients as well as in animal models of depression. In this pathological condition, it exerts its action mainly through activation of type one CRF receptor (CRFR1). We developed a non radioactive *in situ* hybridization method to measure CRFR1 mRNA. We used a cRNA digoxigenin-labeled probe synthesized by the *in vitro* transcription of a rat CRFR1 cDNA subcloned in a vector. Hybridization was performed on rat brain sections and the probe specifically hybridized with the CRFR1 mRNA was detected by a commercial kit. Specificity to CRFR1 mRNA was confirmed in sections hybridized with sense probes, the specificity to RNA was tested digesting the tissue with RNases prior to hybridization and the specificity of the detection procedure was study without using the label probe. We measured the expression of CRFR1 in two hippocampal areas (CA3 and dentate gyrus) of animals after 21 days of exposure to the learned helplessness (LH) paradigm. We compared: a control group (C, rats not exposed to stress), LH+ (rats that develop the behavioural despair after exposure to stress) and LH- (rats that fail to develop the behavioural despair after exposure to stress). LH+ showed a decrease in the mRNA CRFR1 expression versus LH- or C, both in CA3 and in dentate gyrus (p<0.01). We conclude that 3 weeks after exposure to the LH paradigm the expression of CRFR 1 is downregulated.

Supported by: PICT 31953, PIP 5870, UBACYT M013 and M073.

BVI-132.**GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) INCREMENTS ARE OBSERVED IN HIPPOCAMPUS OF CHRONIC RESTRAINT ANIMALS BUT NOT IN ANIMALS EXPOSED TO THE LEARNED HELPLESSNESS PARADIGM**

Cladouchos ML, Sifonios L, Cereseto M, Fernández Macedo GV, Wikinski S.

Instituto de Investigaciones Farmacológicas (CONICET-UBA) Junín 956 piso 5° C.A.B.A., Argentina.

E-mail: mlclado@ffyb.uba.ar

Astrocytic changes in depression and in animal models of the disease are still poorly known. The main goal of this work was to investigate, by immunohistochemistry, the changes in GFAP in two experimental models of depression: the learned helplessness paradigm and the chronic restraint stress. Male adult rats were exposed to a protocol of either restraint stress (RS) (6 hours/day for 21 consecutive days) or inescapable shocks (LH) (one session of 60 footshocks lasting 15 sec each during 1 hour). In this latter model, 4 days after exposure to stress, animals were tested to identify those showing (LH+) or not showing (LH-) despair behaviour. The RS and their controls were sacrificed the day after concluding the protocol and the LH+, LH- and their controls (CLH) were sacrificed 4, 10 and 25 days after exposure to inescapable stress. Only RS animals showed a significant increment compared with their controls (RS: 50,9%, p<0.001, unpaired Student's t test). No differences were found in GFAP on days 4, 10 and 25 between CLH, LH+ and LH- animals. In the literature, experimental models of depression are usually considered to be equivalent. From our results, it seems that caution in these extrapolations should be advisable.

Supported by: PICT 31953, PIP 5870, UBACYT M013 and M073.

BVI-133.**NEONATAL NITRIC OXIDE SYNTHASE INHIBITION MODIFIES NEUROTENSIN EFFECT ON NEURONAL ATPase ACTIVITY**

López Ordieres MG, Alvarez Juliá A, Kemling A, Rodríguez de Lores Arnaiz G.

Instituto de Biología Celular y Neurociencias "Prof. E. De Robertis", Facultad de Medicina, Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, UBA. Junín 956, 1113-Buenos Aires. E-mail: glopez@ffyb.uba.ar

We have previously showed that peptide neurotensin inhibits neuronal Na⁺, K⁺-ATPase activity, an effect which involves high affinity neurotensin receptor (NTS1). Nitric oxide (NO) acts as a neurotransmitter or as a neuromodulator when it is synthesized by neuronal nitric oxide synthase (nNOS). Herein we evaluated neurotensin effect on cortical synaptosomal membranes isolated from female or male Sprague-Dawley rats. Animals were injected at 3, 4 and 5 postnatal days with saline (control) or L-No-Arg, a nitric oxide synthase inhibitor (iNOS) and sacrificed 35 (juvenile) or 50 days (adult) later. The presence of neurotensin at 3.5 x 10⁻⁸-3.5 x 10⁻⁶ M concentration decreased 6%-34% Na⁺, K⁺-ATPase activity in membranes purified from control animals (female or male) whereas it failed to alter the enzyme in membranes obtained after iNOS treatment. The presence of 1.0 x 10⁻⁶ M neurotensin decreased 37-53% [³H]-ouabain binding to membranes isolated from iNOS treated juvenile and adults male rats, respectively. Whereas, the peptide produced only 12% binding decrease in membranes isolated from control animals. In conclusion, early postnatal NO dysfunction may exert a permanent change in neurotensin system that influence Na⁺, K⁺-ATPase activity.

BVI-135.**SEXUALLY DIMORPHIC C-FOS EXPRESSION DURING MORPHINE WITHDRAWAL SYNDROME**

Induni A^{1,2}, Taravini I¹, Bernacchia F¹, Rosselli S¹, Balerio G^{1,2}.

¹ININFA (CONICET), ²Cát. de Farmacología, FFyB (UBA). Junín 956 5° piso (1113), Bs. As. E-mail: gbalerio@ffyb.uba.ar

We have previously demonstrated sex differences during morphine (MOR) withdrawal. We have also shown that the GABA_B agonist baclofen (BAC) was able to prevent the MOR withdrawal in male as well as female mice. The aim of the present study was to evaluate the immediate early gene c-Fos expression in various brain areas in mice of either sex during naloxone (NAL)-precipitated withdrawal and its prevention with BAC. Swiss-Webster prepubertal mice were rendered dependent by i.p. injection of MOR (2 mg/kg), twice daily for 9 days. On the 10th day, dependent mice were divided into two groups: withdrawal group received NAL (6 mg/kg, i.p.) after the last dose of MOR, while prevention group received BAC (2 mg/kg, i.p.) before NAL injection. Animals were anaesthetized and transcardially perfused with paraformaldehyde. Brains were removed and cut to perform immunohistochemical studies. Our results showed a significant decrease in c-Fos expression in CA3 (p<0.01), CA1 (p<0.01) and dentate gyrus (p<0.001) of the hippocampus of MOR withdrawn males vs control group. Conversely, the number of Fos positive nuclei was not modified in any of the areas studied of females. The pretreatment with BAC did not modify the c-Fos expression in MOR withdrawn males. The sexual dimorphism observed herein confirms the greater sensitivity of males in response to MOR. The effect of BAC in preventing the expression of MOR withdrawal would not be related with c-Fos expression.

Supported by UBACYT B021 and B016.

BVI-136.**ROLE OF THE GABAERGIC SYSTEM IN NICOTINE ADDICTION: REWARD AND PHYSICAL DEPENDENCE IN MICE**

Moutinho L¹, Balerio G^{1,2}.

¹ININFA (CONICET), ²Cát. de Farmacología, FFYB (UBA). Junín 956 5°Piso (1113), Bs. As. E-mail: gbalerio@ffyb.uba.ar

Repeated nicotine administration in animals produces several behavioural responses related to its addictive properties, such as reinforcing effects and physical dependence. The aim of the present study was to examine the possible role of the GABAergic system in NIC-induced rewarding effects using the conditioned place preference paradigm (CPP) and in the somatic expression of NIC abstinence. The CPP protocol was performed in three phases. *Pre conditioning*: (Day 1), mice were tested to determine time spent in each compartment for 18 min. *Conditioning*: (Day 2), animals were pretreated with BAC (2 mg/kg, i.p.) or saline injection and immediately confined to one of the choice compartments for 20 min. Six hours later, mice were injected with the alternate condition and confined to the opposite choice chamber. This procedure was repeated on days 3-4-5. *Post conditioning*: (Day 5) the procedure was identical to Day 1. A model of naloxone (3 mg/kg, sc) (NAL)-induced abstinence in chronic NIC-treated mice (25 mg/kg/day, sc) was developed using implanted osmotic minipumps. NIC produced rewarding effects (p<0.01) while BAC did not significantly modify these effects. NAL precipitated NIC withdrawal (p<0.001) and BAC attenuated the severity of this withdrawal (p<0.001). These results demonstrated that the somatic expression, but not the reinforcing motivational effects can be modulated by the endogenous GABAergic system.

Supported by UBACYT B021 and B016.0.

BVI-137.**INVOLVEMENT OF GABAERGIC SYSTEM IN NICOTINE WITHDRAWAL: SOMATIC AND NEUROCHEMICAL VARIATIONS**

Varani A¹, Moutinho L¹, Calvo M¹, Balerio G^{1,2}.

¹ININFA (CONICET), ²Cát. de Farmacología (FFYB, UBA) Junín 956, 5°Piso. Buenos Aires. E-mail: gbalerio@ffyb.uba.ar

Nicotine (NIC) plays a major role in tobacco addiction. The aims of the present study were: a) to evaluate the possible involvement of GABAergic system in NIC physical dependence; b) to analyze the neurochemical variations in various brain regions of mice during NIC withdrawal syndrome and its prevention with baclofen (BAC, GABA_B receptor agonist). Swiss mice received NIC (2.5 mg/kg, sc) 4 times daily, for 7 days. On day 8, dependent mice received the NIC antagonist mecamylamine (MEC; 2 mg/kg, ip) 1h after the last dose of NIC. A second group of dependent mice received BAC (2 mg/kg, ip) before MEC-precipitated abstinence. Somatic signs were measured for 30 min. The levels of DA, 5-HT and its metabolites were determined by HPLC in the striatum, cortex and hippocampus of brains collected 10 min after the last injection on day 8, since NIC withdrawal response reached a maximum between 10 and 15 min. BAC pre-treatment decreased the incidence of some NIC withdrawal signs, such as wet-dog-shakes (p<0.01) and paw tremors (p<0.001). The global withdrawal score was also attenuated by BAC (p<0.001). DA and DOPAC levels decreased in the cortex of the abstinent group (p<0.001 and p<0.01 respectively), while BAC reestablished these levels 10 min after MEC-precipitated NIC withdrawal (p<0.01). We conclude that BAC prevents NIC withdrawal syndrome due to changes in dopaminergic activity measured 10 min after MEC.

Supported by UBACYT B021 and B016.

BVI-138.
LACK OF GABA_{B(1)} RECEPTORS MODIFIES NICOTINE BEHAVIOURAL RESPONSES IN MICE

Varani A¹, Calvo M¹, Balerio G^{1,2}.

¹ININFA (CONICET), ²Cát. de Farmacología (FFYB, UBA) Junín 956, 5°Piso. Buenos Aires. E-mail: gbalerio@ffyb.uba.ar

Nicotine (NIC) is one of the active components of tobacco and some of acute behavioural effects induced by NIC can contribute to its abuse potential in humans. We have demonstrated that the GABA_B receptor agonist baclofen increased the hypolocomotion and decreased the antinociceptive effects induced by NIC. The aim of the present study was to evaluate the possible role of GABA_{B(1)} receptors in responses induced by acute NIC administration by using knockout mice lacking the GABA_{B(1)} receptor and their wild-type littermates. The animals were injected with NIC (0.5, 1, 3 and 6 mg/kg, sc) or saline and locomotor activity was measured 5 min later for a period of 10 min. The antinociceptive responses were determined by tail-immersion and hot-plate tests, 15 and 16 min after NIC or saline injection, respectively. Acute NIC administration decreased locomotor activity and induced antinociceptive responses in the tail-immersion test at the dose of 3 and 6 mg/kg ($p < 0.001$) and in the hot-plate test only at the dose of 6 mg/kg ($p < 0.001$), in wild-type animals. In knockout GABA_{B(1)} mice NIC also induced hypolocomotion at the dose of 6 mg/kg ($p < 0.001$). The antinociceptive effects in the hot plate test were absent, while in the tail-immersion test these responses were lower than in their wild-type mice. These results demonstrated that some acute effects elicited by nicotine can be modulated by the endogenous GABAergic system and support the existence of a physiological interaction between these two systems.

Supported by UBACYT B021 and B016.

BVI-139.
UNDERNUTRITION AT PERINATAL AGE FACILITATES MORPHINE SENSITIZATION AND CROSS-SENSITIZATION TO COCAINE IN ADULT RATS: BEHAVIORAL AND NEUROCHEMICAL STUDIES

Velazquez EE, Valdomero A, Orsingher OA, Cuadra GR.

Depto. de Farmacología, F.C.Q, Universidad Nacional de Córdoba, IFEC (CONICET). C. Universitaria, 5000 Córdoba, Argentina. E-mail: edvelazquez@fcq.unc.edu.ar

To evaluate the influence of early malnutrition on the development of behavioral sensitization induced by repeated morphine administration in different groups of control (C-) and deprived (D) rats motor activity was assessed following different doses of morphine (5, 7.5, 10 or 15 mg/kg, i.p.) or saline every other day for 5 days. At every doses, D-rats showed sensitization and a lower number of sessions were needed to induce this phenomenon as compared to C-rats. Moreover, when a challenge of cocaine (10 mg/kg, i.p) was given 48 h after the last morphine administration, only D-rats showed cross-sensitization in morphine-pretreated animals (7.5 and 10 mg/kg). When extracellular dopamine (DA) were measured in n. accumbens (core and shell) and in dorsal caudate-putamen following a challenge with cocaine in morphine pre-exposed rats (7.5 mg/kg) a higher increase in DA release only in n. accumbens (core) of D-rats was observed. Similar extracellular DA levels were found in the n. accumbens (shell) and in dorsal caudate-putamen of both groups. These results demonstrated that D-rats had a lower threshold to develop sensitization to morphine and cross-sensitization to cocaine as well as a higher responsiveness of the n. accumbens (core) expressed by increased DA release.

BVI-140.
CHRONIC STRESS INDUCES DIFFERENT EFFECT ON LEARNING AND MEMORY IN BALB/C AND C57BL/6 MICE ASSOCIATED TO DIFFERENT REGULATION OF NO PRODUCTION BY PKC ACTIVITY

Palumbo ML, Zorilla-Zubilette M, Cremaschi GA, Genaro AM. CEFyBO-CONICET-UBA, 1° Cátedra de Farmacología, Fac. Medicina, U.B.A. Paraguay 2155, Piso 15, Bs As, Argentina. E-mail: molecula_21@yahoo.com.ar

Stress and imbalance of Th1/Th2 immunity has been implicated in psychiatric disorders. Two genetically different inbred murine strains, C57BL/6 and BALB/c, show different behavioral responses, neurodevelopmental and neurochemical parameters Nitric oxide (NO) has been involved in many pathophysiological brain processes including hippocampal responses to stress. Here, we perform a comparative study on chronic mild stress (CMS) effects upon learning and memory in both strains, analyzing the role of NO production and its regulation by protein kinase C (PKC). Stressed BALB/c, but not C57BL/6 mice, showed a poor learning performance in both open field and passive avoidance inhibitory task. Control C57BL/6 mice showed a higher basal NOS activity than control BALB/c. In CMS BALB/c but not C57BL/6 mice a significant decrease of NOS activity was observed respect to control. PKC inhibition induced appositve effects on NOS activity in stressed mice respect to control depending on the strain studied. Western blot analysis of PKC isoforms revealed that CMS exposition induced an increased in ζ and γ PKC isoforms in BALB/c mice. On the other hand, CMS C57BL/6 mice showed an increase in δ and a decrease in $\beta 1$ PKC isoforms. These results suggest a differential effect of stress, being BALB/c more vulnerable to stress than C57BL/6 mice. This effect could be related to a differential regulation of NOS and PKC isoenzymes.

BVI-141.
IN VITRO ACTIVITY AND EX VIVO PARASITE DIFFUSION, USED TO SELECT AND OPTIMIZE POTENTIAL ANTHELMINTIC AGENTS FOR IN VIVO STUDIES

Munguía B, Espinosa R, Andina MJ, Mendina P, Saldaña J, Manta E, Domínguez L.

Facultad de Química, Universidad de la República, Montevideo, Uruguay. E-mail: munguia@fq.edu.uy

In an interdisciplinary work, search of new broad spectrum anthelmintics, 2-amine-- δ -valerolactam derivatives were obtained, which were more active than Albendazole (ABZ, standard drug with broad spectrum action) against nematode *N. brasiliensis* and no active against cestode *Mesostoides vogae*. Both *in vitro* models were developed by our group. It was designed and synthesized a new derivative, with mixed structure derived from 2-amine-- δ -valerolactam series and 2-amine-ABZ, and the uptake *ex vivo* study was investigated as a tool for chemical optimization of this potential anthelmintic. This work shows the development and comparison of two clean up and extraction methods (solid phase and liquid- liquid extraction) for the parasite diffusion study into *M. vogae* (syn corti). Comparative studies of parasite diffusion were performed using the optimized methodology for ABZ, its metabolite ABZ SX, and the new derivative Product 1. Physicochemical properties (lipophilicity, chemical stability) of the drugs mentioned were also studied.

A					
Abramson D	O-17	Brusco A	BVI-123	De Lucca R	BI-07
Acevedo C	BIII-67	Bucala V	BIII-64	Debattista NB	BII-29, BII-36
Acosta GB	BVI-118	Bucciarelli A	BIII-60, BIII-64	Debenedetti S	BIII-61
Acosta O	BIII-65, O-09	Buitrago E	O-17	del Valle ME	BIII-61
Adler E	C-01	Busch L	BIV-91	Dellicarpini G	O-20
Agostini M	BV-109	Bustillo S	O-09	Delpech G	BIV-86
Aguilar CF	BIII-62	C		del-Rey G	BV-104
Aguirre M	BIV-76, BIV-77, BIV-79, O-03	Cabanillas A	BIV-80	Demurtas S	BIV-74, BIV-94, O-02
Albarellos G	BII-35	Cabrera A	BII-42	Denamiel G	BII-35
Alcalde SM	BIII-72	Cabrera R	BV-107	Denegri G	BI-23, BIV-92, O-12
Alemandi DA	BII-29, BII-40, BII-42, BII-46, BIII-64, BV-105, O-06	Cabreram JL	O-14	Denzoin L	BI-02, BI-05
Almiron R	BVI-121	Caceres LG	BVI-126	Denzoin Vulcano L	BI-12
Alovero F	BIV-87	Cadenazzi G	BI-21	Di Giusto G	BV-98, O-04
Alvarez J	BVI-133	Caldiz C	O-01	Di Gravisaco MJ	BIV-84
Alvarez L	BI-18, BI-21, BII-33, BII-38, BII-41, BII-43, O-12	Calise M	O-14	Di Verniero C	BV-106
Alvarez M	BIV-79	Calvo M	BVI-137, BVI-138	Diab S	BV-108
Alzola R	BI-24	Canzobre MC	BIV-81	Diana-Menéndez S	BV-104
Ambros L	BII-30, BII-37	Capani F	BVI-126	Diaz Aquino V	BIV-75
Andina MJ	BII-141	Cardinali D	C-02	Dib A	BII-42
Anesini C	BIII-53	Cardoso L	BIV-76, BIV-77, BIV-79, BV100	Diez RA	O-14
Aón L	BVI-126	Caruso C	BIV-80	Domínguez L	BII-141
Arany E	C-06	Casas S	BV-107	Donadel O	O-18
Ardusso MS	BV-105	Cassataro J	BIV-73	Duffard R	BI-06, BI-16, BI-17, BVI-125
Arias A	BI-11, O22	Castells D	BII-43	E	
Arnoldi S	BI-03, BIV-78, O-05	Castillo G	BV-103	Echenique CG	BI-09
Aromando R	BI-07	Castro E	BI-05	Elías MM	BVI-128
B		Castro S	BII-42, BIII-64	Elissondo C	BI-23, BIV-92, O-12
Balerio G	BVI-135, BVI-136, BVI-137, BVI-138	Catania VA	BI-09, BI-10, BI-11, O-22	Ennis I	O-01
Ballent M	BI-08, BII-33, O-21	Ceballos FA	BI-14	Enrique A	BIII-57
Barberis M	BIII-55	Ceballos L	BI-18, BII-38, O-12	Entrocasso C	BII-33
Barceló AC	BVI-116	Ceballos M	BV-99	Errasti A	BV-104
Barreiro Arcos ML	BIII-53, BIV-78	Ceci M	BIV-88, O-10	Errecalde C	BII-51
Bazocchini V	BV-107	Cereseto M	BVI-132	Errecalde JO	BI-26, BI-27, BII-50, BII-52, O-13
Becaluba M	BI-02	Ceriani C	BIV-82	Errecalde F	BI-26
Becú-Villalobos D	BV-108	Cerone S	BIV-85	Espinosa R	BII-141
Bellusci C	BII-49	Chantada G	O-17	Esquivel GP	BI-13, BI-14
Beltrame SP	O-07	Chedrese P	BV-109	Esteban E	BIV-82, BIV-85
Benvenuti M	BIII-69	Chiappe de Cingolani G	O-01	Estein S	BIV-73
Berardi A	BIII-70	Chiclana CF	BIII-57	Evangelista de Duffard AM	BI-06, BI-16, BI-17
Bernacchia F	BVI-135	Cholich L	BIII-65	F	
Bertero J	BV-110	Chuluyán E	C-03	Faggiolino P	BII-43
Bertotto ME	BVI-122	Cingolani H	O-01	Fandiño A	O-17
Bettella A	BI-15	Cladouchos ML	BVI-131, BVI-132, BVI134	Farias C	BI-20, BI-25, BII-42
Bignante EA	BVI-124, BVI-127	Clausse M	BIV-73	Farias C	BI-20, BI-25, BII-42
Bilder CR	O-07	Collia N	BI-03, O-05	Farina OH	BII-34
Biolato S	BI-17	Colman E	BIV-84	Felipe A	BI-24
Bisagno V	O-15	Confalonieri A	BIV-88	Fernández H	BIV-89
Bistoletti M	BI-18, BII-41	Confalonieri O	BI-02	Fernández MA	BI-01
Boccio J	BI-03	Consolini AE	BIII-57, BIII-58, BIII-70, BV-101	Fernández Macedo GV	BVI-131, BVI-132, BVI134
Boeris M	BIII-66	Cordiviola C	BII-34	Fernandez RJ	BI-13
Boggio JC	BII-44, BII-51	Córdoba OL	BIII-72	Fernández S	BII-48
Boiero C	BVI-122	Coria L	BIV-73	Ferraro G	BIII-53, BIII-67
Bologna R	BII-49	Correa O	BII-43	Fiel C	BII-48, BIII-69, BIV-93
Bonazzola P	BIII-58	Correa V	O-01	Filia MF	O-20
Borda B	BII-33	Cremschi G	BIII-53, BIV-78, BVI-140	Filinger EJ	BI-01
Borda E	BIV-91	Croci M	O-08	Fillipini B	BVI-116, BVI-117
Bourdet B	BVI129	Cuadro GR	BVI-139	Fioravanti D	BIII-59
Boveris A	C-11	Cuezzo L	BV-103	Fiorentino A	BIV-73
Bramuglia GF	BII-49, BIV-90, O-17	Cumino A	BI-23	Flores ML	BIII-72
Brandan N	BIV-76, BIV-77, BIV-79, BV100, O-03	Curras V	BII-49	Fogel F	BI-05
Breda S	O-11	D		Fonseca C	O-16
Briñón MC	O-19	Dadé M	BIII-59	Formía N	BV-108
Brizuela N	BIII-54, BIII-55, BIII-68, BIV-74, BIV-94, BV-111, O-02	Dallia S	BIII-68	Fossati C	BIV-73
		Daniele M	BI-22, BI-26, BI-27, O-13	Franci R	BI-05
		Davicino R	BIII-53	Fumuso E	BIV-89
		De Battista M	BII-35	Funosas E	BI-07
		De Biasio MB	BIV-83	Fusé L	BII-48, BIV-93
		De Lorenzi P	BVI-125		

- G**
- Gago J BV-104
 Gallo L BIII-64
 García Aseff S BIII-71
 García C BI-21
 García Denegri E BIII-65
 García J BIV-89
 Garcíarena C O-01
 Gay C O-09
 Genaro AM BIV-81, BV-112, BVI-129, BVI-140
 Gershanik O C-13, BVI-113, BVI-114, BVI-115
 Ghanem CI O-20
 Ghersevich S BI-06
 Giambartolomei G BIV-73
 Gil L BIII-71
 Gimeno E BIII-65
 Giordano O BIII-63
 Giuliani F BV-107
 Goin JC O-07
 Goldbaum F BIV-73
 Goldman C BI-03
 Gomez Arévalo G C-13
 González C BIV-89
 González P BIV-80, BVI-119
 Gordon I BIII-69
 Gorzalczy S BIII-67, BIII-72
 Gräve E BII-52
 Gravielle MC BVI-120
 Gravisaco MJ O-08
 Grigorjev C BIII-54
 Guardia Calderón CE BIII-56
 Guardia T BIII-56
 Guelman LR BVI-126
 Guerrini E BIII-55
 Gutiérrez ML BVI-120
 Gutiérrez S BIV-82
 Guzman ML BII-45
- H**
- Haag G BIII-61
 Hallu R BII-37
 Hegoburu M BII-49
 Heredia D O-02
 Himelfarb M BII-44, BII-51
 Höcht C BII-49, BIII-72, BV-106, BVI-117, O-17
 Huber B BI-27, BII-52
 Huergo G BIII-55
- I**
- Iezzi S BI-19, BI-20
 Igarza L BIV-85
 Iglesias L BII-48, BIV-93
 Imperiale F BI-19, BI-20, BI-25, BII-28
 Indart H BIV-85
 Induni A BVI-135
 Inza G BIV-85
- J**
- Jerez S BV-102
 Jimenez-Kairuz A BII-46
 Jones D C-04
 Juaristi J BIV-76, BV100
 Juliarena M BIV-82
- K**
- Kalina E C-14
 Kaliski MA BI-03, O-05
 Kapitulnik J C-10
- Kemling A BVI-133
 Kreil V BII-37
- L**
- Lacau-Mengido I BV-108
 Lacunza J BII-34
 Laffont GV BI-15
 Laino C O-16
 Lambertini A BII-50, BII-52, O-13
 Landi R BIV-85
 Landoni M BII-35
 Lanusse C BI-08, BI-18, BI-19, BI-20, BI-24, BI-25, BII-28, BII-33, BII-38, BII-41, BII-42, BII-43, O-12, O-21
 Larramendy C BVI-113, BVI-114, BVI-115
 Larsen M BI-24
 Lasaga M BIV-80, BVI-119
 Lazarowski A O-05
 Leiva L O-09
 Lemberg A BI-01
 Leonard N BI-03
 Lhez L BII-29, BII-36
 Licoff N BV-108
 Lifschitz A BI-08, BI-19, BI-25, BII-28, BII-33, BII-41, O-21
- Linares LM BV-95, BV-96
 Litterio N BII-44, BII-51
 Llabot J BII-40
 Lloberas M BII-33
 López E BI-07
 López M BVI-123
 López Fernández L BV-103
 López Ordieres MG BVI-130, BVI-133
 López Rivilli MJ BVI-127
 Lorenzo Lopez JR BVI-123
 Lorenzutti M BII-44, BII-51
 Lucas M BII-50
 Luquita MG BI-09, BI-11
- M**
- Madariaga MJ BI-06
 Maestri L BI-07
 Maletto B O-06
 Manazza J BII-33, BIV-73
 Mangano A BII-49
 Mansilla E BIV-75
 Manta E BII-141
 Manuele MG BIII-53
 Manzo R BII-39, BII-40, BII-45, BII-46, BII-47, BIV-87, BV-105, O-11
 Marchetti L BII-50, O-13
 María A BIII-63, O-18
 Mariani E O-19
 Marin GH BIV-75
 Marrassini C BIII-67
 Martijena ID BVI-122
 Martínez A BI-07
 Martire K BIV-75
 Marzi M BV-110
 Maté L BI-08, O-21
 Maur DG BVI129
 Mayer M BV-106
 Mecikovsky D BII-49
 Medina V BIV-78
 Meirovich Y BIV-74, BV-111
 Mejía M BV-108
 Mendina P BII-141
 Merelli A O-05
 Mestorino N BI-26, BI-27, BII-50, BII-52, O-13
 Miceli M BII-32
- Milano G BI-04
 Miño J BIII-67
 Miozza V BIV-91
 Moggi S BIV-85
 Molina VA BVI-122, BVI-124, BVI-127
 Monfrinotti A BII-30
 Mongini C BIV-84, O-08
 Monti C BVI-121
 Montoya L BII-30, BII-35
 Montrull H BIII-68, BIV-74, BIV-94, BV-111
 Moreno F BIII-69
 Moreno L BI-18, BII-38, BII-41, BII-42, BIV-89
 Morón G O-06
 Mosca S O-01
 Mottino AD BI-09, BI-10, BI-11, O-20, O-22, C-09
 Moutinho L BVI-136, BVI-137
 Munguía B BII-141
 Murer GM BVI-113, BVI-114, BVI-115
 Murua D BIV-94, BV-111
- N**
- Nanfaro F BV-107
 Nejamkin P BII-28
 Nicolini J BIV-78
 Niselman V BII-49
 Nocito AL BI-22
 Novak A O-20
 Nuñez M BV-110
- O**
- Obregón GRE BI-13
 Odeon M BVI-118
 Odetto C BV-110
 Olivera M BII-39, BII-45, BII-47, BV-105, O-11
 Opezzo J O-17
 Orce G BV-103
 Orsingher OA BVI-139
 Ortega G O-14
 Ortega I BI-05
 Ortiz JE BII-36
 Otero P BV-99
- P**
- Paciaroni J BVI-128
 Paez MA BV-111
 Paglini G BVI-124
 Palatnik JF BI-22
 Palena M BII-46
 Palma S BII-42, BIII-64, O-06
 Palumbo ML BIV-81, BVI-129, BVI-140
 Paolicchi F BIV-73
 Pappano NB BII-29, BII-36
 Pazo JH BVI-116, BVI-117
 Pedernera AM BIII-56
 Pellegrino JM BI-10, BI-11, O-22
 Pelorosso F BV-104
 Pelzer LE BIII-56, BIII-62, BIII-63, BIII-71, O-18
 Peral de Bruno M BV-102
 Peralta M O-14
 Perazzo JC BI-01
 Perdomo VG BI-22
 Pérez AR BI-09
 Pérez C O-14
 Peroni RN BIV-90
 Perri A BV-108
 Peskin V O-15

- Pino M BI-14
 Piña J BIII-64
 Pistoressi-Palencia MC O-06
 Planells F BV-95
 Pletsch C BI-15
 Pochettino A BI-16, BI-17
 Podestá MF BVI-123
 Prados AP BII-31, BII-37
 Pretti R BIV-75
 Pretto L BI-22, BII-51
 Purslow P BII-48
- Q**
 Quaglia N BV-110, BVI-128
 Quevedo MA O-19
 Quintero M BI-27, BII-52
 Quinteros DA BV-105
- R**
 Radenti J BI-07
 Ragone MI BIII-58, BIII-70, BV-101
 Ramírez AJ C-12
 Ramírez O BVI-121
 Ramírez Rigo MV BV-105
 Ranno G BIV-86, BIV-88, O-10
 Rebuelto M BII-30, BII-31, BII-37, BV-99
 Recavarren M BI-04
 Reinés A BVI-123, O-16
 Revelli SS BI-09
 Revidatti FA BIV-83
 Reyes J BIV-77
 Reyes M O-03
 Reyes Toso CF BV-95, BV-96
 Reyes Toso ML BV-95, BV-96
 Ricarte Bratti JP BIV-74
 Ricci CR BV-96
 Rigalli JP BI-09, BI-10, BI-11
 Ríos H BIV-81
 Rivulgo M BIV-88, BIV-89
 Rizzo N BIV-84
 Rodríguez de Loes Arnaiz G BVI-130, BVI-133
 Rodríguez J O-09
 Rodríguez CB BVI-118
 Romañuk C BII-39, BII-47
 Romay S BI-01
 Romero Arijón A BIII-55
 Romero CR BIV-83
 Romero V BIV-87
 Roque G BIV-75
 Rosin C BVI-130
 Rosón M BV-95
 Rosselli S BVI-135
 Rotelli AE BIII-56, BIII-62
- Rothlin R BV-104
 Rubinstein R BV-112
 Rubio MC BII-49, BIV-90, O-20, C-07
 Ruiz Diaz D O-03
 Ruiz ML BI-09, BI-10, BI-11, O-22
 Ruiz Mostacero J BV-103
 Rule R BII-34
- S**
 Saborido MD BVI-113, BVI-114, BVI-115
 Sagüés F BIV-93
 Sagüés M BII-48
 Salatino AE BVI-118
 Saldaña J BII-141
 Salgueiro MJ BI-03, BIV-78, O-05
 Sallovitz J BI-08, BI-20, BI-25, BII-28, O-21
 San Andrés MI BII-44
 Sánchez Bruni S BII-42, BIV-86, BIV-88, BIV-89, O-10, O-12
 Sanchez M BIV-80
 Sandoval GL BI-13, BI-14, BI-15, BIV-83
 Sansinanea A BI-21
 Saraceno E BVI-126
 Saumell C BII-48, BIII-69, BIV-93
 Scacchi F BV-102
 Scarcella S BI-24
 Schinella G BIII-59, BIII-61, O-01
 Scimonelli T BIV-80, BVI-119
 Sciullo P BIV-84
 Scublinsky D C-05
 Segura-Aguilar J BVI-125
 Sen L BII-49
 Serra H BII-32
 Sierra L BV-102
 Sifonios L BVI-131, BVI-132, BVI134
 Silberman E BV-106
 Sindik M BI-14
 Skliar M BIII-60, BIII-64
 Solana H BI-24
 Sonnemberg M BVI-123
 Soraci A BI-02, BI-05, BI-12
 Sparo M BIV-86, BIV-88, BIV-89, O-10
 Stemberg E BIV-76, BIV-77, O-03
 Sterin-Borda L BIV-91
 Sterin-Speziale N O-16
 Stoyanoff T BIV-76, BIV-77, O-03
 Suárez G BII-42, BII-43
- T**
 Taira CA BIII-72, BV-106
 Tapia O BI-02, BI-05, BI-12
 Taravini IRE BVI-113, BVI-114, BVI-115, BVI-135
- Tarragona L BII-37, BV-99
 Teibler G BIII-65
 Terraes JC BI-13, BI-15, BIV-83
 Todaro J BIV-76, BIV-77, BIV-79, BV-100, O-03
 Tonn C O-18
 Toribio M BIII-66
 Torres AM BV-97, BV-98, O-04
 Toso R BIII-66
 Tournier H BIII-59, BIII-61
- U**
 Ubios A BI-07
 Ughetti R BIV-78
 Urbano FJ O-15
 Urbizu L BIV-88
- V**
 Valdomero A BVI-139
 Varani A BVI-137, BVI-138
 Vega Orozco AS BV-107
 Veggi LM BI-22
 Veksler Hess J BII-37
 Velazquez EE BVI-139
 Velo M BII-35
 Vendrell A BIV-84, O-08
 Viglione PN BV-96
 Villanueva SSM BI-11, O-22
 Villar SR BV-97, BV-98
 Virkel G BI-08, BI-25, BII-28, BII-33, O-21, C-08
- W**
 Wald M BIV-81, BV-112
 Waldner C BIV-84, O-08
 Wallinger ML BV-95, BV-96
 Waxman S BII-30
 Wendel G BIII-63, BIII-71, O-18
 Wikinski S BVI-123, BVI-131, BVI-132, BVI-134, O-15
 Wright A BIII-69
- Y**
 Yranzo GI BVI-127
 Yunes R BV-107
- Z**
 Zalazar A BI-07
 Zarazaga MP BII-44
 Zavaleta R BIV-85
 Zorrilla Zubilete MA BVI-129, BVI-140
 Zubillaga M BI-03, BIV-78, O-05
 Zylberman V BIV-73

