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Paper No.: 2382
FOCUSED CONFERENCE GROUP: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
SUPPRESSION OF THE ANTIGEN-MEDIATED RBL-2H3 CELL DEGRANULATION BY CAFFEIC ACID PHENETHYL ESTER

Eriko Aizu-Yokota, Y Kan, S Tanifuji, N Yoshida, M Funakoshi-Tago, Y Sonoda, T Kasahara
Keio University Fucalty of Pharmacy, Department of Basic Pharmaceutical Chemistry, Tokyo, Japan

Mast cells play a crucial role in allergic inflammatory responses. The cross- linking of high affinity IgE receptor by IgE and antigen on mast cells leads to mast cell activation such as degranulation, synthesis of eicosanoids and cytokines production. These events are initiated by the phosphorylation of a variety of signaling molecules and calcium mobilization. The suppression of degranulation has considered to be a preventive approach for allergic inflammation. Caffeic acid phenethyl ester (CAPE), a component of propolis extracts and a potent inhibitor of NF-κB, is reported to have diverse effects including anti-inflammatory, anti-oxidant and immunomodulatory effects. In this study, we investigated the effects of CAPE on antigen-dependent degranulation, intracellular calcium response and protein phosphorylation. RBL-2H3 cell line has been commonly used as a model of mast cells. For antigen-dependent degranulation experiments, RBL-2H3 cells were sensitized with DNP-specific IgE, and then challenged with antigen DNP-conjugated human serum albumin. Degranulation was determined by measurement of the release of the granule marker, beta-hexosaminidase. Concentration of intracellular calcium was assayed using Fura-2 loading cells. Antigen-induced protein phosphorylation was detected by immunoblotting. CAPE, at 10 to 100 microM, significantly inhibited the antigen-dependent degranulation in a concentration-dependent manner. Moreover, CAPE inhibited antigen-induced phosphorylation of ERK (p44/42 MAPK) and the influx of extracellular calcium. These findings suggest the capability of CAPE as a suppressor of antigen-induced mast cell activation.

Paper No.: 927
FOCUSED CONFERENCE GROUP: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
PROTECTIVE EFFECT OF AMOXICILLIN ON ACETIC ACID- INDUCED COLITIS IN RATS: COMPARISON BETWEEN ORAL VERSUS RECTAL ADMINISTRATION

Mohammad Alhumayyd
King Saud University College of Medicine, Department of Pharmacology, Riyadh, Saudi Arabia

This study was carried out to investigate the therapeutic potential of amoxicillin(AMX) given orally or rectally and its possible mechanism of action on acetic acid-induced colitis in rats. Animals were divided into seven groups, comprising 10 rats each. Groups1-4 were given twice daily doses of AMX (25,50 and 100 mg / kg; p.o.) and once daily dose of sulphasalazine (SPZ) (400 mg/kg; p.o.); respectively for five consecutive days starting 6 hr after induction of colitis by acetic acid. Groups 5-7 (control) were treated orally either with 0.5% methylcellulose(MC) or the lowest dose of AMX(25 mg/kg) after saline or acetic acid pretreatment. In another set of seven groups of animals, the same protocol was followed except that drug treatment were given rectally. Acetic acid – induced colitis was produced in rats and assessment of macroscopic lesions was performed. Biochemical parameters such as reduced glutathione levels, myeloperoxidase activity and vascular permeability were measured. Amoxicillin given orally or rectally, inhibited the severity of colonic lesion. Comparison between the oral and rectal administration of AMX or SPZ showed no significant differences between the values obtained at each dose level. The biochemical changes were reversed and brought towards the control levels by both regimes. The results of this study showed that AMX attenuated acetic acid-induced colitis in rats irrespective of its administration. Furthermore, it suggest that the mechanisms of the beneficial effects of AMX may include prevention of depletion of reduced glutathione, reduction of myeloperoxidase activity and decreased vascular permeability.

Paper No.: 3489
FOCUSED CONFERENCE GROUP: P17 - NEW APPROACHES AND TARGETS IN PSYCHIATRY
NOVEL BIOMARKERS OF DRUG SAFETY RELATED TO DRUG-INDUCED NEUROPSYCHIATRIC ADVERSE EVENTS INCLUDING DRUG-INDUCED SUICIDALITY

Frederic Allemand, L Vincent, L Cavarec, C Plusquellec, C Leborgne, D Weissmann, JF Pujol
BIOCORTECH, Paris, France

Biologic diagnostic of psychiatric disorders is today an unmet medical need since the only diagnostic available is clinic. Patients suffering from psychiatric disorders constitute heterogeneous populations, respectively to prognosis and to disease management. In the vast majority of cases, neither patient heterogeneity nor pharmacogenomics are entirely dependent of genetic differences and functional biomarkers are lacking to stratify patients populations respectively to drug selection and to drug response. Biocortech built a biomarker discovery platform exploiting the properties of RNA editing to discover novel biomarkers to address such needs and initiated clinical validation studies, respectively. Serotonin 5HT2C receptors, as other pharmacologically relevant receptors undergo a post-transcriptional modification known as RNA editing which regulates receptor function by modulating the distribution of isoforms that differ from each other in the combinations of substitutions of certain nucleotides at specific mRNA sites. Editing profiles are altered by disease conditions and by pharmacological agents, and could thus be turned into 'signatures' associated to certain disorders or to drug response. We overcame the technical challenge of measuring editing and fully characterized 5HT2c mRNA receptor editing profile that theoretically encompass 32 isoforms, in cells from various brain structures, and identified 5HT2C receptor editing-related biomarkers that are testable by blood sampling. A significant signature has been determined in suicides attempters from post mortem brain section. We initiated trials in various clinical settings such as in interferon α treated patients, suicide attempters, and patients treated with specific antidepressant regimens.

Paper No. 3480
Focused Conference Group: FC19 - GENERAL SESSION

EFFECT OF SILYMARIN ON INFLAMMATORY MEDIATORS RELEASE IN LPS-INDUCED PARKINSON DISEASE IN RATS

GA Amry(1), Magda Mohamed Hagras(2), M Megalad(3)
(1) King Abdulaziz University Faculty of Science, Department of Biology, Jeddah, Saudi Arabia
(2) King Abdulaziz University Faculty of Medicine, Department of Pharmacology, Jeddah, Saudi Arabia
(3) King Abdulaziz University Faculty of Science, Department of Histology, Jeddah, Saudi Arabia

Degeneration of dopamine (DA)-containing neurons in the substantia nigra (SN) of the midbrain is causes of Parkinson's disease (PD). Although inflammation of the brain has long been speculated to play a role in the pathogenesis of this neurological disorder however the mechanism is still poorly understood. Activation of microglia, the resident immune cells of the brain, and consequent release of proinflammatory factors are believed to contribute to neurodegeneration in PD. The aim of the present study was to examine the effect of silymarin in prevention of inflammatory mediators release and protection of dopaminergic neurons from Lipopolysaccharide (LPS)-induced neurotoxicity. In the present study a single intraperitoneal injection of LPS (15 mg/kg) in adult male Sprague Dawley rats resulted in an increase of midbrain content of neurotoxic factors TNF-α, nitric oxide (NO) and a decrease of DA level at 4, 24 hr, 3 and 7 days compared to the control. In addition, LPS reduced the number and the density of tyrosine hydroxylase-immunoreactive (TH-ir) neurons in the midbrain at 7 days. Pretreatment of silymarin (50 mg/kg) 24 hr before LPS for 7 days decreased TNF-α and NO compared to LPS treated rats. Moreover, it increased DA level and preserved the number and the density of TH-ir neurons. In conclusion, silymarin was found to has a potential therapeutic effect against LPS-induced neurotoxicity via reducing TNF-α and NO inflammatory mediators and preserving DA level in midbrain.
Keywords: Inflammation, LPS, Dopaminergic neurodegeneration, Parkinson's disease, TNF-α, Nitric oxide.

Paper No.: 3504
FOCUSED CONFERENCE GROUP: P04 - PHARMACOEPIDEMIOLOGY, CURRENT CONTROVERSIES AND OPPORTUNITIES
GENERIC SWITCHING DOES NOT INFLUENCE PATIENTS' CONCERNS ABOUT MEDICINES - A PRESCRIPTION DATABASE AND QUESTIONNAIRE STUDY

Morten Andersen(1,2), K Elberg(1), A Foged(1), DG Hansen(1), J Hallas(3), J Søndergaard(1)
(1) Research Unit for General Practice, University of Southern Denmark, Odense, Denmark
(2) Centre for Pharmacoepidemiology, Karolinska Institutet, Stockholm, Sweden
(3) Research Unit for Clinical Pharmacology, University of Southern Denmark, Odense, Denmark

Introduction. Surveys among medicine users have indicated that they experience side effects or lack of effect related to generic substitution, but studies have lacked a control group of patients continuing with the same product. We aimed to assess to what extent generic switching is associated with concerns about medicines substituted. Materials/Patients. A sample of 6000 persons who purchased substitutable antidepressants, antiepileptics and other drugs at pharmacies in the Region of Southern Denmark during September 2008 were identified in the Odense University Pharmacoepidemiologic Database. Physicians excluded 385 persons, 5615 questionnaires were sent out, 2757 were returned, and 2476 patients confirmed purchasing the index medication (overall response rate 41%). Generic switching was determined from dispensing data. Concerns with the index medication was assessed by the Beliefs about Medicines Questionnaire. Results. Generic switching occurred in 31% of patients. Switchers did not express more concerns about their index medication than patients who continued using the same product. The difference between switchers and non-switchers was -0.03 on a scale from 1 to 5 (95% CI -0.10 to 0.05). Patients were more concerned about antidepressants, 0.18 (0.10 to 0.26) and antiepileptics, 0.17 (0.08 to 0.25) than about other drugs. Conclusion. Generic switching did not lead to substantial concerns about the medication. Concerns about medicines were significantly higher among users of antidepressants and antiepileptics than among users of other drugs.

Paper No.: 3493
FOCUSED CONFERENCE GROUP: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
EFFECT OF A SERIES OF BENZOTHIOPHENE Γ-HYDROXYBUTENOLIDE COMPOUNDS ON NF-KB ACTIVATION AND PROINFLAMMATORY CYTOKINES PRODUCTION IN HUMAN PRIMARY KERATINOCYTES

Rosa María Andrés(1), P Navalón(2), MC Montesinos(1), M Payá(1), MC Terencio(1)
(1) University of Valencia and Center of Molecular Recognition and Technological Development (IDM), Valencia, Spain
(2) General Universitary Hospital of Valencia, Department of Urology, Valencia, Spain

Activation of NF-κB and high levels of TNF-α are key features of involved skin in psoriasis. Therefore we have studied how a series of promising benzothiophene γ-hydroxybutenolide (BTH) compounds, which are potent and selective inhibitors of microsomal prostaglandin E synthase 1 (mPGES-1) expression (Guerrero et al., J. Med. Chem., 2007, 50, 2176-84.), affect TNF-α and IL-8 release as well as NF-kappaB activation, in primary human keratinocytes. Cells were isolated from foreskin of healthy donors. Keratinocytes were incubated with keratinocyte basal medium for 24h before the experiments and pretreated for 30 min with compounds or vehicle. After 7h-stimulation with TPA (1 µg/ml), TNF-α and IL-8 levels were determined by ELISA. Stimulated cells released 59.7 ± 9.4 pg/ml of TNF-α and 226.5 ± 12.9 pg/ml of IL-8 compared to 4.9 ± 4.9 pg/ml and 12.5 ± 7.2 pg/ml in basal cells, respectively. At 10 µM, the leader compound BTH produced 100% and 75% inhibition on TNF-α and IL-8 release. Analysis of DNA binding performed in nuclear extracts of 1h-stimulated keratinocytes showed a clear inhibition of NF-κaB activation by BTH. The three analogues tested also reduced significantly all the parameters studied. None of the tested compounds caused cellular toxicity as determined by the MTT assay. Our results showed the ability of BTH and its derivates to inhibit several key biomarkers up-regulated in inflammatory skin diseases such as psoriasis.
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Paper No. 1267
FOCUSED GROUP: FC15 - ENDOTHELIUM IN HEALTH AND DISEASE
INCREASE IN CIRCULATING ENDOTHELIAL PROGENITOR CELLS BY STATINS IN ACUTE CORONARY SYNDROMES

Natália António(1,2), R Fernandes(1), A Paiva(3), L Gonçalves(2), M Freitas(2), LA Providência(2), CF Ribeiro(1)
(1) Coimbra University, Faculty of Medicine, Institute of Pharmacology and Experimental Therapeutics, Portugal
(2) Coimbra University Hospital and Medical School, Department of Cardiology, Coimbra, Portugal
(3) Flow Citometry Laboratory, Histocompatibility Center of Coimbra, Portugal

Endothelial progenitor stem cells (EPCs) are a type of stem cells that are mobilized to the peripheral circulation in response to ischemia, playing a crucial role in vascular repair. Statins have been shown to stimulate EPCs. However, its impact in EPCs response to Acute Coronary Syndromes (ACS) has not been studied. Our work aimed to: 1) assess the levels of circulating EPCs in non diabetic and diabetic patients with ACS; 2) investigate whether previous statin treatment improves EPCs response to ACS. Methods: Human EPCs were analysed in peripheral blood of 20 consecutive patients with ACS and 6 healthy control subjects. Circulating EPCs were quantified by flow cytometry (FACSCANTO II, Becton Dickinson) using 5 directly conjugated antibodies against human FITC-conjugated CD34, PE-conjugated KDR, APC-conjugated CD133, APC-Cy7-conjugated CD45 and PE-Cy5-conjugated CXCR4. We determined the number of CD34/KDR and of CD133/KDR double positive cells. We found a positive correlation between admission troponin I levels and CD34+/KDR+ cells (r= 0.507, p=0.022). Interestingly, there were no significant differences in the number of CD34+/KDR+ or CD133+/KDR+ between diabetics and non diabetics. However, fasting glycemia was inversely correlated with the more immature population of EPCs (CD133+/KDR+) (r=-0.998, p=0.037). Previous statin use was associated with significantly higher levels of CD133+/KDR+ in ACS patients (0.00054 +/- 0.00061% vs 0.00016 +/- 0.00020%, p=0.024). In conclusion, these preliminary results suggest that hyperglycemia deregulates EPCs response. Statin treatment seems to enhance the mobilization of the more immature EPCs what reinforces the importance to start statins as soon as possible in ACS patients.

Paper No. 1645
FocusED Group: FC09 - I INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
THE EFFECT OF PENTOXIFYLLINE ON LEUKOCYTE ACCUMULATION AND ANGIOGENESIS IN AIR POUCH MODEL IN RATS

Mostafa Asadpour(1), M Rasouly(3), M Ziaee(2), S Andalib(4), A Abadpour(1), Y Doustar(3), A Garjani(1)
(1) Tabriz University of Medical Sciences Faculty of Pharmacy, Department of Pharmacology, Tabriz, Iran
(2) Islamic Azad University, Pharmacology Department, Science & Research Branch, Tehran, Iran
(3) Islamic Azad University, Pathology Department, Tabriz, Iran
(4) Islamic Azad University, Young Researchers Club of Tabriz, Tabriz, Iran

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is characterized by marked vascular changes, including vasodilatation, increased permeability, which are induced by the actions of various inflammatory mediators. Studies have shown that pentoxifylline, a methylxanthine derivative, exerts inhibitory effect on vascular permeability by TNF-α inhibition. In this study, we investigated the effect of pentoxifylline on inflammation process such as leukocyte infiltration and angiogenesis. Twenty-four male wistar rats (160–190 g) were divided randomly into control group and three groups treated with pentoxifylline. Inflammation was produced by injection of carrageenan into a newly formed air pouch. Treatment groups were orally administrated for five days with 10, 20 and 40mg/kg/day of pentoxifylline, respectively. Oral administration of pentoxifylline in treatment group with in 40 mg/kg suppressed leukocyte infiltration into the air pouch from (11±1.6)x10(7)

cells/air pouch in control to (5.5±1.1)x10(7)cells/air pouch; *P<0.05*. Meanwhile pentoxifylline exerts anti-angiogenesis effect with 10 mg/kg, 43% (*P<0.001*) and 40 mg/kg 15% (*P<0.05*) significantly but with 20 mg/kg induced angiogenesis 6% with no significance. Pentoxifylline inhibits PMN recruitment and may decrease inflammatory reaction in air pouch model, and suppress angiogenesis dose dependent. Pentoxifylline may be an ideal candidate for use as an anti-inflammatory and anti- angiogenesis agent in most inflammatory disease by specific dosage.

Paper No.: 3456
FOCUSED CONFERENCE GROUP: P04 - PHARMACOEPIDEMIOLOGY, CURRENT CONTROVERSIES AND OPPORTUNITIES
DESIGN, SYNTHESIS, PHOTOCHEMICAL PROBE AND PHYSICO CHEMICAL PARAMETERS OF NORFLOXACINE ANALOGUES USING SOLID PHASE METHODOLOGY VIA APPLICATION VILSMEIER REACTION

Dina Bakhotmah(1), R Abde-Alrahman(1), A Alkordi(2), O Meth-Cohen(1), A Alzahabi(2), P Groundwater(2), R Anderson(2), M Makki(1), M Soliman(1)
(1) King Abdulaziz University, Department of Chemistry, Jeddah, Saudi Arabia
(2) the University of Sunderland, UK

In attempts to synthesize norfloxacin analogues, only 4-(4'-benzylpiperazin-1-yl)-3-haloformamide (2) was synthesis not the isomeric fluorine derivative (3). 7-bromo-6-N-benzyl piperazinyl-4-oxoquinoline-3-carboxylic acid (4) was synthesized when using Vilsmeier Approch to quinolone synthesis. Structure of the bromo products (4) have been elucidated from their elemental analysis and spectral measurements. The antimicrobial and photochemical Probe agents for inhibition of Vitiligo in compared with standard antibiotics have been evaluated. Correlation results showed that lipophilicity, molecular mass and electronic factors might influence the activity.

Paper No.: 818
FOCUSED CONFERENCE GROUP: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
VALIDATION OF AN ANALYTICAL METHODOLOGY FOR DETERMINATION OF OXYTETRACYCLINE RESIDUE IN MILK BY HPLC WITH UV DETECTION

Azam Bakhtiarian, R Khosrokhavar, MJ Hosseini, M Ghazi-Khansari
Tehran University of Medical Sciences, Department of Pharmacology, Tehran, Iran

Oxytetracycline (OTC) is used for the prophylaxis and treatment of a great number of diseases since this antibiotic possesses broad-spectrum activity against many pathogenic organisms. The use of OTC has become a serious problem because of the possible existence of its residues in milk, which can be directly toxic or cause allergic reactions in some hypersensitive individuals. Even low-level doses of antibiotic in milk consumed for long periods can lead to problems regarding the spread of drug-resistant microorganisms. The purpose of the present study was to investigate residual OTC in consuming milk in Tehran using high-performance liquid chromatography (HPLC) with UV detector. OTC residues in extracts obtained from a preliminary cleanup procedure and recoveries from spiked OTC in desire concentrations were between 80% and 97% with appropriate coefficients of variation. The limit of detection (LOD) and limit of determination (LOQ) were 50 and 68.5 ng/mL, respectively. This result shows that this method would be useful for routine monitoring of oxytetracycline residues in bovine dairy milk.

Paper No. 2662
FOCUS GROUP: FC04 - PHARMACOEPIDEMIOLOGY, CURRENT CONTROVERSIES AND OPPORTUNITIES

Does multiple drug exponsure increases the severity of adverse drug reactions?

Francisco Batel-Marques(1,2), N Craveiro(1,2), A Macedo(1,3), P Dinis(1,4), CF Ribeiro(1,4), M Caramona(1,2), T Macedo(1,4)
(1) UFC/AIBILI, Coimbra, Portugal
(2) University of Coimbram School of Pharmacy, Coimbra, Portugal
(3) University of Beira Interior, Faculty of Health Sciences, Portugal
(4) University of Coimbra, School of Medicine, Coimbra, Portugal

Introduction: The extent to which simultaneous exposure to different drugs can worsen the severity of adverse drug events has been subject of research. This issue is of importance, both at clinical and pharmacoepidemiologic levels, due to its implications in both medicines benefit/risk ratio evaluations and patient safety. Aim: To validate the hypothesis that the severity of reported adverse drug reactions (ADR) is increased with multiple and simultaneous drug exposure. Methods: A pilot study was carried out using reported ADR to the regional pharmacovigilance centre of central Portugal. ADR were classified according to their severity (severe/non severe) and patient simultaneous exposure to two or more drugs. Data were collected from January 2001 to October 2009. Results: Of the 1448 reported and validated ADR (69,5% females, median of age 50.6 - 21.9, range 1-99 years) 677 (46.8%) were classified as severe. Of these, 453 cases were exposed simultaneously to more than one drug. For the non-severe ADR (771, 53.2%), concurrent medication was found in 508 cases. The calculated Odds Ratio for severity/non severity was 1.05 (IC 95% 0.84 - 1.3, NS). Conclusions: In the present study increased severity of ADR in the presence of simultaneous exposure to different drugs was not found. Such findings have to be prospectively confirmed with increased simple sizes and adjustments for other variables such as age, gender, number of concomitant drugs and length of simultaneous exposures.

Paper No.: 2823
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
BPC 157 REDUCED POSTOPERATIVE ADHESION FORMATION IN RATS

Lidija Berkopic, M Filipovic, M Sever, R Klicek, S Ilic, I Barisic, B Radic, V Bilic, L Brcic, D Kolenc, S Seiwerth, P Sikiric
University of Zagreb Faculty of Medicine, Department of Pharmacology, Zagreb, Croatia

Peritoneal adhesions even after minor peritoneum injury are common problems after endoscopy or major surgical procedures. We propose for counteraction an orally active, stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419) as anti-ulcer peptide efficient in trials for inflammatory bowel disease (PL-10, PLD-116, PL 14736, Pliva, Croatia) and various wound treatment, no toxicity reported) that diminished adhesions after various intestinal anastomosis in rat (Vuksic T et al, Surg Today 2007;37(9):768-77). Materials and methods: Excision of parietal peritoneum (1x2 cm, 2 cm right from median 3 cm-laparatomy) with underlying superficial layer of muscle tissue was performed in rats. BPC 157 (10 µg, 10 ng/kg i.p., 1 ml/rat)(or an equivolume of 0.9% NaCl) was applied either (i) immediately after surgery; or (ii) once daily throughout 8 days, last application 24 h before assessment on the 9th post-operative day (adhesions scored macro/microscopically (Mazuji's classification)). Results: Rats after surgery exhibited consistent adhesion formation the 9th post-operative day. Macroscopically and microscopically, adhesions were firmly formed between peritoneal lesion and intra-abdominal organs, intestine and uterus, mostly formed of collagen I with a large number of inflammatory cells. Generally, BPC 157 reduced adhesion presentation, either 10 µg or 10 ng/kg, given either immediately after surgery or once daily throughout 8 days, when assessed either macroscopically or microscopically. Adhesions were present only filmy, easy in lysis, and they showed mostly collagen III presentation and few inflammatory cells. Conclusion: In addition to IBD therapy, antagonization of adhesion presentation may provide an additional possibility for this pentadecapeptide therapeutic application.

Paper No.: 3499
FOCUSED CONFERENCE GROUP: P02 - TRANSMEMBRANE TRANSPORT: PERSPECTIVES FOR DISEASE AND DRUG DISCOVERY

DIFFERENTIAL REGULATION OF PURINE TRANSPORT IN PRIMARY HUMAN CARDIAC MICROVASCULAR ENDOTHELIAL CELLS

Derek Bone, J Hammond
University of Western Ontario, Department of Physiology and Pharmacology, London, Ontario, Canada

Nucleoside and nucleobase flux across microvascular endothelial cell (MVEC) membranes is mediated by the equilibrative nucleoside transporter 1 (ENT1) and the equilibrative nucleobase transporter 1 (ENTB1), respectively. Changes in the activity of these transporters may modify vascular function via altered adenosine bioavailability and purine metabolism. Human cardiac MVEC were cultured in serum free medium for 24 hr and then treated with various modifiers at 37°C. ENT1 and ENBT1 function was assessed by the rate of uptake of 10 µM [3H]2-chloroadenosine and 50 µM [3H]hypoxanthine, respectively. Treatment with 100 µM forskolin for 15 min, to directly activate adenylate cyclase, reduced ENT1 function by 40 ± 11% but had no effect on ENBT1 .Activation of PKC with 100 nM PMA for 15 min also decreased ENT1 function by 35 ± 7%. This effect of PMA was blocked by the broad spectrum PKC inhibitor Go6983 but not the alpha/beta isoform selective Go6976, suggesting that PKC-delta mediated this effect. In contrast, ENBT1 was not affected by PMA. Treatment with 25ng/ml VEGF for 24 hr also resulted in a 34 ± 4% decrease in the number of ENT1/cell, whereas ENBT1 function increased by 41 ± 2%. Thus, ENT1 and ENBT1 are differentially regulated by PKC and/or PKA in human cardiac MVECs. This novel finding could allow for selective manipulation of these transporters during periods of altered purine levels such as ischemia/reperfusion.

Paper No.: 3476
FOCUSED CONFERENCE GROUP: P11 - G PROTEIN-COUPLED 7TM RECEPTORS: FROM MOLECULAR TO PHYSIOLOGICAL FUNCTION

STIMULATION OF β-ADRENERGIC RECEPTORS INHIBITS THE PROLIFERATION AND INCREASES THE ADHESION OF MCF-10A HUMAN BREAST CELLS: EVIDENCE FOR CAMP-DEPENDANT EFFECTS VIA DISTINCT SIGNALING PATHWAYS

Ariana Bruzzone(1), H Paris(2), JM Senard(2), IA Luthy(1)
(1) Instituto de Biología y Medicina Experimental - CONICET, Buenos Aires, Argentina
(2) INSERM U858, Team 8, I2MR, CHU-Rangueil, Toulouse, France

The β-adrenergic receptors are known to inhibit the growth of human breast cancer cells but their effects have never been examined in non-tumoral cells. The objective of the present work was to study the effect of β-agonist treatment on the proliferation and adhesion of non-tumoral human breast cells, MCF-10A, and to investigate the signaling pathways involved in these actions. The treatment of MCF-10A with 0.2 ìM isoproterenol caused a significant diminution of cell proliferation (37% reduction after 48h), that correlated with a large decrease of the level of Erk1/2 phosphorylation. This effect was blocked by H-89 (PKA inhibitor) and mimicked by forskolin (direct activator of adenylyl cyclase) or 8-Br-cAMP (activator of PKA and Epac) or 6-Bnz-cAMP (specific activator of PKA) but not by 8-CPT-2'-O-Me-cAMP (specific activator of Epac), indicating involvement of PKA. The exposure of MCF-10A cells to isoproterenol for 4h caused a significant enhancement of cell adhesion (74% vs 12% adherent cells after trypsin-EDTA treatment). Isoproterenol effect was not blunted by H-89. Cells treated with 8-CPT-2'-O-Me-cAMP, but not 6-Bnz-cAMP, exhibited enhanced adhesion, demonstrating involvement of Epac. As assessed by immuno-cytology the effect of isoproterenol was related to a redistribution of â1-integrin and with reinforced cell-cell contacts. The present study demonstrates that â-adrenergic stimulation of MCF-10A cells attenuates cell proliferation and enhances cell adhesion. Both effects are cAMP-dependant, however whereas growth inhibition is mediated via the PKA-Erk1/2 pathway, enhancement of adhesion is due to Epac activation and subsequent redistribution of β1-integrin.

Paper No.: 1374
FOCUSED CONFERENCE GROUP: P17 - NEW APPROACHES AND TARGETS IN PSYCHIATRY
EFFECTS OF RISPERIDONE ON LEARNING AND MEMORY IN THE MORRIS WATER MAZE AND PASSIVE AVOIDANCE TESTS IN RATS

Ýpek K Celikyurt, O Mutlu, G Ulak, FY Akar, F Erden
Kocaeli University Medical Faculty, Department of Pharmacology, Kocaeli, Turkey

Schizophrenic patients suffer from cognitive deficits that are related with functional outcome. Typical antipsychotics lack the ability to improve cognitive dysfunctions in schizophrenia but the effects of atypical antipsychotics on cognitive functions of schizophrenic patients are inconsistent. Ýmprovement, no effect, as well as impairment have been reported in studies. We investigated the effects of an atypical antipsychotic risperidone on learning and memory both in naive and MK-801 (0.15 mg/kg, i.p.) injected rats in the Morris water maze (MWM) and passive avoidance (PA) tests. Two-way ANOVA post hoc Dunnett-t test was used for statistical analysis. In the MWM test, risperidone (0.06, 0.125, 0.25 mg/kg i.p.) didn't alter the distance to platform compared to control group and had no effect on the velocity of rats. Risperidone (0.125 mg/kg) significantly reversed MK-801 induced impairment of learning and memory in MWM test. In the PA test, risperidone alone (0.125, 0.25 mg/kg) had no effect on second day latency (TL2) but it reversed MK-801 induced impairment of learning although this finding failed to reach a statisticly significant level. These results suggest that risperidone might be effective in treating cognitive dysfunctions associated with schizophrenia.

Paper No.: 1850
Focus Group: P19 - GENERAL SESSION
COMMITMENT AND FOLLOW-UP ACHIEVEMENT: CORNER POINTS IN CLINICAL TRIALPERFORMANCE

Antonio Cervera, C Rosso, P Máiquez, D González, JR Castillo
Virgen del Rocío Hospital, Department of Clinical Pharmacology, Seville, Spain

Introduction: inclusion of the patients sample and complete performance of the procedures and visits programmed in a clinical trial should be considered as fundamental goals. In order to obtain results with the power statistically calculated, the number of patient needed is determined in advance. Often, investigators agree in participating and commit the inclusion of a number of patients with the specific inclusion and exclusion criteria. The acceptance in the performance of the trial implies the fully completeness of the procedures and visits scheduled in the protocol. Materials: clinical trials performed through January 2007 to December 2009 in a clinical trial phase I-II unit were analysed. Sample pre-committed by the investigator was compared with the real number of patients included. Follow-up fulfilment, number of visits and procedures specifically asked for the trial were described. Reasons for withdrawal were classified as screening failure, lost of follow-up, adverse event, illness progression, subject choice, investigator decision, death and others. Results: 26 clinical trials were analysed, 14 closed and 12 with recruitment not yet completed at the time of the analysis. Rate of commitment, completeness of follow-up and reasons for withdrawal were described. Conclusion: investigators should be involved in the successful performance of the trial they have agreed to accomplish with. These results will help us to understand reasons of failure and planning a correction programme if needed. We should make a reflection exercise about the role of pharmacologists in improving trials performance.

Paper No.: 1588
Focus Group: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
THE INVOLVEMENT OF CLEC-2 IN LPS-STIMULATED MACROPHAGES

C-H Chang, Tur-Fu Huang
National Taiwan University College of Medicine, Institute of Pharmacology, Taipei, Taiwan

C-type lectin-like receptor 2 (CLEC-2) is a type II transmembrane protein and belongs to the member of the "dectin-1 cluster¨ of C-type lectin-like receptors. Recently, CLEC-2 was found to be expressed on platelets and myeloid cells (i.e. neutrophils, monocytes, and dendritic cells). Monocytes recruitment into subendothelium and differentiation into macrophages plays an important role in inflammatory process. Macrophages are major immune cells and play an important role in modulating homeostasis and immune defense mechanism. In this study, RNA interference (RNAi) technique was used: small interfering RNA (siRNA) of CLEC-2 and short hairpin RNA (shRNA) of TLR-4 were transfected in RAW264.7 macrophage cells. Our data showed that shRNA of TLR-4 significantly reduced TNF-α release in LPS-stimulated macrophages, whereas siRNA of CLEC-2 did not affect LPS-induced TNF-α release in macrophages. Transmigration of macrophages across endothelial cells monolayers and migration of macrophages were quantified. Knockdown of TLR-4 or CLEC-2 in macrophages inhibited macrophages

transmigration across the endothelial cells monolayer barrier induced by LPS. The migration of macrophages induced by LPS was also inhibited by TLR-4 shRNA or CLEC-2 siRNA. Moreover, TLR-4 shRNA but not CLEC-2 siRNA inhibited the phosphorylation of ERK induced by LPS in macrophages. However, the phosphorylation of ERK induced by aggretin (a ligand of CLEC-2) was inhibited by CLEC-2 siRNA. These results indicated that CLEC-2 was involved in LPS-induced migration or transmigration, but not in cytokine release. CLEC-2 may play a role in LPS-stimulated macrophages different from TLR-4.

Paper No.: 3282
FOCUSED CONFERENCE GROUP: P04 - PHARMACOEPIDEMIOLOGY, CURRENT CONTROVERSIES AND OPPORTUNITIES
THE MOROCCAN PEOPLE'S PERCEPTION OF MEDICINE

Maryam Daoudi(1), S Ahid(1,2), H Filali(1), A Tazi(1), R Abouqal(2), Y Cherrah(3), M Hassar(3), F Hakkou(1)
(1) CHU Ibnou Rochd, Service of Clinical Pharmacology and Biochemistry, Casablanca, Morocco
(2) Laboratory of Biostatistics and Clinical Search and Epidemiology, Faculty of Medicine and Pharmacy, Rabat, Morocco
(3) Laboratory of Pharmacology - Toxicology, Faculty of Medicine and Pharmacy, Rabat, Morocco

The Moroccan people's Perception of medicine reflects their degree of knowledge and information about the drugs. The objective of this study is to give a view of the Moroccan people's perception of the medical products and to analyze the fluctuation in their degree of knowledge and behavior.A prospective study was conducted between December, 2008 and May, 2009 among Casablanca population. The study used the quota method and a questionnaire. 1000 interviews were conducted. The average age was of 36.6 years and sex-ratio (M/F) was 0.98. The illiterate interviewees represent 39.9 %. The evaluation of the knowledge of the medicine showed that 67.6 % considered that a good prescription is the one containing one or two medicines. 79.5 % tend to think that the expensive medicine is always the most effective. The evaluation of the behavior towards the medicine showed that self-medication is occasionally resorted to in 75.6 % of cases regardless of their having or not having health insurance (p=0.084). In addition, those not having health insurance buy less of the medicine recommended by their pharmacist ($p < 0.001$). Oral medicine was the most preferred (54.1 %) mainly effervescent tablets by subject aged over 60 ($p < 0.001$). 58 % of the subjects, mainly those with high education, reported to have read the medicines leaflet ($p < 0.001$). The study of the perception of the medicine by the population will allow the professionals of health and the authorities concerned to supply the citizens with effective information about the optimal and rational use of medical drugs.

Paper No.: 1096
FOCUSED CONFERENCE GROUP: P16 - NATURAL PRODUCTS: PAST AND FUTURE?
PHARMACOLOGICAL EVALUATION OF THE ANTISPASMODIC ACTIVITY OF CRUDE EXTRACTS AND PURIFIED FRACTION FROM MEDITERRANEAN MARINE ALGAE

Mehdi Dridi(1, 2), E Majoul(1, 2), A Yacoub(2), O Bouzgarrou(1), R Ben Said(3), A Bouraoui(1)
(1) Military Hospital of Tunis, Department of Pharmacy, Tunis, Tunisia
(2) Monastir Faculty of Pharmacy, Ursam Unit, Department of Pharmacology, Tunisia
(3) INSTM- Salambo, Tunis, Tunisia

The role of marine organisms, essentially algae, in drugs discovery has been greatly enhanced the last few years. As part of our search for a new potential drug, some extracts and purified fractions of marine algae, collected from Tunisian coasts were screened for their antispasmodic activity, *in vitro*, on rat isolated duodenum, in the presence of spasmogenic agents Acetylcholine (Ach) or Barium chloride (BaCl2) and using a Physiograph Narco Biosystems MK III. Of these, aqueous extracts and purified fractions from red and brown algae, exhibited, in a concentration and reversible manner, a significant inhibitory activity against the contractile response induced by Ach and by BaCl2. The percentages of inhibition of aqueous extracts (1mg/ml) of marine algae against the sub maximal contractile response induced by Bacl2 and Ach were respectively : 91.46% and 36% for the extract of the red algae of the genus Hypnea; 72% and 40% for the extract of the brown algae of the genus Dictyopteris. In addition, the purified fraction F3 from the red algae of the genus Hypnea at concentration of 250 µg/ml exhibited an important antispasmodic activity and reduced significantly the sub-maximal contraction induced by BaCl2. The percentage of inhibition was of 87%. The antispasmodic activity of these aqueous extracts and of the fraction F3 were evaluated in comparison to the antispasmodic activity of the reference drug, alverine, and the HPLC profile of the fraction F3 was determined. In order to isolate and characterize the antispasmodic natural product, purification and chemistry studies are under investigation.

Paper No.: 3486
FOCUSED CONFERENCE GROUP: FC18 - NUCLEAR RECEPTOR TARGETS FOR TREATMENT OF DISEASES
ANTIHYPERTENSIVE EFFECTS OF THE PPAR-β AGONIST GW0742 IN SPONTANEOUSLY HYPERTENSIVE RATS

Juan Duarte, R Jimenez, MJ Zarzuelo, P Galindo, M Sanchez, A Nieto, M Romero, AM Quintela-Pousa, M Gomez-Guzmin, E Bailon, J Galvez, F Perez-Vizcano
University of Granada, Department of Pharmacology, Granada, Spain

Activation of nuclear hormone receptor peroxisome proliferator-activated receptor β/δ (PPAR-β) has been shown to improve insulin resistance, adiposity, and plasma HDL levels. In the present study, we hypothesized that chronic treatment with GW0742 might exert antihypertensive effect in spontaneously hypertensive (SHR). The rats were divided into 4 groups: Wistar Kyoto rats (WKY)-control, WKY-treated (GW0742, 5 mg kg-1 day-1), SHR-control, and SHR-treated. Rats were daily administered by gavage for 5 weeks. GW0742 induced a significant reduction in systolic arterial blood pressure and heart rate in SHR but not in WKY rats. SHR rats showed mesenteric vascular structural alterations characterized by a significant increase in media thickness, media cross-sectional area and media-lumen ratio as compared to WKY. In SHR, GW0742 reduced significantly these parameters, being without effects in WKY. This PPAR-β agonist enhanced the endothelium-dependent relaxation to acetylcholine in SHR in isolated aortae. This effect was accompanied with a significant increase in endothelial nitric oxide synthase (eNOS) activity, related to increased eNOS protein abundance and reduced the expression of caveolin-1. Moreover, GW0742 also reduced the increase in NADPH oxidase activity found in SHR control, associated with reduced p22phox and p47phox protein. The increased expression of proinflammatory and proaterogenic genes, IL-1β, IL-6 or ICAM-1 found in SHR, was reduced by GW0742. PPAR-β activation increases the regulators of G protein-coupled signaling (RGS) proteins RGS4 and RGS5, AKT phosphorylation and inhibits ERK1/2 activation in SHR. These studies highlight actions of PPAR-β activation to inhibit functional and structural vascular changes in SHR, which is antihypertensive.

Paper No.: 3116
FOCUSED CONFERENCE GROUP: P16 - NATURAL PRODUCTS: PAST AND FUTURE?
GASTROPROTECTIVE ACTIVITY OF LEMONGRASS (*CYMBOPOGON CITRATUS*) IN ETHANOL-INDUCED GASTRIC ULCERS IN RATS

Isabel Vitória Figueiredo(1), J Sagradas(2), K Cavalcanti(3), R Garcia(2), M Veloso(2), A Rama(2), M Castel-Branco(1), A Cabrita(3), T Batista(4), M Caramona(1)
(1) Center for Pharmaceutical Studies, Coimbra University, Coimbra, Portugal
(2) Laboratory of Pharmacology, Faculty of Pharmacy, Coimbra University, Coimbra, Portugal
(3) Experimental Pathology Institute, Faculty of Medicine, Coimbra University, Coimbra, Portugal
(4) Laboratory of Pharmacognosy, Faculty of Pharmacy, Coimbra University, Coimbra, Portugal

Treatment of gastric ulcers with medicinal plants is quite common in traditional medicine worldwide. Lemongrass (*Cymbopogon citratus*) has been used in folk medicine to treat gastric disturbances. *C. citratus* leaf extracts have shown anti-inflammatory and antioxidant properties, and revealed the presence of flavonoids, tannins and phenolic acids. These properties may be indicative of the potential benefit in the gastrointestinal tract, mainly on ulcerative and erosive lesions generated by free radicals. The aim of this study was to assess the potential gastroprotective activity of *C. citratus* leaf extract in an animal model of gastric lesions. The study was performed on adult male Wistar rats (234.0 ± 22.7 g), fasted for 24 hours but with free access to water. *C. citratus* extract was given orally before (prevention) or after (treatment) intragastric administration of absolute ethanol. Effect of dose (0.2 and 0.4 mg leaf/g) and of contact time with gastric mucosa (1 and 2 hours) were also assessed. Animals were sacrificed, stomach was removed and the lesions were assessed macroscopically and by histopathology. *C. citratus* extract, given orally before or after ethanol, significantly reduced ethanol-induced gastric injury compared with control group. Although not statistically significant, results also suggested that the extract is more effective when contact time with gastric mucosa increases. The effect does not appear to be dose-dependent. The results of this screening assay indicate a gastroprotective activity of *C. citratus* extract against ethanol-induced gastric lesions.

Paper No. 3002
FOCUSED CONFERENCE GROUP: FC19 - GENERAL SESSION
EVALUATIONS IN PHYSIOLOGY AND PHARMACOLOGY – 35 YEARS OF EXPERIENCE

Bjarne Fjalland, M Sheykhzade
University of Copenhagen, Faculty of Pharmaceutical Sciences, Copenhagen, Denmark

The purpose of the study was to examine how various factors influence the marks given in Physiology and Pharmacology over time. These factors include: examinations with/without textbooks, change of textbooks, new evaluation methods, integration of Physiology and Pharmacology and the position of the course within the 5-years curriculum. Two courses of the same size (12 ECTS): Organ related Pharmacology and Physiology (incl. design of bioassays) were investigated. The evaluation method was for all 35 years a 4 hrs written examination and comprised a total of 5836 students. Moving the Physiology course from the 2nd to the 5th year of study did not have any significant effect on the marks. Changing textbook from Danish to English and switching between examinations with and without books did not affect the marks. When the same set off questions was reused a significant difference was observed with the lowest result at the repeated exam. Integration of Physiology and Pharmacology (Organ-related Pharmacology) resulted in a significantly lower result. By changing the evaluation method from a 4 hrs exam with all written materials allowed to a split exam with 2 hrs no material allowed + 2 hrs all written materials allowed, a significant difference was observed with the highest result for the split exam. The survey shows that changing the textbooks or the position of the course in the curriculum did not influence the marks. However, the evaluation methods (+/- books), the homogeneity of the students and the integration of Physiology and Pharmacology significantly influenced the marks.

Paper No. 3141
FOCUSED GROUP: FC19 - GENERAL SESSION
PHARMACOLOGICAL CHARACTERIZATION OF METFORMIN-INDUCED INTESTINAL CONTRACTION

Carlos Fontes Ribeiro(1), A Henriques(2), S Silva(3)
(1) AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal
(2) University of Coimbra, Faculty of Science and Technology, Department of Biochemistry, Coimbra, Portugal
(3) University of Coimbra, Faculty of Pharmacy, Centre of Pharmaceutical Studies, Coimbra, Portugal

Metformin is an oral antihyperglycemic agent used in the treatment of Type 2 Diabetes Mellitus. This biguanide reduces hepatic glucose output, increases glucose uptake in muscle and improves the dyslipidemic profile characteristic of diabetic patients. Besides, it is not associated with hypoglycaemia episodes since the drug does not stimulate insulin secretion. However, diarrhea is one of the gastrointestinal side effects commonly reported with metformin treatment. The cause of diarrhea is unknown but nervous sympathetic blockade can be a mechanistic explanation. The aims of this study were to determine the contractile response of the rat ileon to metformin and the effects of metformin on the rat ileon contractile response to electrical field stimulation (EFS). Rat ileon segments were prepared for isometric contractile concentration-response curves (not cumulative). Exogenous acetylcholine (100 μM) was used to directly stimulate the ileon smooth muscle and to compare the results of both set of experiments. Metformin, used in therapeutic concentrations (6-36 μM), caused concentration-dependent contractions (Emax of 11.86 ± 0.97 mN, n=17; pEC50 of 4.94 ± 0.04, n=10) that were not significantly altered by atropine (10 μM) nor by NG-nitro-L-arginine (250 μM), excluding the involvement of cholinergic receptors or the inhibition of nitric oxide production alone. EFS (1-16 Hz, 100 V, 5 msec pulse width) produced a frequency-dependent contractile response; only the EFS-induced contraction at 16 Hz was significantly reduced in the presence of metformin

(36 μM). Other mechanisms must be involved in the metformin-induced intestinal contraction which means that further studies are needed.

Paper No.: 3461
FOCUSED CONFERENCE GROUP: P08 - DEVELOPMENTS IN THE TREATMENT OF SEXUAL DYSFUNCTION AND DISEASES OF THE LOWER URINARY TRACT
CD151 EXPRESSION : A BIOMARKER FOR PROSTATE CANCER PROGRESSION

Albert Frauman(1), B Newell(1), J Ang(1), M Parker(2), E Williams(3), S Detchokul(1)
(1) University of Melbourne, Department of Medicine, Heidelberg, VIC, Australia
(2) St Vincent Institute of Medical Research, Structural Biology Laboratory, Melbourne, VIC, Australia
(3) Monash Institute of Medical Research, Monash University, Clayton, VIC, Australia

We have shown that the tetraspanin CD151 has prognostic value in prostate cancer (PCa); patients whose cancer has low expression of CD151 have better prognosis than those with high levels1. We are now interested in CD151's role in PCa as a motility and metastasis promoter. Human PCa cell lines LNCaP and PC3 were used in cell migration and invasion assays. The motility and invasiveness of wild-type LNCaP (low endogenous level of CD151) vs. CD151 transfected LNCaP cells and PC3 (high endogenous CD151) vs. CD151 knock-down PC3 cells (KD PC3) was analyzed. LNCaPs transfected with CD151 showed increased motility and invasion compared to control LNCaPs ($P<0.05$), while KD PC3 cells demonstrated reduced motility and invasion compared to control PC3s ($P<0.05$). Paired primary and secondary PCa generated using a SCID mouse model bearing implanted human PCa cell lines are being examined for expression of CD151 and vascular cells, by immunohistochemistry. Although the mechanism is unclear, CD151 appears to promote cell motility and imparts a worse prognosis in prostate cancer. These findings suggest that CD151 could be a useful biomarker for the prognostication of PCa.

1 Ang J et al. Cancer Epidemiol Biomarkers & Prevention (2004) 13: 1717-21

Paper No.: 3475
FOCUSED CONFERENCE GROUP: P11 - G PROTEIN-COUPLED 7TM RECEPTORS: FROM MOLECULAR TO PHYSIOLOGICAL FUNCTION
NEW G PROTEIN ACTIVATION BRET-BIOSENSORS MULTIPLEX REVEALED AN UNEXPECTED EFFICACY FOR THE SAR1ILE4ILE8-ANGII (SII) BIASED-AGONIST

Céline Gales(1), A Sauliere(1), H Paris(1), C Denis(1), M Bellot(1), JL Hansen(2), J-M Senard(1)
(1) INSERM U858, Toulouse, France
(2) Laboratory for Molecular Cardiology, Danish National Research Foundation Centre for Cardiac Arrhythmia, Department of Biomedical Sciences, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

Based on the concept of ligand-directed trafficking, one challenging task in pharmacology is now to optimize cellular assays to appreciate real texture of ligands. Thus, based on our previous work on Galphai1 (Galés, 2006), we developed a BRET assay so measuring for the first time direct activation of all G proteins isoforms from major G protein families (Gi, Gs, Gq/11, G12/13) in living cells. We then explored the activity of Sar1Ile4Ile8-AngII (SII) which has been described as a potent β-arrestin/ G protein independent biased agonist. As already known, we found that AngII was able to activate all G proteins isoforms with high efficacy on Gi and Gq families. Surprisingly, SII did also activate all G protein isoforms but to a lesser extent. When tested in G protein non overexpressing system, AngII and SII both promoted Ca^{2+} production thus validating activation of the Gq pathway. Interestingly, only SII directly inhibited cAMP production thus demonstrating direct cyclase inhibition trough Gi proteins. However, AngII also promoted Gi coupling to AT1-R since PTX treatment revealed AngII-stimulating cAMP production. Thus, SII and AngII both appeared to activate Gi proteins but with different signaling outputs. Differences in G protein signaling for the two AT1 agonists were further reinforced by the observation that they both stabilized different conformations of AT1-R/Gq or Gi complexes. Altogether, these results revealed that SII i/ coupled to G proteins, ii/ stabilized a new AT1-R conformation that signals differently from the natural and physiologic agonist AngII.

Paper No.: 3487
FOCUSED CONFERENCE GROUP: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
ORALLY ADMINISTRATION OF MINOCYCLINE FACILITATES THE RECOVERY OF THE COLONIC DAMAGE IN THE CHRONIC PHASE OF THE DSS MODEL OF MOUSE COLITIS

Julio Galvez(1), N Garrido-Mesa(1), D Camuesco(1), B Arribas(1), M Comalada(2), E Bailon(1), M Cueto-Sola(1), A Zarzuelo(1), A Nieto(3), ME Rodriguez-Cabezas(1)
(1) University of Granada, Department of Pharmacology, Granada, Spain
(2) Institut de Recerca Biomedica, Barcelona, Spain
(3) Anatomic Pathology Laboratory, Granada, Spain

The immunomodulatory properties of minocycline have been reported to contribute to its beneficial effects. In this study we assayed the intestinal anti-inflammatory properties of minocycline in the chronic phase of the dextrane sodium sulphate (DSS)-induced model of mouse colitis. Female C57BL/6J mice were assigned to three groups (n=10): Non colitic and DSS control groups, and MNC treated group (50 mg/kg/day). Colitis was induced by adding DSS in the drinking water (3%) for 5 days. The antibiotic was administered from the day of DSS removal until the sacrifice of the mice, 21 days after. An average disease activity index (DAI) was calculated daily and colonic damage was assessed macroscopically, histologically and biochemically (TNFα, IL-1β and IL-6). *In vitro* studies were also performed in Caco-2 and RAW 264.7 cells stimulated with IL-1β or LPS, respectively. IL-8 production and nitrite levels were determined. MNC administration to colitic mice facilitated the recovery from the DSS-induced colonic inflammation. This was evidenced by reduced DAI values in comparison with untreated colitic mice. A decreased colonic weight/length ratio in minocycline-treated mice was also obtained (32.5 ± 1.3 vs 38.5 ± 1.7 mg/cm, $P<0.01$). This improvement was also observed microscopically. The biochemical analysis showed that minocycline significantly reduced the production of TNFα (35.7%), IL-1β (42.1%) and IL-6 (88.4%). The *in vitro* results revealed that minocycline dose-dependently reduced the production of the markers determined, when compared with untreated cells. In conclusion, the immunomodulatory properties exhibited by minocycline contribute to the amelioration of the intestinal damage induced by DSS in mice.

Paper No.: 3464
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
CANCER THERAPY DRUG DOXORUBICIN, EXACERBATES MYOCARDIAL ISCHAEMIA REPERFUSION INJURY

Ahmad Mayel Gharanei, A Nagra, A Hussain, H Maddock
Coventry University, Biomolecular Sciences Applied Research Group, Coventry, UK

Drug induced cardiotoxicity is a major concern to the pharmacological industry and it is one of the main reasons for non-approval, re-labelling, warnings and withdrawal of pharmaceutical compounds from the market. Doxorubicin is an anthracycline antibiotic used in cancer therapy. Although issues relating to Doxorubicin and cardiac safety have been well established in normal conditions, the effects of this drug on the myocardium during ischaemia-reperfusion have not been investigated in detail to date. Studies were undertaken in Langendorff hearts and adult / neonatal ventricular myocytes. Isolated hearts were subjected to 35 min regional ischemia and 120 min reperfusion. Hearts underwent triphenyl tetrazolium staining for infarct size assessment. Treatment groups (n=7-10) were perfused in the presence or absence of Doxorubicin. Following isolation, neonatal or adult cardiomyocytes were subjected to simulated ischaemia-reoxygenation and Doxorubicin was administered at reoxygenation. Cellular injury was subsequently determined by measurement of live/death ratio and apoptosis using flow cytometry. Administration of Doxorubicin in normoxic conditions or during ischaemia-reperfusion significantly increased infarct size to risk ratio (%) compared to respective non-treated controls (30 ± 5% and 81 ± 6% Doxorubicin vs. 10 ± 2% and 65 ± 3% non-treated control, respectively, $P<0.01$). Doxorubicin also significantly increased apoptosis and decreased cell viability after reoxygenation compared to control. This is the first study to show that the anticancer drug Doxorubicin exacerbates myocardial ischaemia reperfusion injury. Further studies are required to determine the cellular mechanism via which Doxorubicin mediates increased myocardial injury in conditions of ischaemia-reperfusion.

Paper No.: 811
FOCUSED CONFERENCE GROUP: P01 - CLINICAL PHARMACOLOGY IN THE EMERGING COUNTRIES
3D INTERACTIVE ENCYCLOPEDIA FOR THE TEACHING OF PHARMACOLOGY TO MEDICAL STUDENTS

Diego Ospina Gonzales, E Obando, R Escobar, J Estupiñan, T Escobar, MP Usta, LF Cifuentes
Andes University, Faculty of Medicine, Bogota, Colombia

Introduction: the growing number of available medications, the ongoing advances in knowledge around pharmacodynamics, pharmacokinetics, and interactions in the molecular and genomic field make pharmacology a science of continuous growth. It is because of this, that its teachings represent a challenge for both students and teachers, who are always in search for motivation and long lasting learning. Objective: to develop the first 3D interactive encyclopedia (in Spanish and English) for the different therapeutic types of medications, where principles of pharmacodynamics, pharmacokinetics, efficacy, security, pharmacoeconomics, drug interactions and posology are found. Materials: six senior medical students from Universidad de los Andes (Bogota, Colombia) who will develop the first pilot with the therapeutic type anticoagulants, under the coordination of the pharmacology professor. The following steps were included: 1) systematic information research. 2). Development of a monograph and visual material. 3). Arrangements with a 3D animation vendor. Results: during August and November of 2009 the monograph and visual material has been done for six different anticoagulants. In the mean time work is being done with the 3D animation vendor in order to have the first version of the interactive anticoagulant encyclopedia in April 2010. Conclusions: the education in pharmacology has its limitations because of the lack of available teaching material that facilitate understanding. The use of technological tools and the condensation of information in an interactive way will allow students a better understanding in pharmacology as well as educational tools for professors.

Paper No.: 3434
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
PHAMACOGENETICS OF EARLY TOXICITY, PLASMA TROUGH CONCENTRATION AND TREATMENT OUTCOME WITH NEVIRAPINE CONTAINING REGIMEN

C Gozalo(1), L Gérard(2), P Loiseau(3), L Morand-Joubert(4), G Peytavin(5), J-M Molina(6), P Dellamonica(7), L Becquemont(0), J-P Aboulker(2), O Launay(9), Céline Verstuyft(1,8), for the ANRS 081 Study Group
(1) Service de Génétique Moléculaire, Pharmacogénétique et Hormonologie, Assistance Publique-Hôpitaux de Paris, Hôpital Bicêtre, Le Kremlin Bicêtre, France
(2) Inserm SC10, Villejuif, France
(3) Laboratoire d'Immunologie, Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Louis, Paris, France
(4) Laboratoire de virologie, Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Antoine, Paris, France
(5) Service de pharmacologie, Assistance Publique-Hôpitaux de Paris, Hôpital Bichat-Claude Bernard, Paris, France
(6) Service des maladies infectieuses, Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Louis, Paris, France
(7) Service des maladies infectieuses, CHU Nice, Nice, France
(8) Département de Pharmacologie, Univ Paris-Sud, Le Kremlin Bicêtre, France
(9) Université Paris Descartes, EA3620 ; Assistance Publique Hôpitaux de Paris, Groupe Hospitalier Cochin Broca Hôtel Dieu, CIC de vaccinologie Cochin Pasteur, Paris, France

Background: Use of nevirapine (NVP) is hampered by early toxicity often leading to treatment discontinuation. Previous studies led to controversial results concerning associations between single nucleotide polymorphisms (SNPs) and toxicity, drug response, or pharmacokinetic parameters after NVP administration because patients (pts), endpoints, genes and SNPs varied between studies. We investigated in a single homogeneous population relationships between SNPs involved in NVP metabolism (CYP2B6 516G>T, 785A>G and 1459C>T; CYP3A5 6986A>G (*3)), transport (ABCB1 2677G>T/A, and 3435C>T), antigen recognition (HLA-DRB1*0101), and the early toxicity, plasma trough concentration (Cmin) and response to NVP. Methods: Associations were retrospectively investigated among the 72 pts from the ANRS081 trial (n=145 naive pts with HIV-RNA>5000c/ml, CD4<100x106/L, and ALT<2 ULN) randomized in the NVP-d4T-IDV arm who gave their informed consent. Early toxicity to NVP was defined as elevation of ALT>3 ULN, associated or not with rash, within the first 8 weeks of treatment. Cmin was evaluated at week 8, and immuno-virological response at week 24. SNP analysis was realized considering the presence/absence of the variant allele using non parametric tests. Genotyping was performed using the Taqman allelic discrimination assay. HLA DRB1 was determined using a PCR-SSO. Results: Among the 72 pts, 12 presented early toxicity, after a median of 26 days after initiation of NVP. No difference in the

frequency of toxicity could be found between carriers of the CYP3A5 wt allele, nor between carriers of the allelic variants CYP2B6 516T or 785G, and non carriers. No more association was evidenced with the presence of a variant allele of ABCB1. A previous reported association with HLA-DRB1*0101 was not confirmed here. None of the studied SNPs was significantly associated with NVP Cmin, even though there was a trend for increased Cmin in pts carrying the CYP2B6 516 T variant compared to wild-type (wt) pts (P=.17), and in CYP2B6 1459 wt pts compared with carriers of the variant allele (P=.14). No association between studied SNPs and response to therapy was identified, even though a non-significant trend for a higher CD4 increase was observed in carriers of the HLA-DRB1*0101 (P=.09). Conclusion: Relationships between SNPs in CYP2B6, CYP3A5, ABCB1, HLA-DR, and early toxicity to NVP, Cmin, and treatment outcome were not consistently identified in this population.

Paper No.: 681
Focus Group: P16 - NATURAL PRODUCTS: PAST AND FUTURE?
EVALUATION OF THE EFFICACY AND PHARMACODYNAMICS OF UNIM-352, A POLYHERBAL DRUG, IN BRONCHIAL ASTHMA : A REVERSE PHARMACOLOGY APPROACH

Kavita Gulati(1), A Ray(1), MK Siddiqui(2), VK Vijayan(1)
(1) Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India
(2) Central Council for Research in Unani Medicine, New Delhi, India

Integration between traditional and modern systems of medicine has significantly facilitated the drug development process and herbal drugs have emerged as alternative forms of therapy in a variety of pathophysiological states. Further, the concept of "reverse pharmacology" has contributed to the validation of evidence based medicine in the area of phytopharmaceutcals. To highlight this, clinical and preclinical studies were conducted to substantiate the therapeutic efficacy and pharmacodynamics of UNIM-352, a polyherbal Unani formulation, for bronchial asthma. In the clinical study, the efficacy of UNIM-352 was investigated using pulmonary function test parameters, and compared with that of placebo in asthma patients. UNIM-352 significantly enhanced the therapeutic effect of standard anti-asthma treatment as assessed by FEV1, FEV1/FVC ratio, frequency of emergency bronchodilator use and symptomatology, as compared to the placebo group - indicating its efficacy as an adjunct therapy. In the experimental study, immunized rats were treated with UNIM-352 and placebo, and anti-inflammatory and oxidative stress markers were measured using modern laboratory techniques. The polyherbal agent reduced TNF-α, IL-1β and IL-4 levels in blood and BAL fluid, of both normal and stressed rats – an effect that was not seen with the placebo Further, UNIM-352 significantly reduced blood MDA levels and elevated GSH and SOD levels as compared to placebo treated groups. The study suggests that UNIM-352 could be a potential adjunct for treatment of bronchial asthma and emphasizes the complimentary roles of clinical and pre-clinical data in drug development from traditional medicine.

Paper No.: 1386
Focus Group: PW02 - SYMPOSIUM ON ADVANCES IN GI PHARMACOLOGY
AN EVALUATION OF NORMAL SALINE AND RIFAMYCIN AT TWO DOSES ON INTRA-ABDOMINAL ADHESION FORMATION

Ahmed Hakim(1), M Jallouli(2), H Ghozzi(1), Z Sahnoun(1), H Affes(1), S Hammemi(1), K Zeghal(1), R Mhiri(2)
(1) Sfax University Faculty of Medicine, Department of Pharmacology, Sfax, Tunisia
(2) Hédi Chaker Hospital, Department of Paediatric Surgery, Sfax, Tunisia

Introduction: Abdominal infections are associated with intraperitoneal adhesions (IPA), which are the most common cause of intestinal obstruction and important cause of chronic abdominal and pelvic pain. In this studies we hypotheise that peritoneal lavage with rifamycin reduces the number of IPA. Methods: Experimental intra abdominal infection was induced in Wistar rats. After 24 h, the animals underwent relaparotomy and a peritoneal fluid sample was obtained. Animals were randomly assigned to three groups for abdominal cavity lavage: S group (0.9% sodium chloride solution); R25group (rifamycin 25 mg/kg); and R12.5 group (rifamycin 12.5 mg/kg). All animals that died had a necropsy. Surviving rats were sacrificed at the 7th day and underwent a necropsy. At necropsy, IPA were noted and a peritoneal fluid sample was obtained for bacterial analysis. Results: Adhesion formation was significantly reduced in the R25 group and R12.5 group compared with the S group ($P =<0.01$and $P< 0.01$ respectively). There was a greater reduction in neutrophils counts in peritoneal fluid in the R25 and R12.5 group compared to S group ($p=0.037$and $p=0.026$). There was a greater reduction in bacterial counts in peritoneal fluid in the R25 group compared with the S group ($P = 0.003$) but there was no significant difference in the reduction of bacterial count between R25 group and R12.5 group and

between R12.5 group and S group. Conclusion: The present study shows that rifamycin lowers the degree of IPA formation. Neutrophils may have a role to play in modulating IPA formation.

Paper No.: 3496
FOCUSED CONFERENCE GROUP: P04 - PHARMACOEPIDEMIOLOGY, CURRENT CONTROVERSIES AND OPPORTUNITIES RESTRICTING REIMBURSEMENT DECISION TO SPECIFIC DOSES OF STATINS – CONSEQUENCES FOR PATIENTS ON TREATMENT WITH ATORVASTATIN 10 MG

Mikael Hoffmann(1), A Leimanis(2)
(1) The NEPI foundation / Linköping University, Department of Medical and Health Services, Linköping, Sweden
(2) National Board of Health and Welfare, Sweden

The Dental and Pharmaceutical Benefits Agency, TLV, is a government agency in Sweden. One of the consequences of TLV's review of statins in 2009 was restrictions on the coverage for atorvastatin (Lipitor). Previously atorvatstatin could be prescribed without restrictions but since June 2009 it is reimbursed only if generic simvastatin has been tried and the patient has not reached the treatment objectives. In addition atorvastatin 10 mg is no longer reimbursed at all. Method: Data from the Swedish Prescribed Drug Register at the National Board of Health and Welfare. Results: During the first six months of 2009 there were 27,445 individuals with atorvastatin 10 mg dispensed at least twice (38,309 in the corresponding period 2008). Of these 20 % had no statin dispensed during the following six months (9 % in 2008). The first dispensation of a statin during July-December was simvastatin for 37 % of the individuals (1 % in 2008). The most common doses of simvastatin were 20 mg (25 %) or 10 mg (7 %). Almost a fifth or 19 % of the individuals were dispensed atorvastatin 10 mg even though it was no longer covered by the pharmaceutical benefit scheme. An additional 21 % received atorvastatin 20 mg. Discussion: There are several possible explanations why only 5 % of the individuals who were prevalent users of atorvastatin 10 mg were switched to the equi-potent dose of simvastatin 40 mg during the six month studied, among other a possible dose-titration strategy for simvastatin.

Paper No.: 3500
FOCUSED CONFERENCE GROUP: P04 - PHARMACOEPIDEMIOLOGY, CURRENT CONTROVERSIES AND OPPORTUNITIES DRUG PRESCRIBING IN SWEDEN 2001-2008 AND CORRELATION TO DIFFERENT APPROACHES FOR DRUG BUDGET DEVOLUTION AND INFORMATION STRATEGIES

Mikael Hoffmann(1), L-Å Levin(2), D Andersson(2), A Anell(3), E Heintz(2), A Schmidt(2), P Carlsson(2)
(1) The NEPI foundation / Linköping University, Department of Medical and Health Services, Linköping, Sweden
(2) Center for Medical Technology Assessment / Linköping University, Linköping, Sweden
(3) Lund University, Sweden

In Sweden the 21 independently governed counties have chosen different strategies to handle the major reforms in the Pharmaceutical Benefit Scheme in 2002. This study presents some of the major results from a study commissioned by the Department of Health and the Swedish Association of Local Authorities and Regions. Method: Questionnaire to, with a follow-up interview of, key decision makers in the different counties regarding implementation of budget devolution for prescribed drugs and information strategies employed. The different counties were categorized in two dimensions, both to what extent drug budgets had been devoluted, and to the level and scope of information activities, including some aspects of availability of IT for the prescriber. Data of dispensed prescribed drugs from 2001 through 2008 from the Swedish National Corporation of Pharmacies and the Drug register at the National Board of Health and Welfare. Data from Public Performance Reports on Health care and Social Services. The counties were ranked in six different dimensions - volume and cost per inhabitant (both standardized for age and sex); cost-effective prescribing; low level of inappropriate prescribing; introduction of new and innovative drugs; and equity. Results: Counties with a devoluted drug budget had the same volume (DDD/inhabitant) but lower costs (SEK/inhabitants) in 2008, and had a higher level of cost-effective prescribing. No differences were seen in the other dimensions, but this could possibly be explained by low statistical power. A common drug list in the electronic health record within a county correlated with fewer drug interactions.

Paper No.: 2824
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
DICLOFENAC ENCEPHALOPATY, LIVER AND GASTROINTESTINAL LEZIONS IN RAT AND STABLE GASTRIC PENTADECAPEPTIDE BPC 157

Spomenko Ilic, D Drmic, K Zarkovic, D Kolenc, M Coric, L Brcic, R Klicek, B Radic, Z Romic, S Djidic, S Seiwerth, P Sikiric
University of Zagreb Faculty of Medicine, Department of Pharmacology, Zagreb, Croatia

Combined diclofenac encephalopathy, liver and gastrointestinal lesions have not yet been established in rats. The stable gastric pentadecapeptide, BPC 157 (GEPPPGKPADDAGLV, MW 1419, efficient in inflammatory bowel disease trials (PL 14736) and various wound treatment, no toxicity reported) is an anti-ulcer peptide with hepatoprotective effects that may also affect many central disturbances. Diclofenac (12.5mg/kg) was given intraperitoneally once daily for 3 subsequent days. BPC 157 (10µg/kg, 10ng/kg) was given either (i) intraperitoneally immediately after diclofenac or (ii) per-orally in drinking water (0.16 µg/ml, 0.16 ng/ml) up until the end of the experiment. At 3 h following the last diclofenac challenge, we evidenced severe gastric, intestinal and liver lesions, increased bilirubin, AST, ALT serum values, liver weight, prolonged sedation/unconsciousness (after any diclofenac challenge) and finally (hepatic) encephalopathy. Brain edema was particularly present in the cerebral cortex and cerebellum, more in white than in gray matter, damaged (balloonized) red neurons were particularly expressed in the cerebral cortex and cerebellar nuclei, Purkinje cells and less expressed in hippocampal neurons. This was consistently counteracted in diclofenac-rats that received BPC 157 (µg- or ng-regimen, intraperitoneally or per-orally). In conclusion, the successful counteraction of combined diclofenac encephalopathy, liver and gastrointestinal lesions by BPC 157 regimens means that besides inflammatory bowel disease, diclofenac toxicity may be a new domain for possible BPC 157 therapy.

Paper No.: 2825
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
IBUPROFEN HEPATIC ENCEPHALOPATHY, HEPATOMEGALY, GASTRIC LESION AND GASTRIC PENTADECAPEPTIDE BPC 157 IN RATS

Spomenko Ilic, D Drmic, K Zarkovic, D Kolenc, M Coric, L Brcic, M Sever, V Djuzel, A Boban Blagaic, Z Romic, S Seiwerth, P Sikiric
University of Zagreb Faculty of Medicine, Department of Pharmacology, Zagreb, Croatia

The stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419) is an orally active anti-ulcer peptide that may also have an effect on many central disturbances, efficient in inflammatory bowel disease trials (PL14736) and various wound treatment, no toxicity reported, which showed various hepatoprotective effects. Therefore, we challenged rats with chronic ibuprofen application (0.4g/kg intraperitoneally once daily for 4 weeks) and the stable gastric pentadecapeptide BPC 157 therapy. We evidenced a deleterious circuit not reported in ibuprofen-rats as yet: hepatic encephalopathy, gastric lesions, hepatomegaly, increased AST and ALT serum values, with prolonged sedation/unconsciousness and weight loss. In particular, ibuprofen toxicity exhibited itself as brain edema and cyanosis, particularly in the cerebellum, more so in white than in gray mater, damaged (balloonized) red neurons, without any inflammatory reaction, particularly in the cerebral cortex and cerebellar nuclei, less in the hippocampus, dentate nucleus and Purkinje cells. BPC 157 (GEPPPGKPADDAGLV, MW 1419, 10µg, 10ng/kg) was fully effective given as an antidote after ibuprofen: (i) intraperitoneally, immediately after ibuprofen or (ii) in drinking water (0.16 µg, 0.16 ng/ml) until the end of the 4 week-experiment. Thus, apart from its role as an anti-ulcer peptide, efficient in inflammatory bowel disease trials (PL14736) and various wound treatment, with no toxicity reported and which already showed various hepatoprotective effects, either BPC 157 regimen counteracted all of ibuprofen's adverse effects, such as hepatic encephalopathy, gastric lesions, hepatomegaly, increased liver serum values, and BPC 157 rats showed no behavioral disturbances and maintained normal weight gain.

Paper No.: 725
FOCUSED CONFERENCE GROUP: P01 - CLINICAL PHARMACOLOGY IN THE EMERGING COUNTRIES
PHARMACEUTICAL INTERVENTIONS IN ELDERLY HOSPITALIZED PATIENTS

P Jara(1), M Jirón(1), L Escobar(1), Leonardo Arriagada(2)
(1) Universidad de Chile, Departamento de Ciencias y Tecnologia Farmaceutica, Santiago, Chile
(2) Sección Geriatría, Hospital Clinico Universidad de Chile, Santiago, Chile

Pharmaceutical interventions in elderly hospitalized patients. In Chile elderly people have a higher prevalence of non transmissible chronic disease and they are hospitalized 2.6 more times than general population. Hospitalization increase risk for receiving potential inappropriate medications (PIM), as well to develop drug related problems (DRP). The aim of this study was to improve pharmacotherapy use in hospitalized elderly people in an Acute Geriatric Unit (AGU). An intensive prospective follow-up study was carried out in an AGU of a teaching Hospital, developing pharmaceutical interventions to elderly hospitalized patients who met selection criteria. PIM was identified using 2003Beers Criteria and DRP classified by Minnesota Project.Data analyses were performed using STATA 10.1.Seventy (83.3%) of 84 patients met selection criteria. At least one PIM was prescribed in 26 (37.1%) patients, mainly aspirin concomitantly with oral anticoagulant therapy (35.5%), amiodarone (19.4%) and non steroidal anti-inflammatory analgesics concomitantly with oral anticoagulant therapy (19.4%). High severity of PIM was found in 90.3% of cases. Mean number of medication/patient with PIM was significantly higher than patient without PIM, 11.7±5.0 y 8.6±3.7, respectively ($p<0.01$). Sixty seven (95.7%) patient had at least one DRP. Of 568 detected DRP 353(62.1%) were problems associated with compliance. Pharmaceutical intervention performed were accepted in 96.3%(284) of cases (4.2±3.3 interventions/patient) which included stop therapy (14.2%), change of doses (10.2%) and change of administration time (10.2%)Pharmaceutical interventions permitted to resolve DRP as well to improve quality of medication use in elderly hospitalized patients. More studies still are necessary to understand their outcomes.

Paper No.: 1887
FOCUSED CONFERENCE GROUP: P14 - ADDICTION AND DOPING: NEUROBIOLOGICAL AND CLINICAL
SLOW RELEASE ORAL MORPHINE FOR OPIATE SUBSTITUTION THERAPY: A SYSTEMATIC REVIEW

Jérémie Jégu(1,2), P Soler(1), A Gallini(2), J-L Montastruc(2), M Lapeyre-Mestre(2)
(1) University Hospital, Department of Epidemiology, Toulouse, France
(2) University Hospital, Department of Clinical Pharmacology, Toulouse, France

Alternatives to methadone for opiate substitution treatment is still a major concern. Slow Release Oral Morphine (SROM) substitution treatment has been experimented in Austria, Switzerland, Australia and other countries. But today, is there evidences of efficacy of SROM substitution therapy? The aim of this work was to assess the efficacy of SROM substitution therapy by performing a systematic review. All studies about opiate substitution treatment with SROM in adult patients were included. Three independent reviewers assessed the selected articles using a standardized checklist. Retention rate was the main outcome criteria. Data about quality of life, withdrawal symptoms, additional drugs consumption, craving and adverse effects were collected. Study design, study length and number of subjects included were recorded. Twelve articles were selected for qualitative analyses. There was only one randomised controlled clinical trial and one controlled trial without randomisation. The other studies were not controlled. Retention rates were good (from 80.6% to 95%) with SROM substitution but similar retention rates were obtained with methadone substitution treatment. Most of the non comparative studies showed that quality of life, additional drug consumption, craving and withdrawal symptoms were improved under SROM substitution therapy. Only a few studies about SROM substitution therapy are available and most of them are non controlled. There is no evidence that SROM substitution therapy is more or equally effective than the standard methadone substitution therapy. Randomised controlled trials are needed to answer to this question.

Paper No.: 2826
FOCUSED CONFERENCE GROUP: PW02 - SYMPOSIUM ON ADVANCES IN GI PHARMACOLOGY
STABLE GASTIC PENTADECAPEPTIDE BPC 157, SAFE ANTI-ULCER PEPTIDE IN TRIAL FOR INFLAMMATORY BOWEL DISEASE (PL-14736, PLIVA), CAN DECREASE THE INFARCTED AREA AFTER COMPLETE ISCHEMIA OF THE SPLEEN IN RATS

Zeljko Jelincic, A Majerovic, B Radic, L Berkopic, M Sever, R Klicek, D Drmic, L Brcic, S Seiwerth, P Sikiric
University of Zagreb Faculty of Medicine, Department of Pharmacology, Zagreb, Croatia

Aim: Stable gastric pentadecapeptide BPC 157 was shown to recover skin and mucosal ischemic lesions. BPC 157 is safe anti-ulcer peptide (PL-14736, Pliva) in trial for IBD, and wound therapy, no toxicology reported, that healed intestinal anastomoses and fistulas (Skorjanec S et al, Dig Dis Sci. 2009 Jan;54(1):46-56. , Klicek R et al, J Pharmacol Sci. 2008 Sep;108(1):7-17). Spleen ischemia is a condition rarely studied in rats. Therefore, BPC 157 was tested with spleen ischemia in rats. Materials and methods: Spleen ischemia. Wistar rats (200 g b.w.) were subjected to complete occlusion of lienal blood vessels at 12 mm from the spleen. The assessment (the rate of healthy/infarcted areas per 10 visual fields at magnification 63x using VAMS computer system) was after various periods of ligation (3, 6, 9, 12, 18, 24h). Medication, BPC 157 (10 µg, 10ng/kg i.p.) was given immediately before ligation. Results: The severe lienal infarction appeared after 12h, and infarcted areas ware markedly enlarged as time elapsed (i.e., at 18 and 24h). BPC 157 given in either dose significantly decreased infarcted area presentation, and increased the ratio of healthy area in rats with ligated lienal blood vessels. Conclusion: BPC 157 can decrease the infarcted area after complete ischemia of the spleen.

Paper No.: 3501
FOCUSED CONFERENCE GROUP: P15 - ENDOTHELIUM IN HEALTH AND DISEASE
ESTROGEN REGULATION OF BRAIN ENDOTHELIAL MITOCHONDRIA: DIFFERENT EFFECTS IN NORMAL AND HIGH GLUCOSE

Martin F Kemper, D Krause, S Duckles
University of California, Department of Pharmacology, Irvine, CA, USA

Estrogen has a number of protective effects on the vasculature, including increased endothelial nitric oxide production, increased dilation, and decreased inflammation. We have recently shown that estrogen protects the mitochondria in brain blood vessels by reducing reactive oxygen species (ROS) and increasing mitochondrial efficiency. We also find that estrogen suppresses mitochondrial ROS in mouse brain microvascular endothelial cells (bend.3) in culture. Moreover, in these cells, estrogen increases the expression of mitochondrial oxidative phosphorylation complex and carrier proteins, indicating increased oxidative phosphorylation capacity. Specifically, Complex 1 subunit ndufb8, Complex 2 subunit 30kda, Complex 4 subunit 1, ATP synthase subunit alpha and cytochrome c were increased by 30 nM estradiol, as quantified using fluorescence Western blot analysis. A similar effect was seen using the estrogen receptor alpha agonist, PPT. However, when the endothelial cells are cultured in high glucose (25 mM), instead of nomal glucose (5 mM), we found that the ability of estrogen to increase mitochondrial protein expression is blunted or reversed. A number of studies indicate that cardiovascular protective effects of estrogen can be lost in diabetes and/or hyperglycemic conditions. Differing effects of estrogen on mitochondrial electron transport function in normal and hyperglycemia may be a contributing factor in the loss of estrogen protection of the brain endothelium in diabetes.
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Paper No.: 3469
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
DEVELOPMENT AND VALIDATION OF DISSOLUTION TESTING METHOD FOR PIRACETAM TABLETS IN KOREAN PHARMACEUTICAL CODEX

Eun-Jung Kim(1), J-H Lee(1), C-H Park(1), D-S Kim(2), I-K Kim(2)
(1) Gwangju Regional Food & Drug Administration, Hazardous Substances Analysis Division, Gwangju, South Korea
(2) National Institute of Food and Drug Safety Evaluation, South Korea

Although the dissolution test can serve as an effective tool of quality control and predictor *in vivo* performance, there are a number of drugs with no established dissolution specifications in Korean Pharmaceutical Codex (KPC). So, we tried to develop the dissolution testing method of piracetam tablets among commercially available drug preparations which have no dissolution specifications. The analysis method was investigated using HPLC. Chromatography was performed using 10% methanol adjusted with 0.001mol/L (NH4)2HPO4 as mobile phase at a rate of 0.8 ml/min and Capcellpak C18 (5

§-, 4.6¡¿250 mm) as column and monitored at UV 214 nm. To determine the dissolution medium, we studied the dissolution profile observed with various types of dissolution media, pH 1.2, pH 4.0, pH 6.8 solution and distilled water, based on the 'Guidelines on Specifications of Dissolution tests for Oral dosage forms' of Korea Food & Drug Administration(KFDA). In all media, the dissolution rate of piracetam was over 80% in 30min and specificity was confirmed by PDA spectrum of HPLC. Distilled water was selected as the dissolution medium and method validation was studied to verify the newly established method for categories including accuracy, precision, specificity, linearity, quantitation limit and range. The developed method was linear (R2>0.999) in the range of 5.3~53.0ug/mL, precise (RSD%<2%) and accurate (recovery : 98.69~101.60%). These results suggest that this method is simple and suitable to measure the dissolution rate of piracetam. Therefore, the analysis method could be utilized in preparing dissolution specifications of piracetam tablets in the revised version of KPC.

Paper No.: 3497
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
IMPROVING *IN VITRO* METHODS BY DEVELOPING AND USING DEFINED CULTURE MEDIA

Lisbeth Ehlert Knudsen(3), J van der Valk(1), ML Scarino(2), G Gstraunthaler(4)
(1) University of Copenhagen,Department of Public Health, Copenhagen, Denmark
(2) Utrecht University, Faculty of Veterinary Medicine, NCA, Utrecht, The Netherlands
(3) Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Rome, Italy
(4) Innsbruck Medical University, Department of Physiology, Innsbruck, Austria

In vitro methods are widely used to study activities at the cellular level. Furthermore, *in vitro* methods are powerful tools to replace or reduce animal experiments, either as stand-alone, or as part of a testing strategy. Cells are maintained under well-established conditions, which typically involve incubation at 37ºC with a humidified gas mixture of 5% CO2. An often used basal medium is Dulbecco's Modified Eagles Minimal Essential Medium (DMEM). Dependent on the cell type this medium is supplemented with factors essential for proliferation, migration and differentiation of the cells. Usually, serum is used as supplement, often fetal bovine serum (FBS). Chemically defined media should be preferred when using *in vitro* methods with 450 serum free media are available (www.goodcellculture.com). Still, not for every cell type is a defined medium developed. In addition, the formulation of most commercially available media is not released, and these can thus not be regarded as strictly defined. A workshop concluded that the development of serum-free media and cell adaptation processes is an ongoing process in several laboratories, often without knowledge of research processes, experiences or results of other laboratories regarding this topic. This information, particular with regard to precise formulations, should be collected and made publicly available to facilitate the further development and use of defined cell and tissue culture media.

Paper No.: 653
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
EFFICIENCY OF TREATMENT BY ACENOCOUMAROL IN PATIENTS WITH PERMANENT FORM OF ATRIAL FIBRILLATION, WITH USING MODELS OF PERSONALIZED MEDICINE

Ilya Kukes(1), B Shan(2), D Sychev(3)
(1) The Sechenov Moscow Medical Academy, Department of Clinical Pharmacology, Moscow, Russian Federation
(2) The Lomonosov Moscow State University, Moscow, Russian Federation
(3) Institute of Clinical Pharmacology of Science Center of Examination of Medical Applications 'Roszdravnadzor', Moscow, Russian Federation

Introduction: Model of personalized medicine, actively developing now, can reduce the number of side effects during therapy of Acenocoumarol, mainly due to individual genetic characteristics (polymorphisms of genes CYP2C9 and VKORC1). Materials and Methods: In the research we included 84 patients with permanent form of atrial fibrillation, and randomized them in two equal groups with usual way of selecting dosage of acenocoumarol only, and usual way of selecting dosage with pharmacogenetics testing. To assess the efficacy and safety of treatment by acenocoumarol, used the results of a retrospective research, the clinical status of the patient, and laboratory findings, the results of research on blood clotting. Also we analyzed the costs of treating both groups by economic standards of the Russian Ministry of Health Care. Results: In the first group, in terms of 100 patients were nearly 25% of light side effects (nasal bleeding) and nearly 5% of serious side effects (bleeding in the digestive tract). The cost of treatment was 29768,72 ˆ. In the second group, also in terms of 100 patients, light side effects were observed only in nearly 5% of patients, and serious side effects were not observed at all. The cost of treatment was 16904,51 ˆ. Conclusions: Selection of a dose of acenocoumarol, with using models of personalized medicine, reduces

the risk of side effects in 5 times, and also reduces the costs of treating of 100 patients at more than 10000 euros, and reduce side effects from using of oral anticoagulants up to 5 times.

Paper No.: 3466
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
VALIDATION OF ANALYSIS METHOD FOR DISSOLUTION TEST OF BUFLOMEDIL HYDROCHLORIDE

Ryun-Kyung Lee(1), G-R Jeong(1), M-R Jang(1), H-S Oh(1), T-Y Cho(1), I-K Kim(2), D-S Kim(2)
(1) Daegu Regional Korea Food & Drug Administration, Hazardous Substances Analysis Division, Daegu, South Korea
(2) Pharmaceuticals and Medical Devices Research, Department National Institute of Food and Drug Safety Evaluation, South Korea

Despite the fact that the dissolution test can provide the useful information for responding to changes in manufacturing process and prescription, there are a number of drugs in Korea Pharmaceutical Codex(KPC) with no established specifications, as they were developed quite a while ago. Therefore, to secure the good quality of pharmaceutical products, dissolution specification for buflomedil hydrochloride is needed to be established, which is enrolled in KPC with having no appropriate specifications. In this study, the dissolution profile of buflomedil hydrochloride was evaluated by guidance for setting dissolution specification of oral dosage forms, Korean Pharmacopoeia(KP). To determine the dissolution medium, we studied the dissolution profile observed with various types of dissolution medium, pH 1.2, pH 4.0, pH 6.8 buffer and water. And analysis method determined using HPLC. HPLC analysis was performed using an C18 column(250-4.6mm, 3§-), acetonitril - 10mM potassium phosphate monobasic solution (40:60, v/v) as the mobile phase and UV detection at 280nm. Dissolution testing conditions were 900mL of dissolution medium pH 1.2, paddle method and rotational speed at 50rpm. The HPLC method for determination of Buflomedil Hydrochloride was developed and validated by defining the specificity, linearity, accuracy and precision and limit of quantitation.

Paper No.: 3477
FOCUSED CONFERENCE GROUP: PC11 - G PROTEIN-COUPLED 7TM RECEPTORS: FROM MOLECULAR TO PHYSIOLOGICAL FUNCTION
ACTIVATION MECHANISM OF GABAB RECEPTOR THROUGH TRANSACTIVATION OF RTKS

Jianfeng Liu
Key Laboratory of Molecular Biophysics of Ministry of Education, College of Life Science and Technology, Wuhan, PR China

Class-C G-protein coupled receptors (GPCRs) represent a distant group among the large family of GPCRs. This class includes the receptors for the main neurotransmitters, glutamate and GABA, and the receptors for Ca^{2+}, some taste and pheromone molecules, as well as some orphan receptors. Class-C receptors possess a heptahelical domain (HD) involved in heterotrimeric G-protein activation, but most of them also have a large extracellular domain (VFD) responsible for agonist recognition and binding. It is now well accepted that these receptors are dimers, either homo or hetero-dimers. GABAB receptor was the first heteromeric GPCR identified. Indeed, both GABAB1 and GABAB2 subunits appear necessary to get a functional GABAB receptor since agonists bind in GABAB1 VFT domain and GABAB2 HD domain is responsible for G-protein coupling. We show here that the specific activation of GABAB receptor leads to G-protein activation which in turn activate downstream signaling such Erk1/2 and AKT pathway through an RTKs-transactivation mecansims for neuroprotection. Furthermore, we demonstrate that this transactivation may occur via an allosteric communication.

Paper No. 3452
FOCUSED CONFERENCE GROUP: FC04 - PHARMACOEPIDEMIOLOGY, CURRENT CONTROVERSIES AND OPPORTUNITIES
THE EMERGENCE OF MEPHEDRONE AS A RECREATIONAL HIGH

B Lovell, Rachel King, K Willmer
Weat Cumberland Hospital, Department of General Medicine, Whitehaven, Cumbria, UK

Mephedrone (also known as 4-methylmethcathinone or 4-methylephedrone) has emerged as a new recreational drug in the UK. Until its recent criminalisation, it was currently readily available online. It was purchased under the guise of “plant food” and “meow meow” and

was therefore labelled as a 'legal high'. Its relative newness on the recreational scene means that its properties and side effects are not fully understood and therefore there is little awareness of its potential dangers. Users describe effects of euphoria, increased vigilance, excitement and greater confidence. Reported side effects include hallucinations, anxiety, paranoia, fits, delusions and even death. We describe a case series of patients admitted to our acute medical admissions unit after using mephedrone. We encountered our first case in November 2009 and then treated a further eleven patients over the subsequent four weeks. The most common presenting symptoms were dyspnoea, palpitations, anxiety, decreased consciousness and aggression. One young patient suffered a spontaneous pneumomediastinum and surgical emphysema following ingestion of the drug. A second 20 year old male suffered myocardial infarction and life threatening arrhythmias. There is currently very little evidence in the published medical literature regarding this drug. The surge in cases we have seen over the last few months reflects its growing use and popularity. We feel that this is a trend, which is likely to continue and spread to other hospitals. We therefore aim to make other physicians aware of the emergence of mephedrone in the UK so they may vigilant to its potential dangerous effects.

Paper No.: 3503
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
SUBCHRONIC ADMINISTRATION OF DOXORUBICIN TO WISTAR RATS RESULTS IN OXIDATIVE STRESS IN THE ABSENCE OF APOPTOTIC SIGNALING MARKERS

Nuno Machado(1), I Baldeiras(2,3), P Oliveira(1)
(1) Center for Neurosciences and Cell Biology, Department of Life Sciences, Coimbra, Portugal
(2) Coimbra University Hospital, Laboratory of Neurochemistry, Department of Neurology, Coimbra, Portugal
(3) Center for Neuroscience and Cell Biology, Faculty of Medicine, University of Coimbra

Despite the wide range of published data on cardiac toxicity, there is still a shortage of evidence for chronic toxicity of the antineoplastic agent Doxorubicin (DOX) in the lung. The aim of the present work was to determine if DOX causes alterations in selected apoptotic proteins and oxidative stress in the lung, as it does in the heart. For that purpose, lungs from Wistar-Han rats subchronically treated with vehicle or DOX with seven weekly injections were collected and analyzed concerning several proteins involved in mitochondrial permeabilization and apoptotic pathways, including p53, Bax and Bcl-2 and several oxidative stress markers. After subchronic DOX treatment, no alterations in proteins involved in mitochondrial membrane permeabilization were observed. Nevertheless, an increase of malonyldialdehyde levels and a decrease in the lung concentration of vitamin E after DOX toxicity was observed, despite no alterations of tissue reduced and oxidized glutathione. The results obtained indicate for the first time that the lungs of DOX-treated rats appears to be more susceptible to increased lipid peroxidation, which can explain some cases of DOX-induced lung toxicity, summing up to a general deterioration of the cardiovascular fitness in individuals treated with DOX.
Supported by research grant PTDC/SAU-OSM/104731/2008 from the Portuguese FCT (to PO).

Paper No.: 1994
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
WARFARIN HYPERSENSITIVITY BY UNUSUAL DRUG INTERACTION AND GENETIC PREDISPOSITION

Valentina Maggini(1), A Pugi(1), D Coletta(2), B Giusti(3), D Prisco(3), R Abbate(3), A Mugelli(1), F Lapi(1), A Vannacci(1)
(1) University of Florence, Department of Preclinical and Clinical Pharmacology, Florence, Italy
(2) General Practioner AUSL 11 Empoli, Italy
(3) University of Florence – AOU Careggi, Atherothrombotic Disease Center, Department of Medical and Surgical Critical Care, Florence, Italy

Interindividual variability in warfarin response is generally attributed to dietary vitamin K intake, drug interactions, demographic and genetic factors. A suspected interaction between potassium canrenoate (PC) and warfarin was reported in a 77-year-old Caucasic female with atrial fibrillation, cardiac insufficiency and arterial hypertension. During routine monitoring, the INR had been stable (2.4±0.4) with warfarin 35.0 mg/wk since January 2008. Two weeks following the initiation of PC (50 mg/die), an extensive facial haematoma due to a jaw lesion and a marked increase of INR (10.8) occurred, requiring prompt reduction of warfarin to 22.5 mg/wk. The patient denied changes in other drug therapy or in diet. She was genotypized for polymorphisms in CYP2C9 (responsible for S-Warfarin catabolism) and in VKORC1 (warfarin-target enzyme vitamin K epoxide reductase) reported affecting the individual response to

warfarin. She resulted homozygote wild-type for CYP2C9*2 and *3 alleles and homozygote -1639AA (low dose required) for VKORC1. Subjects with VKORC1 -1639AA genotype (frequency=0.182) may be more susceptible to drug–drug interactions enable to modify warfarin pharmacokinetics and pharmacodynamics. Therefore, the addition of PC could potentiate the anticoagulant effect of warfarin by (1) protein-binding displacement of warfarin from albumin in a competitive manner (Takamura N et al, Pharm Res 1997) and (2) competition for metabolism through CYP3A, decreasing warfarin clearance (Kaminsky LS et al, Pharmacol Ther 1997). To our knowledge, an interaction between warfarin and PC has not been previously reported. Although further studies need to be conducted on this purpose, pysicians' knowledge should be enrich with this clinical concern.

Paper No.: 3450
FOCUSED CONFERENCE GROUP: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
INVOLVEMENT OF PGD2, DP1 AND CRTH2/DP2 IN CIA

Nuria Maicas(1), ML Ferrandiz(1), L Ibañez(1), R Brines(1), MI Koenders(2), MJ Alcaraz(1),
(1) University of Valencia Pharmacy Faculty, Department of Pharmacology, Burjassot, Valencia, Spain
(2) Rheumatology Research and Advanced Therapeutics. Radboud University Nijmegen Medical Centre. The Netherlands

Aims: The aim of this study was to investigate the role of PGD2 during the evolution of an arthritic murine model. In addition, we evaluated the contribution of the two PGD2 receptors in the progression of the disease by using an antagonist of each receptor: MK0524 (DP1R antagonist) and CAY10595 (CRTH2/DP2R antagonist). Methods: CIA was induced in DBA/1J mice and the evolution of the inflammatory response was studied from days 21 to 70. In another CIA experiment MK0524 (4 mg/kg/day) and CAY10595 (5 mg/kg/day) were administered p.o. from days 21 to 34. Histological analyses were performed in hind paws. Anti-bovine type II collagen, PGD2 and cytokine levels were determined by ELISA. Time course protein expression in knee joints was determined by immunohistochemical analysis. Results: High levels of PGD2 were detected in arthritic serum samples from days 28 to 70. Immunohistochemical analyses of knee joints showed an expression of h-PGD2S, l-PGD2S, DP1 and CRTH2/DP2 in knee joints during the evolution of this arthritic model. Treatment with MK0524 increased the macroscopic score in paws, the migration of inflammatory cells and the bone remodelling process with new bone formation (osteophytes). CAY10595 did not affect significantly these parameters. MK0524 increased the IgG levels in the serum and the levels of IL-1beta in hind paws, whereas CAY10595 decreased the IgG2a levels in serum and did not affect the IL-1beta. Conclusions: These data suggested an involvement of PGD2 in the progression of rheumatic diseases with a major role of the DP1 receptor.

Paper No.: 3296
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
EVALUATION OF THE VASCULAR REACTIVITY AND OVARIAN FOLLICLES DEVELOPMENT IN FEMALE RATS FED WITH 'PROTEIN DIET'

Elisabeth Marostica, TF Santos, TP Bedo, AS Geraldo, LL da Silva, FCF de Brito, JAS Ribas, VB de Azeredo, Fluminense Federal University, Department of Physiology and Pharmacology, Rio de Janeiro, Brazil

Aim: Our study intends to evaluate the ¡§protein diet¡¨(high-protein, low-carbohydrate, high-fat) (Atkins RC, Avon Book; 1992) effects on the loss of corporal weight, vascular reactivity and ovarian follicles development. Methods and results: 90-day-old female Wistar rats were divided in 4 groups and fed for 30 or 60 days as following: C1-balanced ration with casein and E1-high-protein/low-carbohydrate/high-fat ration, fed ad libitum; C2-balanced ration with casein and E2-high-protein/low-carbohydrate/high-fat ration, with energy-restricted intake (30%). All of them were weighed 3 times a week. The rats were selected in the same phase of estral cycle and after anesthesia, ovaries were obtained and the follicles were analyzed. Concentration-effect curves were obtained from the isolated aorta, using phenylephrine (FNF) and acetylcholine (ACh). The data were express as % of the maximum effect and pD2 (-log EC50) was calculated. Preliminary results showed that weight loss occurred only in C2 (-42.26,,b7.15g) and E2 (-29.20,,b21.88 g). After 30 days, the pD2 value for the ACh in C2 (7.47,,b0.13; n=9) was higher than in E2 (6.91,,b0.10; n=11). Obvious differences were observed after 60-day, regarding the relative percentage of pre-ovulatory follicles among experimental groups (C1: 16%; E1: 18%, C2: 14%, E2: 2%). Conclusion: The results suggest that ¡§protein diet¡¨ without energy-restricted intake does not promote greater loss of corporal weight than the balanced dietary and it is associated with vascular reactivity decrease, increasing the cardiovascular risk. Furthermore, this diet impaired the follicle growth,

maturation and ovulation at the female reproductive tract.
Supported by FAPERJ, CNPq, UFF.

Paper No.: 683
FOCUSED CONFERENCE GROUP: P16 - NATURAL PRODUCTS: PAST AND FUTURE?
EFFECTS OF Z-VENUSOL (*G. PERPENSA*) ON HUMAN PROSTATE AND CERVICAL CARCINOMAS

Lehlohonolo Mathibe, J Botha, S Naidoo
University of KwaZulu-Natal, Department of Therapeutics & Medicine Management, Durban, South Africa

Introduction: *Gunnera perpensa* is a common traditional medicine for various maternal ailments, inflammation and cancerous sores in South Africa. Drewes et al. (2005) reported that the main active ingredient of *G. perpensa*, z-venusol, inhibits the growth of various human pathogens, such as S. epidermidis and B. cereus, *in vitro*. Although there are documented reports of traditional use of *G. perpensa* extracts for the treatment of cancer in (Kuduru et al. 2007), no mechanism of action has been elucidated nor has the anti-cancer potential of venusol been screened. Purpose: To investigate the *in vitro* regulation of cell proliferation by venusol using the human prostate (DU-145) and cervical (HeLa) cancer cell lines. Methods: Commercial DU-145 and HeLa cells were cultured to approximately 50% confluence before incubation for 24 hours with freshly-prepared venusol, at serial dilutions ranging from 2400ug/ml to 2.2ug/ml. The conventional MTT assay was used to determine cell proliferation, measured against non-exposed controls. All dilutions were performed in quadruplicate and the experiments repeated three times. Results: There was a significant decrease in HeLa cell proliferation at concentration of 2400ug/ml (23%, $p < 0.001$) only. None of venusol concentrations had any significant influence on proliferation of the human prostate cancer cell line, DU-145. No gross cytotoxic damage, including cell necrosis, was observed at any venusol concentration for the duration of incubation period, even when extended to 48 hours on both DU-145 and HeLa cancer cell lines. Conclusion: These findings suggest that venusol may have the potential to influence cervical carcinoma cell proliferation.

Paper No.: 3463
FOCUSED CONFERENCE GROUP: FC19 - GENERAL SESSION
RENAL GENE EXPRESSION PROFILE IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

Magdalena Mazagova, R Henning, M Duin, A Buiten, M Sandovici, L Deelman
University Medical Centre Groningen, Department of Clinical Pharmacology, Groningen, The Netherlands

Introduction: Diabetic nephropathy is a chronic kidney disease characterized by the development of tubulointerstitial and glomerular fibrosis. The pathways leading to fibrosis are initiated early after induction of diabetes. In the current study, we aimed at identifying genes associated with renal pre-fibrosis in the first week after induction of diabetes. Methods: Male Wistar rats were sacrificed at day 1, 2, 4 and 7 after induction of diabetes. A non-diabetic control group was included. Total RNA was isolated from rat frozen kidney samples containing both cortex and medulla. Arrays were performed with a dye-swap. Array analysis: Time curves showed expression levels of individual genes within the first week of diabetes compared to non-diabetic controls. The area under the curve (AUC) was calculated to quantify the level of changed gene expression. Array data were confirmed by RT-PCR. In our analysis we focused on genes involved in inflammation, extracellular matrix formation, growth, metabolism and oxidative stress. Results: Our microarray analysis showed that 290 genes were significantly changed in the first week after induction of diabetes. Surprisingly, we did not find any changes in the expression of genes involved in TGF-b signaling. Prominent genes identified to be upregulated included Insulin-like growth factor I, Fgf15, Tieg and GDF-15. These factors may interact with TGF- b induction. Conclusions: We have identified several new renal genes which are associated with the induction of diabetes. Establishment of early genes and their activated pathways may identify targets for novel therapeutic approaches in diabetic nephropathy.

Paper No.: 3427
FOCUSED CONFERENCE GROUP: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
CYCLOOXYGENASE-2 (COX-2) INHIBITION AND CARDIOVASCULAR RISK: A REVIEW AND META-ANALYSIS OF OBSERVATIONAL STUDIES OF CARDIOVASCULAR ISCHEMIC EVENTS WITH NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) 2006-2010

Patricia McGettigan(1), W McGuire(1), D Henry(2)
(1) University of Hull, Hull York Medical School, Hull, UK
(2) Institute for Clinical Evaluative Sciences, UK

Randomised studies of selective cox-2 inhibitors in arthritis and cancer chemoprevention found increased risks of cardiovascular ischemic events. Large observational studies followed. Our review of this literature included 16 case-control and 7 cohort studies published 2002-2006 (McGettigan&Henry JAMA2006;296:1633-1644). In these analyses, rofecoxib was associated with a dose-related risk of cardiovascular ischemic events: doses </=25mg/day OddsRatio(OR)1.35, 95%CI 1.15-1.59; >25mg/day OR2.19, 95%CI 1.64-2.91. No risk was apparent with celecoxib. Diclofenac (OR1.40, 95%CI 1.16-1.70) and indomethacin (OR1.30, 95%CI 1.07-1.60) had risks similar to rofecoxib. Naproxen and ibuprofen were not associated with increased cardiovascular risk. To establish whether subsequent research refuted or confirmed these findings, we reviewed the observational literature published subsequently (2006-2010). Searches returned 33 new studies: 12 case-control, 21 cohorts. Adjusted risk estimates for individual drugs (v non-use) were pooled using StatsDirect Statistical Software2.7.8. Raw data were pooled using ReviewManager5.0.23. The meta-analysis upheld and augmented the earlier findings. Increased cardiovascular risk was associated with use of rofecoxib (doses </=25mg/day: OR1.35, 95%CI 1.18-1.55; >25mg/day: OR2.44, 95%CI 1.71-3.46), diclofenac (OR1.34, 95%CI 1.19-1.52), and indomethacin (OR1.28, 95%CI 1.17-1.41). New findings included: - Dose-related risk with celecoxib: doses </=200mg/day OR1.20, 95%CI 1.06-1.36; >200mg/day 1.60, 95%CI 1.11-1.30. - Increased cardiovascular risk with etodolac (OR1.55, 95%CI1.28-1.87) and etoricoxib (OR 2.05, 95%CI 1.45-2.88) - Low-level risks with naproxen (OR1.16, 95%CI 1.07-1.27) and ibuprofen (OR1.16, 95%CI 1.07-1.27). It appears that all NSAIDs are associated with increased cardiovascular risks. Where they can be studied, the risks appear dose-related. Rofecoxib, etoricoxib, high-dose celecoxib, etodolac and diclofenac are associated with greatest risks.

Paper No.: 638
Focus Group: P01 - CLINICAL PHARMACOLOGY IN THE EMERGING COUNTRIES
COMPARISON OF OUTPATIENT UTILIZATION OF SYSTEMIC ANTIBIOTICS BETWEEN NOVI SAD AND VRBAS, SOUTH BACKA DISTRICT, REPUBLIC OF SERBIA

Vesna Mijatovic, O Horvat, A Sabo
University of Novi Sad, Faculty of Medicine, Department of Pharmacology,Toxicology & Clinical Pharmacology, Novi Sad, Republic of Serbia

Introduction: Antibiotics have been among the most widely used classes of drugs in the world. Monitoring of antibiotic prescribing promotes rational use of these drugs, reduces costs and slows down the progress of resistance. The aim of this study is to compare the outpatient utilization of antibiotics for systemic use in Novi Sad and Vrbas, and to explain their characteristics. Materials/Methods: A retrospective study on drug utilization, according to ATC classification, was conducted on the basis of data received from 33 state-owned and 28 private pharmacies in Novi Sad (323708 inhabitants) and 8 state-owned and 4 private pharmacies in Vrbas (43840 inhabitants), over 3-month period in 2008. Results were presented in terms of DDD/1000 inhabitants/day (DID). Drug utilization 90% (DU90%) method was used to determine the prescribing quality of systemic antibiotics. Results: The overall utilization of systemic antibiotics in Novi Sad was 24.31 DID and 30.33 DID in Vrbas. Penicillins accounted for the highest utilization-8.81 DID in Novi Sad and 17.32 DID in Vrbas, followed by cephalosporins (4.43 DID/4.76 DID), tetracyclines (2.97 DID/1.52 DID) and macrolides (2.57 DID/1.50 DID), retrospectively. In Novi Sad DU90% segment included 11 of 33 antibiotics, while 10 of 28 antibiotics fell within DU90% in Vrbas. Financial expenses for DU90% segment in Novi Sad accounted for 87.73% of overall costs in J01 group, and 87.14% in Vrbas. Conclusion: Antibiotic consumption variety between 2 towns of the same district implies on necessity for improving current guidelines, education (physicians and patients) and incorporating Serbia in existing European projects.

Paper No.: 3180
Focus Group: P01 - CLINICAL PHARMACOLOGY IN THE EMERGING COUNTRIES
EVALUATION OF POTENTIALLY INAPPROPRIATE PRESCRIBING IN ACUTELY ILL ELDERLY COMPARING BEERS AND STOPP CRITERIA

Suzana Mimica Matanovic(1), V Vlahovic-Palcevski(2)
(1) University Hospital Osijek, Department of Internal Medicine, Osijek, Croatia
(2) University Hospital Center Rijeka, Rijeka, Croatia

Introduction : Beers criteria are often used tool for detecting potentially inappropriate medicines (PIMs) in the elderly (Fick DM et al, Arch Intern Med. 2003, 163(22):2716-24.). Recently, a new system-defined tool for detecting PIMs was introduced (STOPP) (Gallagher P et al, J Clin Pharmacol Ther 2008 ;46(2):72-83.). We used both tools in identifying PIMs in acutely ill elderly patients admitted to the Department of Internal Medicine. We also evaluated drug related hospital admissions. Patients : A prospective, observational study of 145 consecutive admissions of elderly patients to the Department of Internal Medicine was performed. Data on concurrent medications, diagnosis and co morbidity were collected. Results : Mean patient age (SD) was 75 (6.1) years. Median number of medicines was 5.1 (range 1-13). Beers criteria detected 51 PIMs in 44 patients (31%); the most common being long acting benzodiazepines in 22 patients (15%) and amiodarone in 14 patients (10%). STOPP criteria detected 47 PIMs in 42 patients (29%), the most common being long acting benzodiazepines in 22 patients (15%) and NSAIDs with ulcer disease history in 5 patients (3%). Adverse drug reactions contributed to 19 admissions (13%). Among drug related admissions, 26% and 21% were attributed to PIMs detected with Beers and STOPP criteria, respectively. Conclusion : Both tools identified similar number of PIMs. STOPP tool covers drug prescribing in the elderly more comprehensively. Incidence of PIMs and drug-related admissions were high, so it is necessary to rationalize drug prescribing in the elderly.

Paper No. 3510
FOCUSED CONFERENCE GROUP: FC17 - NEW APPROACHES AND TARGETS IN PSYCHIATRY
ESTIMATION OF THE AVERAGE WARFARIN MAINTENANCE DOSE IN SAUDI POPULATION

Rajaa Mirghani(1), G Chowdhary(1), T Kashour(2)
(1) King Saud Bin Abdulaziz University for Health Sciences, Faculty of Medicine, Riyadh, Kingdom of Saudi Arabia
(2) King Fahad Medical City,Prince Salman Heart Center, Riyadh, Kingdom of Saudi Arabia

Introduction: Interethnic differences in warfarin dose requirements have been reported by a number of investigators. To our knowledge such dose requirements for Saudi population have not been established and therefore we investigated the average warfarin maintenance dose in Saudis. Patients: One hundered and three adult patients who were visiting the outpatient cardiology clinic at our hospital were considered for inclusion in the study. All Saudi patients who have been on warfarin for at least four weeks and had a stable warfarin dose for at least two consecutive visits and an INR of 2.0-3.0 were included. Data regarding the age, gender, warfarin dose, and indication and duration of warfarin treatment was collected from the medical records of the patients. The average warfarin maintenance dose was calculated. Results: Fifty patients met the inclusion criteria. The mean ± standard deviation (SD) age of the patients was 53.1±18.0 year. The mean ±SD warfarin maintenance dose was 4.6±2.6 mg. Only 16% of the patients had a warfarin stable dose of 5 mg and 58% had a stable dose of less than 5 mg. Conclusion: The average daily warfarin maintenance dose in Saudis seems to be comparable to that reported in Caucasians and lower than that of Asians. However, considering the fact that 58 % of the patients had a warfarin stable dose of less than 5 mg indicates that majority of this patient population require a dose which is lower than the standard dose. The clinical implications of these results have to be further investigated.

Paper No.: 3492
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
WIDE INTER-INDIVIDUAL PLASMA METHADONE CONCENTRATIONS IN ROUTINE MMT PATIENTS IN MALAYSIA

Nurfadhlina Musa(1), R Ismail(1), MH Habil(2), N Mohamad(1), A Al Memam(1), MK Ishak(3), T Soo Choon(1)
(1) University Sains Malaysia, Institute for Research in Molecular Medicine (INFORMM), Kubang Kerian, Kelantan, Malaysia
(2) UMCAS, University Malaya, Kuala Lumpur, Malaysia
(3) General Praticioner, Klinik Dr Khafidz, Semenyih, Selangor, Malaysia

Methadone is an opiate agonist used world-wide for opioid dependence treatment. It undergoes extensive metabolism by several polymorphic enzymes. Its effect also is influenced by binding at receptor sites, specifically OPRM1. The clinical effect of methadone can therefore be variable and unpredictable. Several studies have suggested associations between plasma methadone concentrations and its therapeutics effects. The objective of this study therefore is to assess trough methadone plasma concentrations among routine patients undergoing MMT. This study was approved by the ethics committee at USM. One hundred and forty two plasma samples were collected. Blood was taken by direct venupuncture before patients took their daily doses. Plasma was separated and the concentration of methadone in plasma was determined by HPLC with MS-ion trap detector. The ion signal of m/z 310.2 and m/z407.2 were measured for methadone and carvedilol as internal standard, respectively. Recovery from plasma was 80%. Calibration curve was linear from 5 to 500ng/ml. CV within days and between days were less than 10%. Plasma methadone ranged from 0 to 1495ng/ml and averaged 351ng/ml (90% CI +40.4). The concentrations varied almost 1000-fold although daily doses only varied 8-fold (20 – 160mg + 3). Daily methadone doses poorly predicted plasma methadone concentrations and therefore probably also clinical effects. The wide variability in plasma concentrations suggests a need for plasma methadone concentration monitoring to better adjust methadone therapy.

Paper No.: 2480
Focus Group: P19 - GENERAL SESSION
A REVIST OF BLOOD PRESSURE CONTROL IN NIGERIAN HYPERTENSIVE POPULATION ON PHARMACOTHERAPY

Sunday Ogundele(1,2), O Ogunleye(1,2)
(1) Department of Pharmacology,Lagos State University College of Medicine, Lagos, Nigeria
(2) Department of Medicine,Lagos State University Teaching Hospital, Lagos, Nigeria

Hypertension is a leading non communicable disease in Nigeria with rising prevalence. It remains inadequately treated in the majority of patients with only about 30% achieving satisfactory controls. It therefore becomes necessary to perform periodic management audit of such population. Hence, this study examined the effectiveness of therapy after 3 months of initiation of management in a cohort of Nigerian black hypertensives. This was a cross sectional study of patients seen over a 18 months period between January 2008 and June 2009 at the hypertension clinic of the Lagos State University Teaching Hospital, Ikeja, Lagos. The hospital records of these patients were reviewed and relevant information extracted. The information collected was analysed using commercially available SPSS 15.0. Continuous variables were expressed as means (standard deviation), categorical variables as proportions. Comparisons of means were done with the student't' test. A p value of <0.05 was taken as significant. A total of 372 patients were studied with mean age of 56.81(13.23) years, male; 153(41.1%). 182 patients (48.9%) achieved systolic blood pressure (SBP) below 140mmHg and diastolic blood pressure (DBP) below 90mmHg at 3 months. The mean baseline SBP reduced significantly from 165.72(29.32) mmHg to 143.97(24.85) mmHg at 3 months, $p = 0.00$ while the mean DBP also reduced from 99.79(16.87) mmHg to 84.59(14.33) mmHg at 3 months, $p=0.00$. Most patients were on 2-3 drugs. This showed an improvement over earlier recorded levels of controls in the Nigerian population. A specialized unit for hypertension management produces better blood pressure controls.

Paper No.: 2671
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
SELECTION OF HEALTHY VOLUNTEERS FOR THEIR ENROLLMENT IN EARLY PHASE CLINICAL STUDIES

Giuseppe Pasqualetti, G Gori, F Boresi, C Castiglioni, C Conversano, P Pepe, C Blandizzi, M Del Tacca
University Hospital of Pisa, Clinical Pharmacology Centre for Drug Experimentation, Department of Internal Medicine, Pisa, Italy

Introduction. The medical features of a population of subjects invited to undergo a screening visit were evaluated, in order to create a database of healthy volunteers for conduction of early phase clinical investigations in an Italian Clinical Pharmacology Centre for Drug Experimentation. Patients. Putative healthy subjects were examined, according to standard operative procedures. The eligibility for being included into the database was determined by combined analyses of two distinct clinical and psychological evaluations. First, the subjects were visited by a physician in order to record medical history and perform physical examination. Subsequently, after compilation of MMPI-2 and SCL-90-R#053 psychopathological tests, subjects were evaluated by a clinical psychologist. Results. Study population consisted of 144 putative healthy volunteers (M 64%; F 36%; mean age 27.5 „b7 years) of predominant Caucasian race (99.3%). Subjects were University students (72.2%), employees (18.1%), free lancers (8.3%) or unemployed people (1.4%). There were 38 tobacco and 4 marijuana smokers. We identified 53/144 atopic subjects affected by drug (10.4%), food (2.8%) or environmental antigen (19.4%) allergies. One hundred fourteen subjects (79.2%) were found to be eligible for inclusion in our database as healthy volunteers. Subjects rejected for medical reasons (19/144) presented hepatic, renal and cardiovascular diseases or G6PD deficiency. The exclusions for psychopathological reasons (7/144) encompassed paranoid, somatization, sleeping or obsessive-compulsive disorders. Four subjects were excluded for both clinical and psychological reasons. Conclusion. Appropriate clinical and psychological methodologies are required for selecting healthy volunteers suitable for enrollment in early phase clinical studies.

Paper No. 3507
FOCUSED CONFERENCE GROUP: FC19 - GENERAL SESSION
OPTIMAL SAMPLING STRATEGY AND PHARMACOGENETICS IN PHARMACOKINETIC SIMULATIONS

Jiri Potucek, O Slanar, J Dousa
Charles University, 1st Faculty of Medicine, Department of Clinical Pharmacology, Prague, Czech Republic

The problem of 'Optimal Experiment Design' is very very important part of drug monitoring process in order to adjust optimal load dosage regiment for individual patient. The methods is based on mathematical analysis of measurement time schedule for drug monitoring process. The method is implemented in new generation of MWPharm Windows version project v. 4.xx. Several practical time schedules of recommended measurement time is illustrated on clinical examples. Subsequent vision 5.xx will include intraindividual pharmacokinetic variability due to known pharmacogenetic reasons. This method will further improve validity of PK estimations in specific population subgroups, which are important with regards to potentially different drug response in comparison with the general population.

Paper No. 3484
FOCUSED CONFERENCE GROUP: FC16 - NATURAL PRODUCTS: PAST AND FUTURE?
POTENT ROLE OF PIKRORHIZA KURROA IN PREVENTING "TOBACCO SMOKE" INDUCED ARTHRITIS: *IN VITRO* STUDY WITH THP-1 CELL LINE

Rachana Rachana, M Pant, N Kathuria, V Gnadhi, V Gupta
Biotech, JIIT, Noida, UP, India

The process of apoptosis in macrophages, present or recruited to the site, if imbalanced, can leads towards pathological symptoms of arthritis. Smoking has been reported to have link with arthritis (Tuomi T, Ann Rheum Dis 1990 49: 753-756). In the present study, TPA induced THP-1 cell lines were exposed to tobacco smoke in 96 cell well plate. 0.5% and 1% "smoke concentrate" induced cell death in macrophages (60 and 66 %

resp). Cells pre-exposed to ethanolic extract of Pikrorhiza kurroa (PKE) for 1 hr, were prevented from cell death. 400 µg/ml PKE was also able to enhance the cell viability to 172%, and in presence of serum (in growth media), this was 159%, in comparison to their respective controls. While exposing to smoke, it was observed that, 100 µg/ml of PKE was effective in prevention of cell death in absence of serum but not in presence of serum (with respect to their controls). PKE (400 µg/ml) was able to prevent cell death caused by 0.5% and 1% smoke concentrate, and enhance the cell viability by ~157%, in absence and in presence of serum (with respect to their controls). In past, in Indian traditional medicine PK is used for the treatment of arthritis and other ailments. The future goal of this study is to investigate the mechanism of action of PKE for preventing cell death and also its anti-inflammatory activity (Beukelman, C.J.Annals of Gastroenterology 2002, 15(4):320 - 333), Pikrorhiza kurroa could be a potent source of anti-arthritic molecule in future.

Paper No. 3457
FOCUSED CONFERENCE GROUP: FC15 - ENDOTHELIUM IN HEALTH AND DISEASE
THE NEW NITRIC OXIDE (NO) DONOR CIS-[RU(DCBPY)2(CL)(NO)] (DCBPY-NO) PRESENTS CYCLIC ACTIVITY IN RELEASING NO BY NITRITE

Gerson J Rodrigues(1), SA Cicillini(2), RS Silva(2), LM Bendhack(2)
(1) University of São Paulo School of Medicine of Ribeirão Preto, Department of Pharmacology, São Paulo, Brazil
(2) University of São Paulo School of Medicine of Ribeirão Preto, Department of Physics and Chemistry, São Paulo, Brazil

The NO donor DCBPY-NO, synthesized in our laboratory, is converted to the stable complex DCBPY-NO2 which generates NO forming DCBPY-H2O, that in the presence of nitrite is converted to DCBPY-NO2. This study aimed to investigate NO generation by DCBPY in the presence of nitrite and to identify the NO species involved in the vasodilatation. Concentration-effect curves to DCBPY-NO2, DCBPY-H2O in the presence or absence of 1µM NaNO2 (that does not induce vasodilatation) and NaNO2 were constructed in pre-contracted aortas. To verify the NO specie generated by DCBPY-NO2 and NaNO2, we used scavenger hydroxocobalamin (NO°), L-cysteine (NO-) and Oxihemoglobin (HbO2), extracellular NO. Aortas were relaxed with similar efficacy (ME) by DCBPY-NO2 (ME:103.1±1.2%;n=9) and NaNO2 (ME:105.1±1.4%,n=10). However, the potency (pD2) of DCBPY-NO2 (pD2:5.71±0.06,n=9,P<0.001) was higher than NaNO2 (pD2:4.52±0.07,n=10). As expected, compound DCBPY-H2O had no effect, however with NaNO2 the relaxation was induced, but it was lower (pD2:4.99±0.20,P<0.001,ME:84.8±9.2%,n=5,P<0.001) than DCBPY-NO2. DCBPY-NO2 with NaNO2 presented greater potency (pD2:6.01±0.08,n=6,P<0.01) without changes in the ME. Relaxation induced by DCBPY-NO2 was almost abolished by hydroxocobalamin (ME:14.2±2.0%,n=5;P<0.001) and HbO2 (ME:14.1±1.3%,n=5;P<0.001). L-cystein reduced only the potency to DCBPY-NO2 (pD2:5.03±1.93;n=6;P<0.01). L-cystein and HbO2 did not modify the relaxation induced by NaNO2, but hydroxocobalamin reduced only its potency (pD2:4.03±0.08,n=5,P<0.01). In conclusion, the compound DCBPY has cyclic activity of NO generation in the presence of nitrite. Nitrite from NaNO2 is converted to intracellular NO° and nitrite from DCBPY-NO2 is converted to extracellular NO°. The compound DCBPY-NO2 is not a nitrite donor since its potency is higher than NaNO2.
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Paper No.: 3169
Focus Group: P19 - GENERAL SESSION
QUESTIONNAIRE FOR THE DETECTION OF APPLIED KNOWLEDGE IN PHARMACOLOGY

Jose A Rojas-Ramírez(1), JA Molina-Guarneos(1), N Ramírez-López(2), P Epinoza-Alarcon(3)
(1) UNAM Faculty of Medicine, Department of Pharmacology, Mexico City, Mexico
(2) UNAM Faculty of Medicine, Secretariat of Medical Education, Mexico City, Mexico
(3) Unidad de Investigación Educativa CMN SXX1 IMSS, Mexico City, Mexico

The knowledge that the students of medicine acquires on the preclinical subjects have been designed as a support for applying this knowledge onto the clinical subjects. Nevertheless, so far its known, it has not been elucidated the extent in which the students acquire the

ability to apply this knowledge on clinical subjects. With the aim to evaluate the mentioned ability, an evaluation questionnaire was applied. It was chosen the type of True-False-Not known questions, that has proven to induce a reflection in the student (Viniegra L, Rev Invest Clin 1979; 31:413-20). It was elaborated an evaluation questionnaire of 79 questions that has been applied before and after the pharmacology course to four groups of the second year of medical career, two from high-efficiency program and two from regular program. Additionally it was applied to the students of two groups from humanity areas. In the pre-course application medical students obtained an average score of 27.83±2.57 points, with no significant differences among the groups. In the post-course application only one of the groups high-efficiency program obtained a higher score compared to the regular groups. The score of the humanity areas groups was 13.05±2.0, being significantly lower than the one obtained by medical students. From this results it is concluded that the questionnaire discriminate medical students from students of other areas. Also it distinguishes the abilities obtained among the medical groups. It is proposed that this questionnaire may be useful to measure the repercussion that will be further applied.

Paper No.: 3454
FOCUSED CONFERENCE GROUP: P11 - G PROTEIN-COUPLED 7TM RECEPTORS: FROM MOLECULAR TO PHYSIOLOGICAL FUNCTION
RECEPTORS FOR THE ACTIONS OF PROSTAGLANDIN E_2 IN GUINEA-PIG TRACHEA

Jesper Säfholm, K Stark, L-O Cardell, S-E Dahlén, M Adner
Karolinska Institute, The National Institute of Environmental Medicine and The Centre For Allergy Research, Experimental Asthma and Allergy Research, Stockholm, Sweden

Prostaglandin E_2 (PGE_2) may contract and relax airway smooth muscle via four different receptors (EP_1-EP_4). The receptors involved have remained unclear due to lack of sufficiently selective antagonists. The study characterized the actions of PGE_2 in the guinea-pig trachea by the use of new class of potent and selective drugs. Isometric responses were assessed in tracheal segments from male guinea-pigs kept under a resting tension of 30 mN. The effects of the selective EP_1 (ONO-8130), EP_3 (ONO-AE5-599) or EP4 (ONO-AE3-208) antagonists on responses to PGE_2 or EP_2 agonist ONO-AE1-259-01 were assessed (n=7 for each intervention and concentration of drug). The concentration-response curve for PGE_2 was bell-shaped with contractions at lower concentration and relaxation at higher with potency (pEC50-values) of 8.2±0.2 and 6.9±0.3, respectively. Pre-treatment with EP_1 antagonist (0.1 to 10 nM) resulted in a concentration-dependent reduction of the maximal contraction with no effect on relaxation. The EP_1 antagonist (10nM) also decreased the basal tone (33±5%), similar to that of 3μM indomethacin (31±8%). The pEC50-values for both contraction and relaxation to PGE_2 were unaffected by pretreatment with either EP_3 antagonist (8.1±0.3 and 6.6±0.2, respectively) or EP_4 antagonist (8.3±0.2 and 6.5±0.2, respectively). The EP_2 agonist did not evoke contraction but relaxed carbachol pre-contracted preparations in a concentration-dependent manner that was unaffected by antagonists of EP_1, EP_3 or EP_4 receptors. The EP_1 receptor mediates contraction and maintains basal tone of guinea-pig trachea. The EP_2 receptor mediates relaxation and is a potential target for bronchoprotective therapy.

Paper No.: 3384
FOCUSED CONFERENCE GROUP: P05 - TRANSLATIONAL SCIENCE IN THE METABOLIC SYNDROME: BASIC AND CLINICAL PHARMACOLOGY

ABCC8 POLYMORPHISM (SER1369ALA): INFLUENCE ON SEVERE HYPOGLYCEMIA DUE TO SULFONYLUREAS

Ryosuke Sato(1), R Genma(2), N Inui(3), K Takeuti(3), H Nakamura(1), M Maekawa(4), H Watanabe(3)
(1) Hamamatsu University School of Medicine, Department of Endocrinology and Metabolism, Hamamatsu, Japan
(2) Seirei Hamamatsu General Hospital, Department of Endocrinology and Metabolism, Hamamatsu, Japan
(3) Hamamatsu University School of Medicine, Department of Clinical Pharmacology, Hamamatsu, Japan
(4) Hamamatsu University School of Medicine, Department of Laboratory Medicine, Hamamatsu, Japan

Background: Sulfonylureas are widely used for the treatment of diabetes mellitus and categorized according to their binding sites of the ATP-sensitive K^+ channel (K ATP channel) complex. The binding sites are classified into A, B, and A+B site, respectively. Ser1369Ala variant in the ABCC8 gene that encodes sulfonylurea receptor 1, a subunit of the K ATP channel, has been shown to be associated with the hypoglycemic effect of gliclazide, A site binding sulfonylurea. However, the clinical influence of Ser1369Ala variant on the treatment with A+B site binding sulfonylureas, such as glimepiride or glibenclamide, is still uncertain. Method: In a case-control study, 32 patients with type 2 diabetes admitted

to the hospital with severe hypoglycemia and 125 consecutive type 2 diabetic outpatients without severe hypoglycemia were enrolled. We determined the genotypes of the ABCC8 polymorphism (Ser1369Ala) in the patients with or without severe hypoglycemia. All of the patients were taking glimepiride or glibenclamide. Results: In the patients treated with glimepiride or glibenclamide, we found no significant differences in the distributions of Ser1369Ala genotype between the patients with or without severe hypoglycemia (p-value 0.26). Moreover, Ala1369 minor allele tended to be less frequent in the hypoglycemic group (31% vs. 43%; OR: 1.65; 95% CI: 0.92-2.96; p-value 0.09). Conclusion: Our finding suggests that Ser1369Ala variant is not a major predictive factor of severe hypoglycemia due to A+B site binding sulfonylureas, such as glimepiride or glibenclamide.

Paper No.: 3471
FOCUSED CONFERENCE GROUP: P17 - NEW APPROACHES AND TARGETS IN PSYCHIATRY
ASSESSMENT OF THE ATYPICAL NEUROLEPTIC RISPERIDONE IN PRECLINICAL MODELS FOR SCHIZOPHRENIA AS COMPARED TO ITS CENTRAL EXPOSURE AND MOTOR IMPAIRMENT IN RATS

Chantal Schlumberger(1), M Pietraszek(1), D Schäfer(1), S Greco(2), J Nagel(1), W Danysz(1),
(1) Merz Pharmaceuticals, R&D CNS, In vivo Pharmacology, Frankfurt, Germany
(2) Merz Pharmaceuticals, R&D CNS, Biological Analytics, Frankfurt, Germany

Schizophrenia is a psychiatric disorder treated by typical (e.g. haloperidol) and/or atypical neuroleptics (e.g. risperidone (RISP)). Here, we assessed the concentrations of our research tool RISP in the brain following i.p. administration using brain microdialysis in Sprague-Dawley rats. Behavioural effects of RISP were measured in the conditioned avoidance response (CAR) and stimulant-induced hyperactivity in the open field (OF) as well as in catalepsy in order to compare central exposure to behavioural effects. Microdialysis revealed that i.p. administration of RISP (0.3, 1 and 3 mg/kg) led to a peak brain concentration of 9.86 ± 2.24; 53.58 ± 8.35 and 210.57 ± 45.69 nM respectively, thus after 0.3 mg/kg, it exceeds by c.a. 6-fold the reported affinity for D2 or 5HT2a receptors (Horacek et al., 2006; Arnt & Skarsfeldt, 1998). RISP disrupted the CAR at doses of 0.3 & 1 mg/kg (escape failures: 1 mg/kg RISP only). At doses of 0.1 – 3 mg/kg it inhibited amphetamine (3 mg/kg i.p.)-induced hyperlocomotion and at 1 and 3 mg/kg ameliorated the increase in vertical activity (rearings). Phencyclidine (5 mg/kg i.p.)-induced hyperlocomotion was inhibited at 1 and 3 mg/kg i.p. RISP alone decreased spontaneous horizontal exploration at all doses. Finally, RISP elicited cataleptic behaviour at (1 and) 3 mg/kg as measured by the descent latency from podium, bar and grid. In summary, atypical neuroleptic RISP shows effectiveness in models for schizophrenia however sometimes at doses already eliciting adverse effects underlining the necessity for multiple tests to yield a reliable assessment of a compound's therapeutic index.

Paper No. 2651
FOCUSED GROUP: FC09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
IMPACT OF SOLUBLE ADENYLYL CYCLASE ON APOPTOSIS OF B LYMPHOMA CELLS

Martina Schmidt(1), M Grandoch(2), A-A Weber(2), J Fischer(2)
(1) University of Groningen, Department of Molecular Pharmacology, Gronningen, The Netherlands
(2) University of Essen Hospital, Institute of Pharmacology, Essen, Germany

In murine immature WEHI-231 cells we recently reported that activation of extracellular signal-regulated kinases ERK1/2 and the phosphoinositid 3-kinase effector protein kinase B (PKB)/Akt by the B cell receptor (BCR) was regulated by the novel cAMP effector Epac (exchange protein directly activated by cAMP). Consequently, we studied the role of cAMP and Epac on BCR-induced early and late responses in the human B lymphoma cell line Raji. As cAMP and Epac act as mediators in this signaling cascade in both cell lines, we aimed next to identify the adenylyl cyclase (AC) being responsible for the BCR-induced cAMP production in B lymphocytes (transmembrane AC (tmAC) versus soluble AC (sAC)). We demonstrated the expression of sAC by immunostaining with a specific antibody directed against mammalian sAC using western blot and immunofluorescence. Furthermore, studies with the sAC-specific inhibitor KH7 permitted to distinguish between sAC and tmAC-mediated effects. Preincubation of both cell types, WEHI-231 and Raji, with the inhibitor significantly diminished BCR-induced ERK1/2

phosphorylation and Rap1 activation. Importantly, inhibition of sAC by KH7 enhanced BCR-induced apoptosis, whereas inhibition of ACs by SQ22536 had the opposite effect. Our results indicate an involvement of sAC and Epac in BCR-induced responses in murine as well as in human B lymphocytes. In addition, our data indicate that cAMP and Epac might exert pro-and anti-apoptotic signaling properties in B lymphocytes. Funded by an Rosalind Franklin Fellowships and the DFG.

Paper No.: 3459
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
DOES DEEP BRAIN STIMULATION OF SUBSTANTIA NIGRA SUPPRESS TONIC EPILEPTIC SEIZURES?

Safa Shehab, A Al- Nahdi, F Al-Sharif Al-Zaabi, F Al-Mugaddam, M Al-Sultan, M Ljubisavljevic
United Arab Emirates University, Faculty of Medicine & Health Sciences, Al-Ain, United Arab Emirates

A new method to treat epileptic patients is deep brain stimulation (DBS). Recent studies showed that DBS of the substantia nigra reticulata (SNr) in rats has an anticonvulsant effect on forebrain clonic seizures. The aim of this study was to determine whether DBS of SNr could also suppress tonic motor seizures evoked in hindbrain structures. The precise mechanism of DBS is not yet fully understood but electrical stimulation with high frequency often mimics the effects of ablation of a particular area of the brain. Consequently, in the first experiment the effects of different stimulation frequencies (80, 130, 260 and 390 Hz) on neuronal activation induced specifically in SNr, using c-fos immunocytochemistry, were determined. The results showed that the stimulation of the SNr with 80 Hz has no inhibitory effect while stimulation with 130, 260 and 390 Hz produced a remarkable suppressive effect compared with the control unstimulated side. The aim of the second experiment was to determine whether the inhibition of SNr bilaterally with DBS could suppress tonic seizures induced by electrical shock. Statistical analysis showed that the mean tonic seizure scores following stimulation at either 130 or 260 Hz were not significantly different from scores following the application of the electrode without the current. The data suggest that DBS of the SNr produced neuronal inhibition but failed to suppress tonic seizures. In conclusion, DBS of SNr with current frequencies used in this study might not be the best choice for treating patients who suffer from epileptic tonic seizures.

Paper No.: 3465
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
CHARACTERISING THE PHARMACOLOGICAL CARDIOVASCULAR SAFETY PROFILE OF IPRATROPIUM FOR THE TREATMENT OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Katherine Lindsay Simmons, J Khan, A Hussain, H Maddock
Coventry University, Biomolecular Sciences Applied Research Group, Coventry, UK

Chronic Obstructive Pulmonary Disease (COPD) is a disease characterised by inflammation of the airways which accounts for the 4th highest cause of global death. The functional role of Muscarinic receptor antagonists such as Ipratropium Bromide (Atrovent®) and Tiotropium Bromide (Spiriva®) have been shown to successfully improve pulmonary function in COPD patients. Recent studies have associated an increased risk of myocardial infarction or stroke in COPD patients currently receiving treatment with Muscarinic receptor antagonists such as Ipratropium. The aim of the study was to profile the effects of Ipratropium on the myocardium subjected to ischaemia-reperfusion conditions. Langendorff hearts were subjected to ischaemia followed by reperfusion where the non-specific muscarinic receptor antagonist Ipratropium was administered throughout reperfusion (1nM, 10nM or 100nM). Hearts underwent triphenyl tetrazolium staining for infarct size assessment. In further studies cardiomyocytes were exposed to simulated ischaemia-reoxygenation in the absence or presence of Ipratropium (1fM-1mM) and cellular injury was determined by measurement of live/death ratio and apoptosis. Administration of Ipratropium (10nM or 100nM) throughout reperfusion significantly increased infarct size to risk ratio (%) compared with controls (62±2% and 74±4% vs. 52±3% Control $P<0.01$ respectively). In isolated cardiomyocytes, Ipratopium treated groups were observed to significantly increase apoptosis and cell death compared to non-treated controls. This is the first pre-clinical study to indicate that Muscarinic receptor antagonists like Ipratropium significantly increase myocardial injury when administered during ischaemia-reperfusion. Further studies are required to determine the cellular mechanism via which muscarinic antagonists mediate myocardial injury in conditions of ischaemia-reperfusion.

Paper No.: 1538
FOCUSED CONFERENCE GROUP: PW23 - APPLYING PHARMACOGENOMICS (PGX) FROM RESEARCH INTO CLINICAL PRACTICE: PRESENT AND FUTURE METHODS FOR DETERMINATION 6 β-HYDROXYCORTISOL/CORTISOL RATIO IN HUMAN URINE BY LC-MS

Valery V Smirnov, GV Ramenskaya, DA Suchev, AU Savchenko
Sechenov Moscow Medical Academy, Pharmaceutical Department, Moscow, Russian Federation

The given methods have been developed for the determination of isoenzyme CYP 3A4 activity. Endogenous 6 beta-hydroxycortisol appear from cortisol under the action of CYP 3A4 only. The ratio of 6 beta-hydroxycortisol/cortisol in urine speaks about its activity. Low values of the 6 beta-hydroxycortisol/cortisol ratio correspond to low activity CYP3A4, and high values correspond to high activity. Change of CYP3A4 activity can have clinical consequences at drug application. Determination of CYP3A4 activity allow for choose a medicine and adjust its dose. For determination 6 beta-hydroxycortisol and cortisol has been chosen LC/MS. For the assay from patients was selected first void urine before and during treatment. Liquid-liquid extraction from 2 ml of urine was then performed with an 4 ml ethyl acetate / isopropanol mixture (85/15), repeated twice. The assay spent on Agilent 1200 LC/MS. The mix the acetonitril /water acidified by formic acid (45/55) has been chosen as mobile phase. Quantitative definition was spent by absolute calibration. The validation of methods has been made. As a result of the work the validate method of definition endogenous cortisol and 6 beta-hydroxycortisol in urine has been developed. The given method excludes necessity of administration of any medicine; therefore it's safe for the patient (and even at pregnant and breast - feeding women and infants). Advantage of this method is also possibility of collection samples (first void urine) in home conditions.

Paper No.: 3439
FOCUSED CONFERENCE GROUP: P02 - TRANSMEMBRANE TRANSPORT: PERSPECTIVES FOR DISEASE AND DRUG DISCOVERY
MINERALOCORTICOID RECEPTOR DEGRADATION IS PROMOTED BY HSP90 INHIBITION AND THE UBIQUITIN-PROTEIN LIGASE CHIP

Olivier Staub, N Faresse
University of Lausanne, Department of Pharmacology & Toxicology, Lausanne, Switzerland

The mineralocorticoid receptor (MR) plays a crucial role in the regulation of Na^+ balance and blood pressure, as evidenced by gain of function mutations in the MR of hypertensive families. In the kidney, aldosterone binds to the MR, induces its nuclear translocation, and promotes a transcriptional program leading to increased transepithelial Na^+ transport via the epithelial Na^+ channel ENaC. In the unliganded state, MR is localized in the cytosol and part of a multiprotein complex, including Hsp90, which keeps it ligand binding competent. 17-AAG is a benzoquinone ansamycin antibiotic that binds to Hsp90 and alters its function. We investigated whether 17-AAG affects the stability and transcriptional activity of MR and consequently Na^+ reabsorption by renal cells. 17-AAG treatment lead to reduction of MR protein level in epithelial cells *in vitro* and *in vivo*, thereby interfering with aldosterone-dependant transcription. Moreover, 17-AAG inhibited aldosterone-induced Na^+ transport, possibly by interfering with MR availability for the ligand. Finally, we identified the ubiquitin-protein ligase CHIP as a novel partner of the cytosolic MR, which is responsible for its polyubiquitylation and proteasomal degradation in presence of 17-AAG. In conclusion, 17-AAG may represent a novel pharmacological tool to interfere with Na^+ reabsorption and hypertension.

Paper No.: 3502
FOCUSED CONFERENCE GROUP: P02 - TRANSMEMBRANE TRANSPORT: PERSPECTIVES FOR DISEASE AND DRUG DISCOVERY

DEVELOPMENT OF A HIGH THROUGHPUT PEPT1 ASSAY USING VOLTAGE-SENSITIVE FLUORESCENCE

Mohammed Ullah, K Luetchford, K Fenner
Pfizer Global R&D, Sandwich, Kent, UK

The mammalian proton-coupled peptide transporter, PepT1, is expressed in the intestinal apical barrier

where it is responsible for the absorption of dietary di- and tri-peptides, and is a major route for oral drug absorption. Several classes of compound are known to interact with PepT1, including the beta-lactam antibiotics and angiotensin-converting enzyme inhibitors, as well as some pro-drugs. We have developed a high throughput, fluorescence based PepT1 functional assay, allowing the identification of potential substrates and inhibitors of PepT1. This assay is based on the detection of membrane depolarization that occurs as a result of PepT1 transport activity, which involves the translocation of protons across the cell membrane along with substrate. In order to achieve the required sensitivity for this assay, we transfected HeLa cells with human PepT1. A high level of stable PepT1 expression was achieved by using a Lentiviral transduction approach, allowing miniaturization of the assay to 384-well format. Control (non-transfected) cells are used to determine non-specific membrane potential changes. Upon PepT1 activation, dose-dependent membrane depolarization was observed using test substrates. This assay may thus be of use for many projects that require assessment of PepT1 interaction.

Paper No.: 1837
FOCUSED CONFERENCE GROUP: P16 - NATURAL PRODUCTS: PAST AND FUTURE?
ADVERSE EVENTS AND INTERACTIONS DUE TO CHINESE HERBAL DRUGS IN ITALY. A THREE-YEAR PHARMACOEPIDEMIOLOGY, PHARMACOGENOMIC AND PHARMACOVIGILANCE SURVEY

Alfredo Vannacci(1,2), F Lapi(1,2,3), E Gallo(1,2), A Pugi(1,2), E Lucenteforte(1,2), F Firenzuoli(4), V Mascherini(4), R Raschetti(5), A Mugelli(1,2), F Menniti-Ippolito(5)
(1) University of Florence, Department of Preclinical and Clinical Pharmacology, Florence, Italy
(2) Tuscan Regional Centre of Pharmacovigilance, Florence, Italy
(3) Regional Authority for Healthcare Services of Tuscany, Epidemiology Unit, Florence, Italy
(4) Centre for Natural Medicine, ASL 11 Hospital, Empoli, Italy
(5) National Institute of Health, National Centre of Epidemiology, Rome. Italy

Chinese herbal medicine is traditionally one of the more important treatment modalities used in Traditional Chinese Medicine (TCM). Usually, Chinese Herbal Medicines (CHMs) are composed of different herbs tailored to the individual patient. Despite its difficult standardization, popularity of CHM in western countries largely increased in the last years and several studies were conducted to assess its efficacy and safety. Aside with its therapeutic potential, several safety concerns were arisen, and although massive databases of genomic, proteomic and chemical data are now available to study and identify the structure of active compounds from herbal drugs, still little is known on pharmacognosy and pharmacogenomics of most CHMs. Here we report preliminary results of a three-year research aimed to assess pharmacoepidemiology of CHM in Italy, conducted by means of a Prescription Event Monitoring (PEM) survey. Phases of the research include: 1.Realization of a National Registry for CHM practitioners. 2.Epidemiological survey on the prevalence of use of CHM. 3.Short-, medium- and long-term evaluation of the efficacy and safety of CHMs in the frame of the PEM Survey. 4.Pharmacological researches aimed at describing pharmacodynamic, pharmacokinetic and pharmacogenetic issues of main CHMs. Results of the first half year of the project will be reported in the current communication, with particular regard to the set up of a national registry of CHM qualified practitioners, and to preliminary results of the epidemiological survey on the prevalence of CHM use Italy. Research supported by a Young Researchers' grant from Italian Ministry of Health.

Paper No. 3490
FOCUSED CONFERENCE GROUP: FC09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
GENE THERAPY WITH 2,3-INDOLEAMINE DIOXYGENASE (IDO) DIMINISHED ACUTE REJECTION AND PRE-FIBROSIS FOLLOWING ALLOGENEIC KIDNEY TRANSPLANTATION

Diana Vavrincova-Yaghi, LE Deelman, H van Goor, M Seelen, IP Kema, A Smith-van Oosten, D de Zeeuw, RH Henning, M Sandovici
University Medical Center Groningen, Department of Clinical Pharmacology, Groningen, The Netherlands

IDO, the rate limiting enzyme in the tryptophan catabolism, has recently emerged as an important immunosuppressive molecule, involved in the regulation of both physiologic, as well as pathologic processes. Accumulating evidence exists that cells expressing IDO can suppress T-cell responses and promote tolerance. Here we investigate the effects of IDO on the acute rejection of the transplanted kidneys, using an adenovirus-mediated gene delivery approach. The experiments were performed in a rat Fisher to Lewis acute rejection model of renal

transplantation. RGD modified adenovirus carrying IDO gene (RGD-AdTIDO, n=9/group) or RGD modified adenovirus carrying gene for GFP (RGD-AdTL, n=8/group) were injected into the renal artery of the donor kidney before transplantation. A group receiving no treatment (saline, n=8/group) served as a control. Rats were sacrificed after 6 days. Enzymatic activity of IDO and its expression was assesed using HPLC and western blotting. Succesful gene delivery was confirmed with real time PCR and immunohistochemistry. Local IDO gene therapy significantly decreased elevated plasma creatinine compared to the saline injected group and RGD-AdTL group (93.7±18.9 μmol/l for RGD-AdTIDO, 228.3±46.4 for saline and 248.2±43.6 for RGD-AdTL). Moreover, therapy with IDO reduced the infiltration of cytotoxic CD8+ T cells and macrophages into the graft and diminished renal interstitional pre-fibrosis. Also, IDO gene therapy limited up-regulation of IL-2, KIM-1 and TGF-β mRNA, compared to the saline and RGD-AdTL groups. The present study demonstrates for the first time that IDO (over)expression in the renal graft improves renal function and morphology in a clinically relevant model of acute rejection.

Paper No. 3491
FOCUSED CONFERENCE GROUP: FC19 - GENERAL SESSION
IMPROVED MYOGENIC CONSTRICTION OF MESENTERIC ARTERY AFTER EGF RECEPTOR BLOCKER TREATMENT IN 5/6 NEPHRECTOMIZED RAT

Peter Vavrinec, N Ulu, S Landheer, H Buikema, R van Dokkum, R Henning
University Medical Center Groningen, Department of Clinical Pharmacology, Groningen, The Netherlands

Introduction: Myogenic constriction (MC) refers to ability of small arteries to constrict with elevated pressure. It is important part of the autoregulation of vascular system. Our group has previously shown decreased MC of mesenteric arteries after 5/6 nephrectomy (5/6Nx) in rat, whereas therapy with ACE-inhibitors and AT1 receptor blockers is effective in preventing loss of MC. Epidermal growth factor receptor (EGFR) is involved in calcium signaling and even more, EGFR can transactivate the AT1 receptor. We wanted to test whether chronic blocking of EGFR is able to lower blood pressure after 5/6Nx and whether this would lead to prevention from loss of MC of mesenteric artery. Methods: Wistar rats underwent 5/6Nx (5/6Nx group) or sham (sham group) operation and where treated with an EGFR-blocker - PKI-166 (PKI group), ACE-inhibitor-lisinopril (lis group) or vehicle. Twelve weeks after 5/6Nx, mesenteric arteries were mounted in a perfused vessel setup for determination of MC. Furthermore, systolic blood pressure (SBP) and proteinuria were assessed. Results: EGFR-blocker treatment decreased SBP (159±9 vs 181±4 mmHg for PKI and 5/6Nx respectively; $p<0,05$) and restored MC to values of sham operated or ACE-inhibitor treated animals (% of max MC: 4,6±1 for 5/6Nx; 10,6±1,6 for PKI; 11,9±,9 for lis and 13,7±1,1 for sham; all $p<0,05$ vs 5/6Nx). However, indicators of renal function (proteinuria and creatinine clearance) were unaffected by EGFR-blocker treatment. Conclusion: We have shown for the first time, that EGFR antagonism lowers increased blood pressure and protects mesenteric artery from loss of myogenic constriction after 5/6Nx in rat.

Paper No.: 3472
FOCUSED CONFERENCE GROUP: P11 - G PROTEIN-COUPLED 7TM RECEPTORS: FROM MOLECULAR TO PHYSIOLOGICAL FUNCTION
STRUCTURE-ACTIVITY RELATIONSHIPS FOR NEGATIVE EFFICACY AT DELTA AND MU OPIOID RECEPTORS

Vanessa Vezzi(1), R Guerrini(3), G Balboni(3), P Molinari(2), S Salvadori(3), T Costa(2)
(1) University of Rome 'Sapienza', Department of Biochemistry, Rome, Italy
(2) Istituto Superiore di Sanità, Rome, Italy
(3) University of Ferrara, Italy

The delta opioid (DOP) receptor exhibits a considerably greater level of constitutive activity than the mu opioid (MOP) receptor. Although a number of ligands that have efficacy in suppressing the spontaneous activity of DOP receptor (e.g.inverse agonists) are known, a full understanding of the structural determinants that are important for negative efficacy in DOP and MOP pharmacophores is still missing. In this study we have used an assay based on resonance energy transfer (RET) between luciferase-tagged MOP or DOP receptors and fluorescently-tagged

Gbeta1 subunits to measure precisely the negative efficacy of ligands based on the 2,6-dimethyltyrosine-1,2,3,4-tetrahydroquinoline-3-carboxylate (Dmt-Tic) pharmacofore. Basal RET signal was enhanced by agonists and inhibited by negative antagonists, and both effects were prevented in the presence of a neutral antagonist such as naltrindole. GDP abolished constitutive receptor signals both at DOP and MOP receptor, thus marking the maximal level of negative efficacy of the system. Of the 35 analogues analysed in this study, 17 were inverse agonists displaying a wide range of negative efficacy for DOP receptors, but only two exhibited negative antagonism also at MOP receptors. N,N-dimethylation of the Dmt moiety of the molecules or the C-terminal substitution with certain amino acid residues bearing a free carboxyl group, are modifications capable to convert DOP agonists into powerful inverse agonists, and indicate two major independent determinants where the emergence of negative and positive efficacy can be controlled and modulated.

Paper No.: 3488
FOCUSED CONFERENCE GROUP: P17 - NEW APPROACHES AND TARGETS IN PSYCHIATRY
***IN VITRO* DIAGNOSTIC TESTS TO STRATIFY POPULATIONS OF PATIENTS SUFFERING FROM PSYCHIATRIC DISORDERS AND DRUG COMPANION TESTS TO PREDICT CNS DRUG SAFETY RESPONSE**

Dinah Weissmann, L Vincent, L Cavarec, C Plusquellec, C Leborgne, F Allemand, JF Pujol
BIOCORTECH, Paris, France

Biologic diagnostic of psychiatric disorders is today an unmet medical need since the only diagnostic available is clinic. Patients suffering from psychiatric disorders constitute heterogeneous populations, respectively to prognosis and to disease management. In the vast majority of cases, neither patient heterogeneity nor pharmacogenomics are entirely dependent of genetic differences and functional biomarkers are lacking to stratify patients populations respectively to drug selection and to drug response. Biocortech built a biomarker discovery platform exploiting the properties of RNA editing to discover novel biomarkers to address such needs and initiated clinical validation studies, respectively. Serotonin 5HT2C receptors, as other pharmacologically relevant receptors undergo a post-transcriptional modification known as RNA editing which regulates receptor function by modulating the distribution of isoforms that differ from each other in the combinations of substitutions of certain nucleotides at specific mRNA sites. Editing profiles are altered by disease conditions and by pharmacological agents, and could thus be turned into 'signatures' associated to certain disorders or to drug response. We overcame the technical challenge of measuring editing and fully characterized 5HT2c mRNA receptor editing profile that theoretically encompass 32 isoforms, in cells from various brain structures, and identified 5HT2C receptor editing-related biomarkers that are testable by blood sampling. A significant signature has been determined in suicides attempters from post mortem brain section. We initiated trials in various clinical settings such as in interferon α treated patients, suicide attempters, and patients treated with specific antidepressant regimens.

Paper No. 1791
FOCUSED GROUP: FC19 - GENERAL SESSION
STEREOTYPY IN THE DEER MOUSE, CORRELATION WITH CORTICO-STRIATAL OXIDATIVE STATUS AND DOPAMINE TURNOVER, AND RESPONSE TO COMBINED FLUOXETINE/RISPERIDONE TREATMENT

De Wet Wolmarans(1), M Guldenpfennig(1), B Harvey(1), D Stein(2), J du Preez(1)
(1) North-West University, Department of Pharmacology, Potchefstroom, South Africa
(2) University of Cape Town, South Africa

The deer mouse presents with stereotypical movements, that are variable within a population, and which closely resemble behaviours seen in OCD. OCD is linked to altered redox status. Since dopamine can promote oxidative stress and also increase stereotypies, we postulated that deer mouse stereotypy may be correlated with abnormalities in this regard. Deer mice were separated into different stereotypical cohorts. Frontal DOPAC and HVA, as well as oxidized GSSG and reduced GSH glutathione were determined by tandem LC-MS. SOD activity was determined using a SOD kit. Saline, fluoxetine (5mg/kg/day), risperidone (2mg/kg/day), and a

combination of these, were administered to high stereotypical mice for 3 weeks. Behavioural analyses were performed, animals sacrificed, and the above mentioned neurochemical parameters determined. While chronic treatment with fluoxetine separated from control in the 3rd week, with risperidone being completely ineffective. The combination though was effective in attenuating stereotypy. This provides evidence for the predictive validation of the model. Cortical GSH and GSSG levels were lower in HSB mice, compared to NS mice, and showed a significant correlation with the severity of stereotypy, suggesting a deficient glutathione system in this stereotypy.

Paper No.: 1120
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION - HEPATOLOGY

EFFECTS OF HYPERIN ON REPLICATION OF HBV IN HEPG2.2.15 CELL AND IMMUNOREGULATORY

Xin-Bo Yang(1), K Huang(1,3,4), Z-M Huang(2), M Geng(1), H-Y Chen(1), J-H Wang(1), J-R Yang(3)
(1) Institute of Geriatrics, General Hospital of PLA, Beijing, PR China
(2) Hospital 302 of PLA, Department of Pharmacy, Beijing, PR China
(3) Wannan Medical College, Anhui Wuhu, PR China
(4) Institute of Materia Medica, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, PR China

Introduction: The present study is to examine hyperins inhibitive effects of replication of hepatitis B virus (HBV) in vitro and the impact on the immunological function in vivo. Methods: 1. In vitro test: The HepG2.2.15 cells were treated by hyperin for 8d, the cells and medium of the 4th, 8th day and 4th day following drug withdrawal were collected, respectively. The contents of HBeAg and HBsAg in the medium were measured, the levels of HBV-DNA and cccDNA in HepG2.2.15 cells were all detected by Quantitative real-time PCR, respectively. 2. *In vivo* tests: The acute hepatic injury model was reproduced by injection of ConA into mice.In order to observe the protective effect of hyperin against the acute immunologic liver injury in mice, the percentages of CD3+, CD4+ and CD8+ in the peripheral blood,the levels of cell factors and the contents of AST and ALT in serum were measured. Rerults: 1. In vitro test: The inhibition rates of Hyp on HbeAg and HBsAg in the HepG2.2.15 cells were 73.9% and 64.6% on day 8, respectively. Hyperin had inhibitive effect on HBV-DNA and cccDNA in HepG2.2.15 cells $P<0.05$ to various degrees. 2. In vivo tests: Compared with the model group, the percentages of CD3+, CD4+ and CD8+were greatly increased and ratio of CD4+/CD8+ and the levels of cytokines (IL-2,IFN-r, IL-4,and TNF-alpha)were remarkably reduced in hyperin groups $P<0.05$ or $P<0.01$. The content of AST and ALT in serum was decreased in hyperin groups. Conclusion: Hyperin has significant effects on anti-hepatitis B virus and improving immunologic liver injury.

Paper No.: 3460
FOCUSED CONFERENCE GROUP: P01 - CLINICAL PHARMACOLOGY IN THE EMERGING COUNTRIES
DEVELOPMENTAL ORIGINS OF ADULT HEALTH AND DISEASE: THE ROLE OF PERICONCEPTIONAL AND FOETAL NUTRITION

Paulette Merline Youadeu, IC McMillen
Polyclinique de la Renaissance, Department of Pharmacy, Belabo, Cameroon

The 'developmental origins of adult health and disease' hypothesis stated that environmental factors, particularly maternal undernutrition, act in early life to programme the risks for adverse health outcomes, such as cardiovascular disease, obesity and the metabolic syndrome in adult life. Early physiological tradeoffs, including activation of the foetal hypothalamo–pituitary–adrenal (HPA) axis, confer an early fitness advantage such as foetal survival, while incurring delayed health costs. We review the evidence that such tradeoffs are anticipated from conception and that the periconceptional nutritional environment can programme the developmental trajectory of the stress axis and the systems that maintain and regulate arterial blood pressure. There is also evidence that restriction of placental growth and function, results in an increased dependence of the maintenance of arterial blood pressure on the sequential recruitment of the sympathetic nervous system and HPA axis. While the 'early origins of adult disease' hypothesis has focussed on the impact of maternal undernutrition, an increase in maternal nutritional intake and in maternal body mass intake has become more prevalent in

developed countries. Exposure to overnutrition in foetal life results in a series of central and peripheral neuroendocrine responses that in turn programme development of the fat cell and of the central appetite regulatory system. While the physiological responses to foetal undernutrition result in the physiological trade off between foetal survival and poor health outcomes that emerge after reproductive senescence, exposure to early overnutrition results in poor health outcomes that emerge in childhood and adolescence.

Paper No.: 2987
FOCUSED CONFERENCE GROUP: P01 - CLINICAL PHARMACOLOGY IN THE EMERGING COUNTRIES
APPROPRIATENESS OF SERUM LEVEL DETERMINATION OF VALPROIC ACID

Khaled Zeghal(1), A Hakim(1), H Ghozzi(1), H Affes(1), S Hammemi(1), C Mhiri(2), Z Sahnoun(1)
(1) University of Sfax Faculty of Medicine, Department of Pharmacology, Sfax, Tunisia
(2) Habib Bourguiba Hospital, Department of Neurology, Sfax, Tunisia

Introduction: The role of valproic acid (VPA) therapeutic drug monitoring is to improve compliance, seizure control and avoiding side effects. The aim of this study is to asses the appropriateness of the determination of serum levels of VPA in epileptic patients. Methods: We performed a retrospective analysis of 180 VPA serum level determinations. Appropriateness criteria regarding indication and timing were defined a priori using existing criteria from the literature [Affolter N, Krähenbühl S, Schlienger RG.Appropriateness of serum level determinations of antiepileptic drugs. Swiss Med Wkly. 2003 Nov 22; 133(43-44):591-7.]. Results: Of 180 levels assessed, 128 (71.1%) had an appropriate indication, the majority (52%) were performed for suspected toxicity or concentration dependent adverse drug reaction. Of 52 levels assessed as having inappropriate indications, most (79%) were identified in patients with routine monitoring. Conclusion: Over than quarter of VPA measurements did not meet the criteria of appropriate VPA levels determinations. This is causing great loss of money and time. Indiscriminate use of blood level determinations of VPA is not recommended and clinical judgment must always be used to decide when the measurements are performed and how to interpret the information obtained

Paper No.: 555
FOCUSED CONFERENCE GROUP: P09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
THE EFFECTS OF PERIPHERAL ADMINSTRATION OF GABA ON LPS-INDUCED INFLAMMATION IN LIVER

M Zendehdel(1), M Sayyah(2), B Khoshkholghsima(2), M Motaghinejad(1), M Shahabi(1), Majzoob Zanjani Sayyah (3)
(1) University of Tehran Faculty of Veterinary Medicine, Department of Basic Sciences, Tehran, Iran
(2) Department of Physiology and Pharmacology, Institute Pasteur, Tehran, Iran
(3) Neda Pharmaceutical Co, Research and Development, Tehran, Iran

It has been reported that GABA, as an inhibitory neurotransmitter, has an anti-inflammatory effect. We investigated whether GABA attenuates liver injury in LPS- treated rats. Wistar rats were injected (ip) with LPS(5 mg/kg) and/or GABA (1 g/kg). Serum leveles of aspartate and alanine transaminases were measured as an index of liver damage. Hepatic TNF-α were evaluated as possible mediators of GABA action. LPS increased hepatic TNF-α. GABA adminstration attenuated the effects of LPS on transaminases and TNF-α in vivo. The GABA prevented an endotoxin- induced increase in serum transaminases and hepatic TNF-α. In summary, these data indicate that GABA has a protective effect on the liver in LPS-injected rats that seems to be mediated by TNF-α.

Paper No.: 3435
FOCUSED CONFERENCE GROUP: P19 - GENERAL SESSION
COMBINATION EFFECTS OF TS-1 AND RADIATION ON H441/5HRE-LUC CELL XENOGRAFTS CANCER MODEL

Lihua Zeng, Y Zhang, X Miao, H Lang, J Guo, M Yang, Y Li, D Ren, G Guo
Fourth Military Medical University, Department of Radiation Medicine, Xi'an, PR China

TS-1 is an orally administered antitumor drug composed of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and oteracil potassium in a molar ratio of 1:0.4:1 . The therapeutic effect of concurrent chemoradiotherapy with TS-1 has been confirmed in various solid tumors; however, the detailed

mechanism of action has not yet been fully elucidated. Here, we identified hypoxia-inducible factor-1 (HIF-1) as one of the targets of TS-1 in chemoradiotherapy. In growth delay assays using a tumor xenograft of non-small-cell lung carcinoma, H441, TS-1 treatment enhanced the therapeutic effect of single gama-ray radiotherapy (14 Gy) and significantly delayed tumor growth tripling time by 1.94-fold compared to radiotherapy alone ($P < 0.01$). An optical in vivo imaging experiment using a HIF-1-dependent 5HREp-luc reporter gene revealed that TS-1 treatment suppressed radiation-induced activation of HIF-1 in the tumor xenografts. The suppression led to apoptosis of endothelial cells resulting in both a significant decrease in microvessel density ($P < 0.05$; vs. radiotherapy alone) and a significant increase in apoptosis of tumor cells ($P < 0.01$; vs. radiotherapy alone) in tumor xenografts. All of these results indicate that TS-1 enhances radiation-induced apoptosis of endothelial cells by suppressing HIF-1 activity resulting in an increase in radiosensitivity of the tumor cells. Our findings strengthen the importance of HIF-1 as a therapeutic target to enhance the effect of radiotherapy.

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Paper No. 2726
FOCUSED GROUP: FC18 - NUCLEAR RECEPTOR TARGETS FOR TREATMENT OF DISEASES

Effects of berberine on expression of xenobiotic nuclear receptor CAR and metabolic enzyme CYP3A4 in HepG2 cells *in vitro*

Guo-Liang Zhang, L Zhang, H-H Pan, S-J Yin, X Wang, D Li
Beijing University Basic Medical School, Health Science Center, Department of Pharmacology, Beijing, PR China

Objective: To examine the effects of berberine on the expression of constitutive androstane receptor (CAR) and metabolic enzyme cytochrome P450 3A4 (CYP3A4) in HepG2 cells in vitro. Methods: The viability and proliferation of cells were measured by MTT test and alamar blue assay using the spectrophotography. The expression of CYP3A4 mRNA was determinated by quantitative real-time PCR (qPCR). Results: Compared with control groups, the inhibit ratios of proliferation by berberine in the concentration range of 2-50 μM were 4.7%, 19. 1%, 22.3%, 31.3% and 44.9 % respectively, and IC50 value was 68.9 μM after incubated 24h. Treatment of HepG2 cells with rifampicin (50μM) up-regulated expresson of CYP3A4 mRNA from 12 -fold to 32-fold (22.16±9.23) as compared to the control cells. Incubation with 3.6μM berberine for 24h and 48h induced the gene expression by 18.22-fold and 2.19-fold respectively. Conclusion: The present study indicated that berberine inhibited the proliferation of HepG2 cells and up-regulated the expression level of CYP3A4 in vitro, however, the underlying mechanism needs to be further discussed.

Paper No. 775
FOCUSED GROUP: FC19 - GENERAL SESSION
***IN VIVO* [11C]PK11195 POSITRON EMISSION TOMOGRAPHY IMAGING AS A BIOMARKER TO ASSESS LIPOPOLYSACCHARIDE-INDUCED LUNG INFLAMMATION IN RAT**

Aijun Zhu(1), K Zasadny(1), S Katugampola(2), MJ Callahan(1), MB Skaddan(1), L Chen(1), K Kuszpit(1), C Brown-Proctor(1), R Harris(1), T Bocan(1)
(1) Bio-Imaging CoE, Pfizer Global R&D, Groton, CT, USA
(2) Experimental Bio-Sciences, Sandwich, Kent, UK

Introduction: Chronic obstructive pulmonary disease (COPD) is a major public health problem. As COPD progresses, polymorphonuclear neutrophils, macrophages and B cells increase. Therefore, detecting inflammatory processes of lung non-invasively is our primary motivation for using positron emission tomography (PET) imaging. To access the usefulness of [11C]PK11195 PET imaging in evaluating acute lung inflammation induced by aerosol Lipopolysaccharide (LPS) exposure. Materials: Four groups of jugular-cannulated (JVC) male Sprague-Dawley rats were studied: treatment by aerosol exposure of phosphate buffered saline (PBS), treatment by aerosol exposure (1mg/ml) of LPS, no treatment, and rats without JVC. All animal study procedures conducted were approved by an institutional review committee (IACUC). At

8 hours post LPS treatment, dynamic PET imaging was conducted for 90 min after iv administration of [11C]PK11195 with serial blood sampling. At completion of PET, lung was collected for histology. Lung sections were stained with hematoxylin and eosin (H&E stain) for general morphology assessment. PET imaging of lung was quantified using Logan plot analysis yielding distribution volume (DV) of tracer. Results: H&E stain results show increases in neutrophils of LPS treated lungs, while [11C]PK11195 PET imaging did not show any significant change in [11C]PK11195 DV across groups. JVC surgical procedure did not produce a detectable signal associated with possible inflammation. Conclusions: Macrophage and neutrophil densities at 8 hours induced by 1 mg/ml LPS exposure, though physiologically relevant to levels associated with COPD in patients, is not sufficient to be detectable by [11C]PK11195 PET imaging.

Paper No.: 3152
FOCUSED CONFERENCE GROUP: P13 - MAXIMISING BENEFITS AND MINIMIZING HARMS FROM DRUGS
POSTGRADUATE PHARMACOTHERAPY PROGRAM BASED ON ESSENTIAL MEDICINES CONCEPT FOR PRACTICING PHYSICIANS LEADS TO MORE RATIONAL PRESCRIBING IN INTERNAL MEDICINE

Lilia Ziganshina, A Bevz, V Khaziakhmetova, A Titarenko, A Kuchaeva, T Abakumova, O Pasynkova, A Korableva, R Gamirova, A Filimonova, I Burashnikova, E Aleksandrova
Kazan State Medical Academy, Department of Clinical Pharmacology and Pharmacotherapy, Kazan, Russian Federation

The irrational use of medicines remains to be a serious problem in health systems globally with detrimental effect on quality of care and public health. Positive effects of problem-based pharmacotherapy teaching of medical students have been well documented. However studies of effects of pharmacotherapy teaching in postgraduate education systems on prescribing practices are limited. The aim of this study was to investigate the effect of postgraduate pharmacotherapy teaching of medical doctors (internal medicine) on the quality of prescribing in real life clinical practice. In order to improve medicine use in internal medicine a comprehensive problem-based pharmacotherapy learning module was developed and implemented in the regular clinical pharmacology post-graduate training program of practicing physicians at the Kazan State Medical Academy (KSMA) for postgraduate training, Kazan, the Russian Federation. As part of this module, internists learned how to design and use their personal formulary – how to rationally select, prescribe, and monitor medicine use. Physicians were randomly allocated to this module (study group, n=37); traditional teaching with existing standard treatment guidelines was provided to physicians of control group (n=34). Medicine use of internists was measured with WHO prescribing indicators. Data were collected within a three-year period, 751 medical charts by participating physicians were blindly analyzed. Thus, post-graduate pharmacotherapy active learning centered on designing personal formulary resulted in more rational prescribing by internists in their practice. This effect was obtained across various fields of internal medicine by far exceeding the list of studied model situations thus implying the transfer effect of acquired skills.

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