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**ABSTRACT BOOK
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Paper No.: 3512
FOCUSED CONFERENCE GROUP: P09 –
GENERAL SESSION
PHYSICAL AND CHEMICAL STABILITY
OF TAXOTERE® 1-VIAL (20MG/ML)
INFUSION SOLUTION FOLLOWING
REFRIGERATED STORAGE

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Background: Taxotere 1-vial is expected to simplify preparation by no longer requiring the initial dilution step that was necessary with the 2-vial presentation, offering greater convenience with an easier and quicker preparation. Purpose: The physical and chemical stability of a new one-vial formulation of docetaxel (Taxotere® 1-vial, sanofi-aventis, Dagenham, United Kingdom) in a large number of infusion solutions was studied following refrigerated storage. Methods: Taxotere 1-vial infusion solutions were prepared by adding 200mg of Docetaxel, using a single introduction of 10ml docetaxel concentrate (20mg/ml) into 250mL polyethylene bags filled with 0.9% saline. Infusion bags were stored under refrigeration at 5°C and removed after either 2 (n=60 bags) or 7 (n=60 bags) days and assessed immediately and at intervals after removal and storage for a further 24 hours at ambient temperature (about 20°C). Crystallization was assessed by a careful visual examination. The chemical stability was assessed by determination of the docetaxel content and degradation impurities in several of the infusion bags used in the physical stability study by HPLC. The chemical stability was investigated on the same solution resulting from the physical stability study. Results: No visible crystallisation was observed in any Taxotere 1-vial infusion bags following refrigerated storage for either 2 or 7 days and subsequent storage for 24 hours at approximately 20°C. There was no significant evolution of pH, docetaxel content, or degradation impurities in the infusion bags during this time. Conclusions: The new Taxotere 1-vial formulation is associated with reduced risk of precipitation of docetaxel via elimination of the premix solution (required for the Taxotere 2-vial presentation) and a one-shot preparation technique (ie, only one intervention into the infusion bag). Moreover, refrigerated storage has been significantly shown to extend the physico-chemical stability for up to

7 days. However, only physico- chemical stability has been demonstrated in this study.

Paper No.: 2365
FOCUSED CONFERENCE GROUP: FC11 - G
PROTEIN-COUPLED 7TM RECEPTORS:
FROM MOLECULAR TO
PHYSIOLOGICAL FUNCTION
INFLAMATION CAUSES THE JNK-
DEPENDENT UPREGULATION OF CX43
GAP JUNCTION CHANNEL AND
HEMICHANNEL EXPRESSION

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Hyperthermia or inflammation activates a number of stress kinases which may modify properties of membraneous and intercellular channels and cause the upregulation of their expression. In HeLa cells expressing connexin43 fused with green fluorescent protein (Cx43GFP) we measured the number of gap junction (GJ) channels in junctional plaques using conventional fluorescent microscopy (Palacios-Prado N et al, J. Physiol. 2009; 587: 3251-3269) and the number of hemichannels in cell membranes by TIRF microscopy. The relative fluorescence intensity of a single GJ channel or hemichannel was estimated from horizontal GJ plaques between overlapping cells assuming channel density in the plaques was approximately 10000 channels/ μm^2 (Lal R et al, Am. J. Physiol. 1995; 268: C968-C977). The Cx43GFP channel and hemichannel number was determined in control (37°C) and after preincubation in 42°C for 2, 6 and 24 hours in the absence or presence of XG102 (4 μM), an inhibitor of c-Jun-N-terminal kinase (JNK). Hyperthermic conditions caused near 2-fold reduction of Cx43GFP channel and hemichannel number. The maximal effect was achieved in approximately 6 h. Preincubation of HeLa cells in 37°C with XG102 for 2 h had no effect on Cx43GFP channel and hemichannel number, however completely prevented the subsequent effect of hyperthermia. The total amount of Cx43GFP protein was examined by Western blotting using a specific antibody against Cx43 in all experimental conditions, as well. Our results suggest that in hyperthermic conditions, JNK may regulate trafficking of Cx43 hemichannels to the

membrane and GJ plaques, the process involving the rearrangement of cytoskeleton proteins.

Paper No.: 818

**FOCUSED CONFERENCE GROUP: FC19 -
GENERAL SESSION**

**VALIDATION OF AN ANALYTICAL
METHODOLOGY FOR DETERMINATION
OF OXYTETRACYCLINE RESIDUE IN
MILK BY HPLC WITH UV DETECTION**

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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 desired 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.: 3511

**FOCUSED CONFERENCE GROUP: FC13 -
MAXIMISING BENEFITS AND
MINIMIZING HARMS FROM DRUGS
ROLE OF M4 MUSCARINIC
ACETYLCHOLINE RECEPTORS IN
COCAINE-MEDIATED EFFECTS**

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Many central actions of acetylcholine are mediated by muscarinic (M1–M5) receptors and disturbances in the central muscarinic cholinergic system have been implicated in several pathophysiological conditions, including drug addiction and schizophrenia. Behavioral and neurochemical studies of M4 receptor knockout mice have shown that the M4 receptor subtype plays an important role in the regulation of dopamine neurotransmission. We wanted to further investigate the involvement of muscarinic M4 receptors in the behavioural and neurochemical effects of cocaine. To this end we investigated the effect of a potent and selective allosteric potentiator of M4 receptors (VU0152100) in cocaine-induced hyperlocomotor activity, acute cocaine self-administration and cocaine-induced striatal dopamine release. The selective allosteric potentiator of M4 receptors VU0152100 inhibited cocaine-induced hyperactivity, cocaine self-administration and cocaine-induced striatal dopamine release. The present data support a role for the M4 muscarinic acetylcholine receptor as a possible new target in the treatment of drug addiction and psychosis.

Paper No.: 1103

**FOCUSED CONFERENCE GROUP: FC16 -
NATURAL PRODUCTS: PAST AND
FUTURE?**

**THE EFFECT OF *DILLSUN* EXTRACT ON
THE GUINEA PIG ILEUM MOVEMENT IN
IN VITRO MODEL**

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Previous studies indicated that *Dillsun* is a plant which is gaining decreasing acceptance as a folk remedy for treatment of the irritable bowel disease although the mechanism of this action is not clear yet. For this purpose we studied the effect of *Dillsun* extract in guinea pig ileum. Male guinea pig (220-250 gr) were used. Pieces of ileum (2-3

cm) were mounted in a 50 ml organ bath containing tyrode solution with temperature (37 °C).and bubbled with (95%O₂,5%CO₂). the ileum was stimulated at 0.1 Hz and contraction was recorded by physiograph.Then were added *Dillsun* extract in five concentration and calculated change in contraction. it has been shown that 0.1 Hz stimulation of guinea pig ileum induced contractions which are depressed by Atropin. Addition of aqueous extract of *Dillsun* extract to the organ bath during 0.1Hz stimulation decreased contraction in a Dose dependent manner (Ec50%=0.7mg/ml). it seams that *Dillsun* extract shows its effect by inhibition cholinergic system. Because the contraction of Acetylcholine decreased by addition of *Dillsun* extract.

Keywords: *Dillsun* extract, guinea pig ileum movement

Paper No.: 3128

**FOCUSED CONFERENCE GROUP: FC19 - GENERAL SESSION
PACLITAXEL-EVOKED PAIN AND ITS THERAPY; *IN VIVO* VOLTAMMETRIC STUDY**

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Introduction: We investigated the character of chemotherapy-evoked neuropathic pain using rats treated with paclitaxel. The novel method of *in vivo* glutamate voltammetry was utilized to determine if changes in extracellular glutamate levels and release in rats occur after chronic paclitaxel therapy. Peripheral neuropathy is the dose-limiting side effect of chemotherapeutics in the taxane. The cause of the pain is not known. But β-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. This study investigated whether changes in glutamate homeostasis occur after paclitaxel treatment in specific brain areas. Materials: Analgesiometer (Randal- Selitto) was used to measure the mechanical allodynia. 200 micromolar glutamate was given to the areas and uptake activities were recorded with *in vivo* voltammetry system. Results: Paclitaxel-evoked pain detected in analgesiometer test on 14th day. And it continued to 60th day. Pain thresholds were came from 34±36gr and 18,5±5,3gr to 27,6±26,5gr and

36,7±19,6gr respectively in that days. In voltammetric records amplitudes and glutamate uptake rates were decreased in pain areas. Uptake rates in somatisensorial cortex and posterior intrathalamic nucleus changed from 0.04±0.01 to 0,45±0,11 and 0.51±0.18 to 1,2±0,4 respectively in ceftriaxon-treated rats. Conclusion: This study showed that glutamate uptake was decreased in paclitaxel-treated animals, and ceftriaxon was increased this uptake in pain areas of brain cortex and thalamus. The resultant elevated extracellular glutamate comprises a new putative mechanism and therapy for the iatrogenically induced neuropathic pain of the taxane class of chemotherapeutic agents.

Paper No.: 3468

**FOCUSED CONFERENCE GROUP: FC09 - INFLAMMATION AND IMMUNOPHARMACOLOGY: NEW TOOLS FOR OLD DISEASES
NEUROPEPTIDE COMBINATION TARGETING MS – NOVEL AUTOIMMUNITY TREATMENT APPROACH**

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Background: Combination of two peptide components: alpha 1-13 ACTH (a-MSH-like,deacetated and deamidized a-MSH) and 1-5 adrenorphine (metenkephaline) in 1/5 proportion is shown to be efficacy and safe new way of treatment potentially disabling and devastating condition as Multiple Sclerosis in some forms is. Both peptide components belong to neuropeptide class and are derived from same precursor, what seems to be adequate hypothesis for their synthetic merge. A Pharmacodinamic property of this combination includes anti-inflammatory, anti-oxidative, analgesic, and antipyretic cytological responses. Selective immunomodulatory peptide response is current potential option in some immunological and malignant diseases. This method is focused on long term remission of disease, without potential toxicity and adverse effects which characterized current immunosuppressive drugs and treatment options. Methods: To test safety and efficacy of two

peptide combinations following studies were performed: Preclinical studies of acute and subacute toxicity on animal models, studies of pharmacokinetics and pharmacodynamics (healthy volunteers), and I, II, III phases of clinical trials with included MS patients. Results: Preclinical data suggested extremely safe profile of neuropeptide combination. Results derived from clinical trials (all III phases) confirmed same favorable safety profile for mentioned combination, and also outcome treatment efficacy which is proven by sophisticated NMR screenings as well as with standardized clinical examination and symptom reduction scales.

Paper No.: 839

**FOCUSED CONFERENCE GROUP: FC19 -
GENERAL SESSION
THE ROLE OF THE ADENOSINE
RECEPTORS ON MECHANISM OF
CITALOPRAM-INDUCED
CARDIOVASCULAR TOXIC EFFECTS**

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This study was designed to clarify the role of adenosine receptors on mechanism of citalopram-induced cardiovascular toxic effects. The cardiovascular toxic dose of the citalopram was found as 4mg/kg/min (n=18). Rats were randomized into four groups. After the stabilization period, sodium cromoglycate (A₃ receptor antagonist, 20mg/kg, i.v) was administered to all groups. 5% dextrose (n=7), DPCPX (selective adenosine A₁ receptor antagonist, 20µg/kg/dk, n=7), CSC (selective adenosine A_{2a} receptor antagonist, 24 µg/kg/min, n=7) or 10% DMSO (n=3) was administered for 20 minutes, respectively. Following the infusions, we administered citalopram (4mg/kg/min/60 minutes). Mean arterial pressure (MAP), heart rate (HR), QT and QRS durations were recorded. In the dextrose group, citalopram infusion caused a significant decrease in the MAP ($P<0.001$) and HR ($P<0.01$) after the 20th minute and caused a significant prolongation in the QRS ($P<0.05$) and QT durations ($P<0.01$) after the 30th minute. The citalopram infusion in the DPCPX group, caused a significant decrease in the MAP after the 20th minute ($P<0.001$) and caused a significant

decrease in the HR after the 30th minute ($P<0.01$). It caused a significant prolongation in the QRS duration at the 60th minute ($P<0.05$). It did not cause any significant difference in the QT duration. DPCPX infusion significantly prevented the prolongation of the QT duration induced by citalopram after the 20th minute when compared to the control group ($P<0.05$). In the CSC and DMSO groups, there was not any significant change in citalopram-induced cardiovascular effects. The adenosine A₁ receptor stimulation may be responsible for the citalopram-induced QT prolongation.

Paper No.: 2651

**FOCUSED CONFERENCE GROUP: FC09 -
INFLAMMATION AND
IMMUNOPHARMACOLOGY: NEW TOOLS
FOR OLD DISEASES
IMPACT OF SOLUBLE ADENYLYL
CYCLASE ON APOPTOSIS OF B
LYMPHOMA CELLS**

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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.: 3465

**FOCUSED CONFERENCE GROUP: P19 -
GENERAL SESSION
CHARACTERISING THE
PHARMACOLOGICAL
CARDIOVASCULAR SAFETY PROFILE OF
IPRATROPIUM FOR THE TREATMENT
OF CHRONIC OBSTRUCTIVE
PULMONARY DISEASE**

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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, Ipratropium 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.: 1877

**FOCUSED CONFERENCE GROUP: FC13 -
MAXIMISING BENEFITS AND
MINIMIZING HARMS FROM DRUGS
POTENTIAL STATIN-DRUG
INTERACTIONS: PREVAILANCE OF PRE-
HOSPITAL AND
HOSPITALCOPRESCRIPTIONS TO
CARDIAC PATIENTS**

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Drug-drug interactions substantially increase the risk of adverse drug reactions. Hydroxymethylglutaryl CoA-reducetase inhibitors (statins) are widely used in cardiovascular prevention. The aim of the present study was to assess the use of statins and the incidence of potential statin-drug interactions (pSDIs) in hospitalized patients. We compared the prevalence of statin coprescriptions at hospital entry and discharge. Medical records of 1641 cardiac patients were retrospectively examined. Two groups of pSDIs were looked for: 1) with drugs that can alter the effects and toxicity of statins (inhibitors and inducers of cytochrome P-450 (CYP), fibrates); 2) with drugs that may have increased toxicity in the presence of statins

(coumarin anticoagulants, digoxin). We identified 218 patients receiving a statin therapy at admission and 491 at discharge. The statin prescribed most often was simvastatin. The total number of pSDIs was 57 (26.1 %) at admission and 120 (24.4 %) at discharge. The rate of pSDIs with CYP-inhibitors was 6 % and 4 %, respectively. Amongst them, amiodarone was most commonly involved. Combinations with calcium antagonists were infrequent. Coprescriptions with acenocoumarol affected overall 86 patients in both settings, 9 of them having an associated increase of $INR > 3$. It is concluded that a relatively high proportion of our cardiac patients are exposed to potentially adverse SDIs, with roughly the same incidence at hospital admission and discharge. Combinations leading to increased risk of statin toxicity are not very common. Patients receiving acenocoumarol along with a statin should be closely monitored for possibly enhanced anticoagulation.

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