

EPHAR 2008 CONGRESS
13 – 17 JULY
MANCHESTER, UK



LATE BREAKING & HOT TOPIC
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C081

Merkel cells as putative new targets of anti-inflammatory drugs

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Inflammation is characterized by tissue acidification, cells swelling and histamine release from mast cells. Merkel cells (MC) are epidermal neuroendocrine cells implicated in touch perception and neuropeptides release. Interestingly, they also express the proton-gated osmoreceptor transient receptor potential vanilloid 4 (TRPV4) and the histamine-H3 receptor. We achieved an original culture model wherein enriched porcine Merkel cells were exposed to various substances. We studied the release of vasoactive intestinal peptide (VIP), a neuropeptide with anti-inflammatory properties in the skin. MC from pig snout were retrieved by positive magnetic cells sorting as confirmed by immunocytochemistry and electron microscopy. After 3 days in culture, cells were exposed to histamine, 4 α PDD, a phorbol-derivative agonist of TRPV4 and acetylcholine. VIP release was assessed by ELISA. In our culture conditions, MC extended many cytoplasmic processes suggesting a good viability. More than 60% of the cell populations were MC. Following exposure to histamine or the TRPV4 agonist, VIP release was enhanced. Conversely, exposure to acetylcholine led to decreases VIP exocytosis. MC are endowed with ion channels sensitive to inflammation and for which activation enhances VIP release as demonstrated here. Interestingly, VIP is known to down-regulate pro-inflammatory mediators like IL6, IL12, TNF α , inducible nitric oxide synthase and it can up-regulate anti-inflammatory mediators like IL10. Thus, these new findings suggest that MC can take place in regulation of inflammation. The opposite effect of histamine and acetylcholine also argues for a putative role in hypersensitivity and probably atopic dermatitis.

C082

Liver injury caused by ischemia-reperfusion in the rat is attenuated by pre-treatment with synthetic and endogenous inhibitors of glycogen synthase kinase-3beta

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Glycogen synthase kinase 3beta (GSK-3beta) is a serine/threonine kinase involved in the modulation of the inflammatory response playing a pivotal role in the regulation of NF-kappaB activation. Here we investigate the effects of two GSK-3beta inhibitors on the liver injury caused by ischemia-reperfusion injury, in the rat: a synthetic inhibitor, TDZD-8 and the endogenous inhibitor, insulin. Male Wistar rats were randomly allocated into 6 groups as described: (1) Sham Group; (2) I/R Group: rats which were subjected to liver ischemia for 45 minutes interrupting blood supply to 3/4 of the liver, followed by reperfusion for 2 h; (3) TDZD-8 + I/R Group: rats which received TDZD-8 (1 mg/kg i.v.) 30 minutes prior to liver I/R; (4) Insulin + I/R Group: rats which received insulin (1,4 UI/kg i.v.) 30 minutes prior to liver I/R; (5) TDZD-8 and (6) Insulin Control Groups. Blood samples were obtained at the end of reperfusion for determination of biochemical markers of liver injury and tissue samples were also collected for histology. Our results show that both TDZD-8 and insulin administration reduced the increase in the level of biochemical markers for liver injury when compared to I/R group: AST (1132 \pm 61 vs. 541 \pm 254 and 555 \pm 10 IU/l, for TDZD-8 and insulin, respectively) and ALT (1941 \pm 92 vs. 583 \pm 90 and 174 \pm 27 IU/l, for TDZD-8 and insulin, respectively). We demonstrate here that TDZD-8 and insulin cause a substantial reduction in the ischemia-reperfusion-induced increase in the serum levels of hepatic injury markers, providing, to our knowledge for the first time, the evidence that inhibition of GSK- 3 β reduces the liver injury induced by ischemia-reperfusion, in the rat. We propose that GSK-3beta inhibitors, may be useful in the therapy of liver injury associated with ischemia-reperfusion of the organ.

C083

Generation of endogenous hydrogen sulphide by cystathionine gamma-lyase limits renal ischaemia/reperfusion injury and dysfunction

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The generation of hydrogen sulphide by cystathionine gamma-lyase (CSE) may either limit (Sivarajah *et al.*, 2006) or contribute (Qu *et al.*, 2006) to the degree of tissue injury caused by ischaemia/reperfusion. Seventy-four male Wistar rats (200-320 g) were used to investigate the effects of endogenous and exogenous hydrogen sulphide in renal ischaemia/reperfusion injury (IRI). Rats were subjected to 45 min ischaemia and up to 72 h reperfusion. Administration of the irreversible CSE inhibitor, dL-propargylglycine, prevented the recovery of renal function (serum creatinine) following ischaemia and 72 h reperfusion. The hydrogen sulphide donor sodium hydrosulphide attenuated renal, tubular and glomerular dysfunction and renal injury caused by ischaemia and 6 h reperfusion. Western blot analysis of kidneys taken at 30 minutes reperfusion showed that sodium hydrosulphide significantly attenuated phosphorylation of p-38, JUN 1/2, and ERK 1/2, and activation of NF- κ B. At 6 h reperfusion, sodium hydrosulphide significantly attenuated acute tubular necrosis (histology), the activation of caspase-3 and Bid, the decline in the expression of anti-apoptotic Bcl-2, and the expression of NF- κ B dependent proteins (iNOS, COX-2, and ICAM-1). These findings suggest that (i) the synthesis of endogenous hydrogen sulphide by CSE is essential to protect the kidney against IRI and aids in the recovery of renal function following IRI, (ii) hydrogen sulphide generated by sodium hydrosulphide reduces IRI and morphological changes of the kidney, and (iii) the observed protective effects of hydrogen sulphide are due to both anti-apoptotic and anti-inflammatory effects.

Qu K, *et al.* (2006). *Stroke* 37, 889-893; Sivarajah A, *et al.* (2006). *Shock* 26, 154-161

C084

Late administration of a novel EPO analogue in the presence of immunosuppressants protects the mouse kidney from ischaemia/reperfusion injury

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Erythropoietin (EPO) is the major hormone stimulating the production and differentiation of red blood cells. EPO is used widely for treating anaemia of renal disease or anaemia induced by chemotherapy. EPO is beneficial in various models of ischaemia/reperfusion injury (IRI) such as stroke, myocardial infarction, hind-limb ischaemia, acute kidney injury, haemorrhagic shock and liver ischaemia. There are two biologically distinct functions of EPO through its interaction with two very different types of receptors: EPO receptor homodimer (erythropoiesis) and beta common heterocomplex (tissue protection). The novel peptide ARA290 has been modelled upon the 3D structure of the region of EPO presumed to bind to and initiate signalling of the beta common heterocomplex. Thirty-five male C57/B6 mice (20g) were subjected to 30 min bi-lateral renal ischaemia and 24 h reperfusion. ARA290 (10 μ g/kg) was administered in saline 6 h after the onset of reperfusion. ARA290 significantly attenuated both the renal dysfunction and injury associated with renal IRI. Kidney transplantation is the best available therapy for patients with end-stage renal disease. Kidneys subjected to pre-retrieval warm ischaemia are at risk of developing IRI after transplantation, which is the most important antigen-independent negative factor associated with chronic allograft nephropathy. Forty "immunosuppressed" male C57/B6 mice (20g) were subjected to 30 min bi-lateral renal ischaemia and 48 h reperfusion. ARA290 (10 μ g/kg) was administered in saline 6 h after the onset of reperfusion. A single administration of ARA290 6 h into reperfusion attenuates the increased renal dysfunction and injury caused by immunosuppressive therapy.

C085

Relative importance of the calcium-sensing receptor and GPRC6A in responses to calindol in the guinea pig common carotid artery

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Calindol activates the endothelial extracellular calcium-sensing receptor (CaR) and the related GPRC6A which opens IK_{Ca} channels followed by smooth muscle hyperpolarization and relaxation (Weston *et al.* 2005; Harno *et al.* 2008). The present study aimed to determine whether CaR and GPRC6A have a functional role in the guinea pig carotid artery, a typical conduit artery. Male Duncan-Hartley guinea-pigs (~500g) or Wistar-Kyoto rats (~300g) were used. Guinea-pig carotid artery myocyte hyperpolarizations to calindol (300nM, 0.4 ± 0.3 mV; 1μ M, 3.6 ± 0.7 mV, n=4) were smaller than those in rat mesenteric arteries (300nM, 8.0 ± 1.7 mV; 1μ M, 12.8 ± 1.0 mV, n=4). The GPRC6A-selective agonist, L-ornithine (300 μ M), induced a small hyperpolarization of guinea-pig and rat myocytes (3.0 ± 0.8 mV, n=4 and 4.5 ± 0.3 mV, n=4, respectively). In both vessels, hyperpolarizations to 300nM calindol in the presence of L-ornithine were larger than in its absence ($P < 0.05$). In tension studies (carotid arteries pre-contracted with 1μ M phenylephrine), 3μ M calindol produced a small relaxation ($16.0 \pm 10.8\%$) despite an intact endothelium (subsequent relaxation to 3μ M acetylcholine, $92.5 \pm 4.0\%$). Western blotting detected GPRC6A but failed to detect CaR protein in carotid artery lysates. These data suggest that the CaR may be absent from the carotid artery. In rat mesenteric arteries, calindol may cause hyperpolarisation of myocytes through GPRC6A activation (Harno *et al.*, 2008) and we thus propose that the effects of calindol in carotid arteries may be due to the activation of GPRC6A alone.

C086

In vitro cardiac safety assessment of antidepressant drugs in intact rat hearts and after ischemia-reperfusion

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In this study different noradrenalin and serotonin reuptake inhibitor antidepressant drugs were used. Their dose dependent effects on isolated hearts were compared with increasing concentrations in intact hearts and following ischemia-reperfusion in order to get cardiac safety profile data. The animals were divided into nine groups of six rats each. The hearts were perfused in Langendorff systems. In intact groups, after a stabilisation period, six different concentrations (1×10^{-7} to $1 \times 10^{-4.5}$ mol/L) for amitriptyline, fluoxetine, maprotiline, and trazodone were applied for five minutes in increasing concentrations. For ischemia-reperfusion experiments the hearts were subjected to normothermic global ischemia for 15 minutes where the hearts were kept in hypoxic normothermic Tyrode solution. The hearts were then reperfused for 30 minutes before drug application in the same setting as intact hearts. Haemodynamic variables and bipolar electrocardiograms were recorded continuously. Amitriptyline, fluoxetine and maprotiline depressed left ventricular developed pressure (respectively 8.3 ± 6.9 ; 26.7 ± 10.3 and $65.2 \pm 25.0\%$ of baseline value for normoxic experiments; 15.1 ± 4.7 ; 14.3 ± 5.3 and $13.6 \pm 4.1\%$ of baseline for ischemia-reperfusion experiments at the highest concentration) and $+dp/dt_{max}$ in a dose dependent manner, whereas the depression was moderate with trazodone (left ventricular developed pressure values; 94.0 ± 5.0 ; $59.5 \pm 10.4\%$ of baseline respectively for normoxic and ischemia reperfusion groups), in both normoxic and ischemia reperfusion groups. All drugs decreased heart rate in higher concentrations. In conclusion trazodone seems to be a safer agent in this setting regarding all parameters measured.

C087**4-aminopyridine contracts pulmonary artery in voltage-dependent and voltage-independent manner**

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Voltage-gated K^+ (K_V) channels are predominantly expressed in pulmonary vasculature and have been thought to play an important role in regulation of membrane potential (V_m) and vessel contractility. The main aim of this project was to investigate the role of K_V channels in intrapulmonary arteries (PAs) isolated from male Wistar rats (200-250 gm) using small vessel wire myography. Mesenteric arteries (MAs) were used as a representative of systemic circulation. Our results demonstrate that the effect of 4-AP, a specific inhibitor of the K_V channels, was significantly potentiated by 20 mM KCl that causes membrane depolarization. 4-AP-induced contraction of PAs was only partly ($35.0 \pm 4\%$, $n=6$) blocked by the inhibitor of L-type Ca^{2+} channels diltiazem (10 μ M), whereas in MAs contraction was nearly completely blocked ($94.0 \pm 2\%$, $n=6$). Similar partial block by diltiazem was observed in PAs but not in MAs for contraction induced by 80 mM KCl. 4-AP induced contraction in PAs was also blocked by Rho-kinase inhibitor Y-27632 (10 μ M). The effects of diltiazem and Y-27632 were additive in PAs. The role of Rho-kinase in 4-AP-induced contraction was confirmed with Western blot analysis. Pretreatment with 4-AP (10 μ M) increased the levels of phosphorylated myosin light chain (p-MLC) in PAs. This effect was reversed by pretreatment of tissues with Y-27632 (10 μ M). These results suggest that 4-AP induced contraction involves voltage-dependent and voltage-independent mechanisms and that Rho-kinase signalling pathway contributes, at least in part, to 4-AP-induced pulmonary vasoconstriction.

C088**Effect of the estral cycle on central 5-HT levels in intact female rats and that of estrogen replacement treatment in ovariectomized rats**

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Extensive clinical evidence suggests a close link between ovarian hormone fluctuations during the menstrual cycle, the serotonin (5-HT) system and migraine. The aim of this work was to analyze the central levels of serotonin (5-HT), as compared with those of noradrenaline (NA) and dopamine (DA), during the estral cycle of intact female rats, and in ovariectomized rats submitted to chronic (14 days) estrogen replacement treatment. The neurotransmitters were quantified by HPLC in the dorsal raphe (DRN) and paraventricular nuclei (PVN) dissected from 300 μ m-thick brain slices. Two consecutive 4 day long estral cycles were determined in intact female rats; animals with longer lasting cycles were excluded. Ovariectomized rats were subcutaneously implanted with silastic capsules containing vehicle (cholesterol) and 17β -estradiol for 14 days. A significant increase in the levels of 5-HT, NA and DA was found in the DRN and the PVN during the proestrus, as compared to the other phases of the estral cycle. In ovariectomized rats, chronic estrogen replacement treatment failed to induce any significant change in the content of NA and DA in the DRN and the PVN, but it evoked a significant increase in the content of 5-HT in both nuclei; acute estrogen withdrawal (i.e. 2 days after removing the capsules) induced a significant decrease in 5-HT levels to pretreatment values. These data support the hypothesis: 1) that estrogens play an important role in the modulation of central 5-HT levels; 2) that migraine attacks might be related to a sudden decrease of 5-HT levels in the brain following an estrogen-induced maximum; and 3) that fluctuations of ovarian hormones may be crucial for migraine to occur. The relevance of estrogen-induced changes in the central levels of NA and DA remains to be determined.

C089**Influence of rearing condition on 5-HT_{1A} receptor responsiveness**

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Rearing conditions in the early stages of life have been reported to alter the behavioral and neurochemical effects in the later life and modify the responsiveness to many drugs. The aims of these experiments were to investigate the effects of difference rearing conditions on anxiety and depression and to compare the effects of the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT in isolation and socially reared rats. Male Wistar rats were obtained weaning, and reared either alone (isolation rearing) or in groups of six rats/cage (social rearing). After four weeks, these rats were tested for their sensitivity to 8-OH-DPAT using the elevated plus-maze and the forced swimming tests. The results from the plus-maze test showed that the plus-maze behaviors of drug free isolation reared rats were not significantly difference from socially reared rats. Pretreatment of 8-OH-DPAT (0.05, 0.1 and 0.5 mg/kg s.c.) dose-dependently produced an anxiogenic profile, indicated by a decrease in the percentage of open arm entries and the percentage of time spent on the open arms. These effects of 8-OH-DPAT were more pronounced in isolation than socially reared rats ($P < 0.05$). The results from the forced swimming test demonstrated that drug free isolation reared rats showed significantly less immobility time and more struggling than the socially reared rats. Pretreatment of 8-OH-DPAT (0.1, 0.25, 0.5 and 1 mg/kg s.c., 24, 5 and 1 h before a 5 min test) in both isolation and socially reared rats produced a dose-dependent reduction of immobility and elevation in struggling compared to the saline treated group. These effects were greater in the isolation reared rats than the socially reared rats. The results suggest that rearing in isolation may produce some of its behavioural effects through central serotonergic mechanisms.

C090**Lipid rafts but not putative receptors mediate anandamide inhibited hepatic stellate cell proliferation**

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An increasing body of evidence about anandamide (AEA) has indicated its role in the systemic circulation disturbance in cirrhosis. However, its precise physiological role in liver fibrosis remains to be elucidated. This study determined the mechanism of AEA on hepatic stellate cell (HSC). By using MTT, we investigate the effect of AEA and its CB1 and CB2 receptor antagonists (SR141716A and AM630) on the proliferation of HSC. Meanwhile, we adopted nuclear staining of Hoechst 33258, Western-blot and flow cytometry (FCM) to detect the mechanism of AEA on HSC. Because AEA could interact with lipid rafts (LRs) on cell membrane, the LR on HSC were detected by confocal microscopy. We also studied if the membrane cholesterol depletory-methyl- β -cyclodextrin (MCD) exhibits an inhibitory effect on anandamide-induced HSC death. We present evidence that AEA trigger potently growth inhibition in HSC, in a concentration-dependent manner (5 μ mol/L -20 μ mol/L), showed by MTT. Nuclear staining, Western-blot and Annexin V-PE/7AAD binding assay showed that AEA induce HSC necrosis but not apoptosis. Whereas, cannabinoid receptors antagonists failed to block anandamide-induced cell death. Moreover, LR on HSC were shown by confocal microscopy, and MCD to counteract the anandamide-induced HSC death ($P < 0.05$). In conclusion AEA could potently suppress the proliferation of HSC via the membrane cholesterol-LRs but not putative receptors, LR may be functioning as anandamide receptors in HSC. (Supported by National Nature Science Foundation of China NO.30571627)

C091

7 β -hydroxycholesterol is antiapoptotic and induces proliferation in human endothelial cells by a ROS-independent ERK-dependent mechanism

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Atherogenic potential of oxidized low density lipoproteins (oxLDL) has been correlated to their content in 7 β -hydroxycholesterol (7 β OHC) (Brown and Jessup, 1999). oxLDL have a dual effect on endothelial cell viability, inducing apoptosis at high concentrations and proliferation at low concentrations (Galle *et al*, 2001). Considering that 7 β OHC is apoptotic for endothelial cells at concentrations ≥ 20 μ g/mL (Lizard *et al*, 1999), a study on the effect of lower concentrations of 7 β OHC on human umbilical vein endothelial cells (HUVEC) was undertaken. 7 β OHC (1-10 μ g/ml) significantly increased viability (+150% after 24 h) of growth factor-deprived HUVEC. This effect was due to an increase in proliferation, determined by [³H]thymidine assay, as well as a reduction in HUVEC apoptosis, suggested by a decrease of caspase-3 activation and annexin V+ staining. 7 β OHC protected also against staurosporine treatment. Determination of intracellular ROS with CM-H₂DCFDA showed an increase in ROS production by 7 β OHC that was reduced by the NAD(P)H oxidase inhibitor hydralazine, however the antiapoptotic and proliferative effects were independent on ROS. Both antiapoptotic and proliferative effect of 7 β OHC were blocked by inhibition of MEK with PD98059 or U0126, nevertheless 7 β OHC was unable to induce an increase of ERK phosphorylation. The results show that concentrations of 7 β OHC below 20 μ g/mL are antiapoptotic and induce proliferation in HUVEC. These effects are ROS-independent and are regulated by the MEK/ERK cascade.

Brown and Jessup (1999) *Atherosclerosis* 142: 1-28.

Galle *et al.* (2001) *Kidney Int.* 59: S120-S123.

Lizard *et al.* (1999) *Arterioscler Thromb Vasc Biol.* 19: 1190-1200.

C092

Regulation of axonal and dendritic growth by the extracellular calcium-sensing receptor (CaSR)

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The extracellular calcium-sensing receptor (CaSR) monitors the systemic extracellular free ionized calcium level ($[Ca^{2+}]_o$) in organs involved in systemic $[Ca^{2+}]_o$ homeostasis. However, the CaSR is also expressed in the nervous system where its role is unknown. We studied the CaSR in the mouse superior cervical ganglion (SCG), an experimentally tractable population of neurons from the peripheral nervous system and a well established model of neuronal development. The morphology of individual neurons was analysed after a range of manipulations by quantification of total neurite length, and neuritic arbors by Sholl analysis. Comparison of the sympathetic innervation of CaSR mice was by quantification of tyrosine hydroxylase staining of the iris. Statistical analyses were carried out using either Student's *t*-test or one-way ANOVA with Tukey's *post hoc* test. We found high levels of the CaSR in perinatal (embryonic day 18 – postnatal day 0) mouse sympathetic neurons when their axons are innervating and branching extensively in their targets. Manipulating CaSR function in these neurons by varying $[Ca^{2+}]_o$, using CaSR agonists (NPS R-467) and antagonists (NPS-89636) or expressing a dominant-negative CaSR (R185Q) markedly affects neurite growth *in vitro* ($n \geq 3$; $P < 0.001$). Sympathetic neurons lacking the CaSR have smaller neurite arbors *in vitro* ($n \geq 3$; $P < 0.001$), and sympathetic innervation density is reduced in CaSR-deficient mice *in vivo* (postnatal day 1; $n = 3$; $P < 0.001$). Hippocampal pyramidal neurons, which also express the CaSR, have smaller dendrites when transfected with dominant-negative CaSR in postnatal organotypic cultures ($n = 5$; $P < 0.001$). Our findings reveal a crucial role for the CaSR in regulating the growth of neural processes in the developing peripheral and central nervous systems.

C093

Epigenetic mechanisms of irinotecan sensitivity in colorectal cancer cell lines

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Irinotecan is a topoisomerase-I (Top-I) targeting drug employed for colorectal cancer treatment. DNA demethylating agents, including 5-azacytidine, displayed synergistic antitumor activity with several chemotherapy drugs. The aim of the present study was to investigate whether 5-azacytidine may enhance irinotecan cytotoxicity by at least one of the following mechanisms: 1) Top-I promoter demethylation 2) activation of genes involved in Top-I transcriptional regulation (p16, Sp1, p53) 3) modulation of cell cycle and apoptosis after DNA damage. Growth inhibitory effect of irinotecan active metabolite (SN38), 5-azacytidine and their combination was studied in four colorectal cancer cell lines (HT29, SW620, WiDr, LS174T). The effects of these treatments on the cell cycle was analyzed by flow cytometry, and apoptosis was measured by fluorescence microscopy. Top-I and Sp1 mRNA modulation by 5-azacytidine were measured by real-time PCR. Methylation of Top-I, p16, 14-3-3 σ , hMLH1 promoters before and after 5-azacytidine treatment was measured through Methylight PCR. Results demonstrated that low dose 5-azacytidine significantly enhances the apoptotic effect of irinotecan in all colorectal cancer cells. However, 5-azacytidine enhanced irinotecan growth inhibitory effect only in p53-mutated cells, with a 22.1-fold (HT29), 10.3-fold (SW620) and 2.9-fold (WiDr) IC₅₀ reduction. Top-I up-regulation by 5-azacytidine was significantly correlated with this effect, and was coupled to p16 demethylation and Sp1 up-regulation. P16 demethylation by 5-azacytidine was also associated with an enhanced cell cycle arrest after irinotecan treatment. In p53-wt LS174T cells, 5-azacytidine downregulated Top-I expression, thereby reducing SN38 cytotoxicity. In conclusion, 5-azacytidine modulates Top-I expression in a Sp1-, p16- and p53-dependent manner. Based on these findings, 5-azacytidine-irinotecan combination appears to be particularly effective against p53-mutated, p16-methylated colorectal cancers.

P205

Effect of various agonists and antagonists on mobility of glucocorticoid receptors measured by fluorescence correlation spectroscopy

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Glucocorticoids (GCs) are anti-inflammatory agents. Their receptor, GC receptor (GR), is one of the nuclear receptors and its transcriptional activity is regulated by ligands. Effects of agonists and antagonists on GR have been examined by various assays containing receptor binding and reporter gene assays. Here, we propose a novel parameter, diffusion constant of GR, to examine function of GR ligands. Fluorescence correlation spectroscopy (FCS) is a powerful method used to measure mobility of fluorescent molecules in subfemtoliter observation volume. We transfected EGFP-hGR α cDNA into Hela cells. EGFP-hGR α was translocated from cytoplasm into nucleus by addition of ligands tested. The diffusion constant of EGFP-hGR α in the nucleus was measured by FCS with a LSM510-ConfoCor2 system (Carl Zeiss, Germany). Fluorescence autocorrelation function was calculated according to a two-component fitting model and we selected second diffusion constant for analysis of ligands. High affinity ligands, dexamethasone, prednisolone, deltafludrocortisone and desoxymetasone dramatically decreased the diffusion constant to about 10% of that in the absence of ligands. In contrast, cortisone and progesterone known as low affinity ligands did not affect the mobility of EGFP-hGR α . GR is reported to associated with various cofactors and specific DNA sequence. The difference of the diffusion constant between high and low affinity ligands might reflect different association profile of GR. This work was performed as a part of a research and development projects of the Industrial Science and Technology Program supported by the New Energy and Industrial Technology Development Organization (NEDO).

P206**Menthol derivative WS-12 selectively activates transient receptor potential melastatin-8 (TRPM8) ion channels**

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Transient receptor potential melastatin-8 (TRPM8), a cationic ion channel is involved in detection of normal cooling-sensation in humans and rodents. Previous studies show that TRPM8 activation by cooling or chemical agonists produces profound, mechanistically novel analgesia in chronic pain states such as neuropathic pain in rodents. Known TRPM8 agonists such as menthol and icilin have a relatively low potency and cross-activate nociceptors like TRPA1; thus bearing a limited therapeutic usefulness. For that reason, characterising ligands, which selectively activate TRPM8, presents a clinical and experimental need that requires urgent attention. Using *Xenopus laevis* oocytes as expression system, we evaluated WS-12, a menthol derivative, for its potential interaction with all thermo-sensitive TRP ion channels. Oocytes were superfused with the test and standard substances respectively. Evoked responses were measured by two-electrode voltage clamp technique and the magnitudes of currents were compared with baseline values. WS-12 robustly activated TRPM8 in low micromolar concentrations (EC_{50} 12 ± 5 μ M) thereby displaying a higher potency and efficacy compared to menthol (EC_{50} 196 ± 22 μ M). Any of the other described thermo-sensitive TRP ion channels including TRPV1, TRPV2, TRPV3, TRPV4 and TRPA1 were not activated at a concentration (1 mM) saturating for TRPM8 responses; a characteristic which is in sharp contrast to menthol as it activates TRPA1 and TRPV3 in addition to TRPM8. Unlike icilin, WS-12 does not desensitise TRPM8 channels to repeated exposure of 1 mM saturating doses. In addition, acidosis or variations in extracellular calcium have no influence on potency/efficacy of WS-12 for TRPM8. The selectivity profile of WS-12, its several-fold higher potency and around two-fold increase in efficacy compared to menthol warrants its potential utility for therapy in chronic neuropathic pain states and as a diagnostic probe in prostate cancer.

P207***In vitro* generated cardiomyocytes - a novel tool to assess the cardiotropic actions for drug development and safety pharmacology**

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Scientists in cardiac science and toxicology suffer from the lack of standardised, pure *in vitro* cardiac cell and tissue models. Up-to-date, models for cardiotoxicity comprise recombinant cell systems e.g. injected oocytes and mammalian cell lines expressing ion channels or primary preparations of cardiomyocytes, cardiac tissues (e.g. papillary muscle) and Langendorff-perfused explanted hearts or *in vivo* animal models, respectively. Here we present data that *in vitro* generated, fibroblast-free cardiomyocytes derived murine embryonic stem cells (mESC-CM – Cor.At[®]) can be produced in large-scale and are a novel pure *in vitro* model to solve critical issues of cardiac ion channel modulation and humoral regulation. mESC-CM keep their functional integrity in culture for weeks after thawing. Voltage clamp experiments revealed expression of typical cardiac currents like I_{Na} , $I_{Ca,L}$, and I_K . Block of I_{Kr} currents by E-4031(hERG) was demonstrated in current and voltage clamp experiments and extra-cellular recording from multi electrode arrays (MEAs) revealing the capability of the mES-CM as an *in vitro* model for non-clinical assessment of the potential for delayed cardiac repolarisation. Application of typical ion channel blockers lidocaine (I_{Na} blocker) and nifedipine ($I_{Ca,L}$ blocker) as well as the beta-adrenergic and muscarinic agonists supramen and carbachol, respectively, in voltage clamp and MEA experiments display the functional integrity of the cells. Due to their electrical coupling evidenced by synchronous beating in 2D tissue cultures on MEAs and the expression of Cx43, the mESC-CM represent the first standardised *in vitro* cardiac tissue model to assess drug-induced arrhythmias. Furthermore, we were able to demonstrate cardiomyocyte specific cytotoxicity and the induction of hypertrophy.

P208**Pharmacologic induction of vascular extracellular superoxide dismutase expression *in vivo***Tatsiana Suvorava¹, Marc Oppermann¹, Vera Balz², Georg Kojda¹¹*Institute of Pharmacology, University Hospital, Duesseldorf, Germany*, ²*Otorinolaringology Clinic, University Hospital, Duesseldorf, Germany*

Pentaerythritol tetranitrate (PETN) treatment reduces progression of atherosclerosis and of endothelial dysfunction and decreases oxidation of LDL in rabbits. These effects have been linked to decreased vascular superoxide production but the underlying molecular mechanism remains unknown. We investigated the effect of (PETN) and of overexpression of endothelial nitric oxide synthase (eNOS⁺⁺) on expression and activity of extracellular superoxide dismutase (ecSOD). Methods: C57BL/6 mice were randomized to receive placebo or increasing doses of PETN for four weeks and eNOS⁺⁺-mice with a several fold higher endothelial-specific eNOS-expression were generated. Expression of ecSOD was determined in lung and aortic tissue by real time-PCR and Western blot. ecSOD activity was measured using inhibition of cytochrome-C reduction. Results: There was no effect of PETN treatment or eNOS overexpression on ecSOD mRNA, while ecSOD protein expression increased from 2.5-fold to 3.6-fold (p<0.05) by 6 mg PETN/kg BW/day and 60 mg PETN/kg BW/day, respectively. A similar increase was found in aortic homogenates. eNOS⁺⁺ lung cytosols showed an increase of ecSOD protein level of 142±10.5% as compared to transgene negative littermates (P<0.05), which was abolished by nitro-L-arginine treatment. In each animal group the increase of ecSOD expression was paralleled by an increase of ecSOD activity. Conclusions: Increased expression and activity of microvascular ecSOD are induced by an increased bioavailability of vascular NO. Upregulation of ecSOD likely represents a mechanism underlying antioxidative and antiatherosclerotic effects of PETN.

P209**Effect of oral organic nitrates on expression and activity of vascular soluble guanylyl cyclase**Thao-Vi Vu Dao¹, Suvorava Tatsiana¹, Kumpf Stephanie¹, Bas Murat², Weber Martina³, Oppermann Marc¹, Kojda Georg¹¹*Institute for Pharmacology and Clinical Pharmacology, Düsseldorf, Germany*, ²*Department of Otorhinolaryngology, Heinrich-Heine-University, Düsseldorf, Germany*, ³*Division of Cardiology, Emory University, Atlanta, GA, USA*

The regulation of vascular soluble guanylyl cyclase (sGC) expression by NO is still under discussion. *In vitro*, nitric oxide (NO) has been shown to downregulate the expression of sGC but it is unclear if this mechanism is operative *in vivo* and occurs during nitrate treatment. We investigated whether high dose isosorbide mononitrate (ISMN) or pentaerythritol tetranitrate (PETN) treatment changes vascular sGC expression and activity *in vivo*. New-Zealand White rabbits received a standard diet, 2mg or 200mg ISMN/kg BW/day for 16 weeks and C57BL/6 mice received standard diet, 6mg, 60mg or 300mg PETN/kg BW/day for four weeks. Absorption was checked by drug/metabolite plasma level determination. A new strain of transgenic mice with a vascular specific overexpression of eNOS was used to observe the effect of endogenous NO. Western blots of rabbit aortic rings showed similar protein levels of sGC- α_1 (P=0.2790) and sGC- β_1 (P=0.6900) in all groups. Likewise, ANOVA showed no difference of sGC expression in lungs of PETN-treated mice (P=0.0961 for sGC- α_1 and P=0.3709 for sGC- β_1 subunit). The activities of isolated sGC in response to SNAP (1 μ M-1mM) were identical in aortae of ISMN-treated rabbits (P=0.0775) and lungs of PETN-treated mice (P=0.6348). The aortic relaxation response to SNAP slightly decreased at high ISMN but not at high PETN. Transgenic mice showed a strong blood pressure reduction sensitive to eNOS-inhibition but again no change in sGC protein levels. However, sGC activity and phosphorylation of vasodilator-stimulated phosphoprotein was reduced. These data refute the hypothesis that therapeutic treatment with long acting NO-donors has a significant impact on regulation of vascular sGC expression and activity *in vivo*.

P210**The role of beta3-adrenoceptors and muscarinic receptors in the response of the heart to stress**

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We have shown previously that not only beta-adrenoceptors participate on response to stress in the lung tissue. The aim of this study was to elucidate the role of other heart receptors: beta3-adrenoceptors (beta3-AR) and muscarinic receptors (MR) in stress response. We studied the effects of cold and immobilization (IMO) stress treatment on the amount of the alpha1, beta1, beta2, beta3-AR, MR binding sites, on the mRNA levels and receptor protein concentrations, on the basal and stimulated adenylyl cyclase activity in specific heart regions (in the left (LV) and right (RV) ventricles) in rat and in mice lacking M2 MR. We used radioligands for receptor binding sites determination. Gene expression was determined using RT-PCR, the amount of respective receptor protein was determined using Western blots. Gene expression of rat beta3-AR was significantly affected by immobilization stress in rat LV, RV. 7 IMO caused the increase in the amount of beta3-AR mRNA in LV and the same trend was observed on protein levels and in the density of beta3-AR binding sites. In RV, there was an increase in the amount of mRNA for beta3-AR, but there were no changes in the protein concentration and in the number of receptor binding sites. On the other hand, 1 IMO induced the increase in the amount of the mRNA for beta3-AR in LV but there was decrease in the quantity of beta3-AR binding sites. Single and repeated cold treatment caused the analogical effects. The activity of adenylyl cyclase was affected by stress as well. The experiments with M2 KO mice showed that M2 MR are not essential for the stress reaction and that beta-AR are important players in stress response. We conclude that beta-AR are mainly important in the heart response to stress in variance to lung. This work was supported by Grant GAUK 11/06

P211**Chronic cannabis use does not affect the normalization of hypothalamic-pituitary-adrenal (HPA) axis induced by methadone in heroin addicts**

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The hypothalamic-pituitary-adrenal (HPA) axis activity is usually altered by heroin use (Nava et al., 2006; Stimmel & Kreek, 2000). In the present study we evaluated in one hundred and twenty-one heroin addicts the effects of marijuana smoking on the normalization of the HPA axis upon methadone treatment. The study was approved by Local Ethical Committees and was conducted according to the principles of Helsinki Declaration. The study showed that in heroin addicts who are chronic cannabis smokers, a treatment with methadone lasting 12 months was able to normalize both plasma corticotropin (ACTH) and cortisol levels, as well as to control both heroin withdrawal symptoms and opioid craving. As expected in the same group of patients, marijuana smoking and its craving were not reduced by methadone treatment. Our data confirm that methadone treatment outcomes are not modified by cannabis use and they add in the literature the evidence that chronic cannabis use is not able to affect the normalization of HPA axis upon methadone treatment in heroin addicts. The above evidence fits with the consolidate data that chronic cannabis use does not facilitate heroin consumption and relapse.

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Stimmel, B. & Kreek M.J. (2000). *Mt. Sinai J. Med.*, 67, 375-380.

P212**Cyclic nucleotide phosphodiesterase (PDE3 and PDE4) limit inotropy and cAMP production induced by the β_2 -adrenoceptors (β_2 -AR), salbutamol, in rat ventricular myocardium**

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We studied the effects of the non selective PDE inhibitor IBMX, as well as the inhibitors of PDE3 (cilostamide) and PDE4 (rolipram) on contractility and cAMP tissue levels induced by the β_2 -AR agonist salbutamol (SAL) in rat right ventricle. The role of Gi, in regulating inotropic response to SAL was also evaluated. Sprague-Dawley rats (230-250g) were used. Cumulative concentration-response curves were constructed in right ventricular strips (in Tyrode Solution at 37°C, pH 7.4, and electrically driven at 1 Hz, 1 ms), for SAL alone and in the presence of CGP 20712A (0.3 μ M) and ICI 118551 (50nM), antagonists of β_1 - and β_2 -AR, respectively as well as IBMX (30 μ M) and cilostamide (0.3 μ M) or rolipram (1 μ M). Some animals were treated with Pertussis toxin (PTX, 30 μ g/Kg. ip.). cAMP levels were determined by immunoassay. Data presented as mean \pm s.e.m (n) and analysed by Students t-test or ANOVA. CGP 20712A abolished the inotropic effect of SAL (0.1 μ M -1mM), which was restored by either IBMX (Emax= 43.6 \pm 7.5%;-log EC50=6.2 \pm 0.03;n=5) or rolipram+cilostamide (Emax=24.6 \pm 5.4%,n=4). These effects were virtually abolished by ICI 118551. SAL, in the presence of CGP 20712A, increases contractility by 9.1 \pm 1%, n=4, P<0.05) in rats pretreated with PTX. IBMX enhances by \approx 150%, cAMP production induced by SAL (0.5 μ M) in the presence of CGP 20712A and this effect was abolished by ICI 118551. Also, rolipram combined with cilostamide increases by \approx 60% cAMP levels caused by SAL+CGP 20712A. This study has demonstrated that both, PDE3 and PDE4, limit contractile responses and cAMP production induced by β_2 -AR activation in rat ventricular myocardium. PDEs are more important than Gi proteins in blunting inotropic effect mediated by β_2 -AR in this tissue. Supported by grant 05/2338 from the "Ministerio de Sanidad y Consumo" (Spain).

P213**The effect of paroxetine on ouabain-induced arrhythmia in isolated guinea-pig atria**

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It is generally accepted that the selective serotonin reuptake inhibitors (SSRIs) do have some cardiac effects. The aim of the present study was to investigate the effect of paroxetine on spontaneously beating isolated guinea-pig atria and its effects on ouabain-induced arrhythmia in atria as well. Guinea-pig of either sex weighing 400-500g was anaesthetized by ether and exsanguinated. Seven atria were used for each experiment. Results were expressed as mean \pm SE of seven guinea-pig atria in each group. Statistical significance was determined by using Student's *t*-test for paired data. P value < 0.05 was considered significant. PX (1-16 μ g/ml) caused a dose-dependent decrease in the rate of contractions (14-70%) but the changes of contractile force were negligible (8-16%). Ouabain alone (1.2 μ g/ml) produced arrhythmia at 7.2 min and asystole at 20 min. Pretreatment with PX (4 μ g/ml) significantly increased the time of onset of arrhythmia to 19.8 min. These findings indicate that PX produces a direct cardiac action, probably due to the inhibition of cardiac Na⁺ and K⁺ channels. The observed effects were dose-dependent. These effects are similar to those of fluvoxamine, citalopram and sertraline, but did not show a significant change in force of contractions. On the other hand, our results suggest that PX may reduce the membrane conductance through inhibition of ionic channels which decrease ouabain-induced arrhythmia

P214**The effect of fasudil, RhoA/Rho-kinase inhibitor, on phenylephrine-induced contractions in lung strips of chronic ethanol treated-mice**

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Recent studies indicated that the RhoA/Rho-kinase pathway plays an important role in the contraction of smooth muscle and is involved in Ca^{2+} sensitization (Uehata *et al.*, 1997; Somlyo *et al.*, 1998). The Ca^{2+} sensitization plays an important role in maintenance of the contractile response to in airway smooth muscle (Yoshii *et al.*, 1999). RhoA/Rho-kinases are expressed in rat bronchial smooth muscle (Chiba *et al.*, 1999), and participate in contraction to phenylephrine, endothelin, angiotensin and norepinephrine. Several factors modulate bronchial smooth muscle contraction signaling associated with the RhoA/Rho-kinase pathway. However, to our knowledge, it has not yet been investigated the effect of chronic ethanol treatment on the Rho-kinase pathway in the lung. Therefore, in the present study, the effect of chronic ethanol treatment on the phenylephrine-induced contractions of lung strips was investigated in mice. Phenylephrine (10^{-8} - 10^{-4} M) and KCl (10-80 mM) induced concentration-dependent contractions in lungs strips. Chronic-ethanol treatment (14 days) significantly inhibited contractions to phenylephrine compared to the control group. Fasudil (10^{-4} M) induced relaxation in the lung tissues contracted with phenylephrine. In ethanol treatment groups, fasudil (10^{-4} M)-induced relaxations were significantly decreased. These results suggest that chronic ethanol treatment may cause desensitization to contraction with phenylephrine and relaxation with fasudil in lung strips of mice, and this phenomenon may involve the RhoA/Rho-kinase pathway.

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Somlyo AP *et al.* Acta Physiol. Scand 1998, 164; 437-448

Uehata *et al.*, Nature 1997, 389; 990-994.

Yoshii *et al.*, Am. J. Respir. Cell. Mol. Biol. 1999, 20; 1190-1200

P215**Vascular oxidative stress inhibits mobilization of circulating stem cells with endothelial progenitor capacity in mice.**

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We sought to investigate the effects of increased vascular oxidative stress on exercise-induced endothelial progenitor capacity (EPCs) mobilization. Methods: Transgenic mice with a vascular-specific overexpression of catalase and reduced vascular levels of oxidative stress (cat^{++}) and their transgene negative littermates (cat^n) were assigned to a sedentary group and a group undergoing moderate forced exercise training (15 m/min, 30 min, 5 days a week, 3 weeks). The number of EPCs in peripheral blood was measured by Fluorescence-Activated Cell Sorting using anti-mouse CD3, Flk-1 and CD34, CD133 or Sca-1 antibodies. Results: There was no difference in circulating EPCs between sedentary and freely moving C57BL/6 mice ($p > 0.05$, $n = 5$). Three weeks of forced exercise training failed to mobilize EPCs defined as double positive for Flk-1 and CD34 or CD133 ($p > 0.05$, $n = 5-9$). Similarly, the number of EPCs was not different between sedentary and voluntary exercised groups ($n = 5-8$, all $p > 0.05$). FACS analysis of cat^{++} and cat^n peripheral blood revealed no effect of catalase overexpression on the basal level of circulating EPCs ($p = 0.68$, $n = 8$). Inhibition of catalase by 2 week treatment with catalase inhibitor aminotriazole (670 mg/kg in drinking water) strongly reduced the number of endothelial progenitors in blood of sedentary cat^n , and to a lesser extent also in cat^{++} ($p < 0.05$, $n = 5-8$). When mice with vascular specific overexpression of catalase were subjected to the forced exercise training, the number of circulating EPCs was strongly increased ($n = 4-8$, $p < 0.05$). Furthermore, exercise-induced increase of circulating EPCs was completely abolished in cat^{++} treated with aminotriazole. Conclusion: Hydrogen peroxide, an important component of vascular oxidative stress inhibits exercise-induced mobilization of EPCs.

P216**New formulation for management of pain and inflammation related disorders: development, characterization and pharmacological evaluation**

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Non-steroidal anti-inflammatory drugs (NSAIDs) are the “Gold Standards” in the treatment of various pain and inflammation related disorders like arthritis. However, oral administration and newer selective COX-2 NSAIDs are known to induce severe intolerance. Thus, the present work aims to develop and evaluate a novel carrier for targeted epicutaneous delivery of a potent NSAID, diclofenac. Flexible-Vesicles (FVs) are new generation liposomes designed to deliver drug to deep-seated inflamed and painful locations through topical route. Various formulations with different compositions were prepared and characterized to select the best formulation. The selected vesicular system was characterized and evaluated for *ex-vivo* drug permeation and drug deposition employing suitable membranes. The biological activity of final optimized formulation was evaluated using the carrageenan induced rat paw edema model with simultaneous radiographic analysis. FVs selected were found have best desired characteristics among all the prepared FVs. The *ex-vivo* permeation and skin-deposition of the diclofenac loaded FVs were found to be higher compared to other tested formulations. The *in-vivo* pharmacodynamic result was found to be in agreement with the *ex-vivo* findings as FVs have shown faster onset and prolonged anti-inflammatory effect, while radiographic analysis ratifies the above result. The results of present study demonstrated superior efficacy and safety of topically applied FVs loaded formulation. Hence, it can be concluded that diclofenac containing FVs can be a very good proposition for management of pain and inflammation related disorders

P217**Liver injury caused by ischemia-reperfusion in the rat is attenuated by pre-treatment with simvastatin**

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Simvastatin is a member of the therapeutic family of statins. Recent experimental studies demonstrate that treatment of animals with statins reduces the injury caused by ischemia-reperfusion of the heart, brain and kidney. There is evidence that statins interfere with several signalling pathways involved in inflammation such as: NF-kappaB, peroxisome proliferator-activated receptors, and heme-oxygenase. Here we investigate the effects of simvastatin, on the liver injury caused by ischemia-reperfusion (I/R) injury, in the rat. Male Wistar rats were randomly allocated into 4 groups as described: (1) Sham Group; (2) I/R Group: rats which were subjected to liver ischemia for 45 minutes interrupting blood supply to $\frac{3}{4}$ of the liver, followed by reperfusion for 2 h; (3) Simvastatin + I/R Group: rats which received simvastatin (1 mg/kg i.v.) 30 minutes prior to liver I/R; and (4) Simvastatin Control Groups. Blood samples were obtained at the end of reperfusion for determination of biochemical markers of liver injury and tissue samples were also collected for histology. Our results show that simvastatin reduced the increase on the level of biochemical markers for liver injury when compared to I/R group: AST (965±52 vs. 740±27 IU/l) and ALT (1000±63 IU/l vs. 535±35 IU/l). We demonstrate here that simvastatin, an inhibitor of HMG-CoA reductase and a ligand of PPAR- α and PPAR- γ , causes a substantial reduction in the ischemia-reperfusion-induced increase in the serum levels of hepatic injury markers, providing, to our knowledge for the first time, the evidence that statin administration reduces the liver injury induced by ischemia-reperfusion, in the rat. We propose that statins, such as simvastatin, may be useful in the therapy of liver injury associated with ischemia-reperfusion of the organ.

P218**Kallikrein inhibitors limit B₂ antagonist-induced progression of oedematous to haemorrhagic pancreatitis in rats**

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Exocrine hyperstimulation with caerulein is an established model for oedematous acute pancreatitis. Prevention by kinin B₂ receptor antagonists of oedema formation induces a progression to a haemorrhagic course in this model. We have investigated whether increased kallikrein activity in the pancreas is responsible for vascular damage and whether this could be prevented by selective kallikrein inhibitors. Caerulein was infused i.v. in anaesthetized Sprague-Dawley rats (200–250 g) and vascular damage was assessed by histological evaluation and determination of haemoglobin accumulation in the tissue. In addition, oedema formation, tissue and plasma kallikrein activities and the endogenous kallikrein inhibitors α_1 -antitrypsin and α_2 -macroglobulin were measured. Haemorrhagic lesions induced by icatibant were associated with a pronounced reduction of α_1 -antitrypsin ($P<0.01$) and α_2 -macroglobulin ($P<0.05$) in the pancreas and a concomitant augmentation of tissue kallikrein activity ($P<0.01$). The tissue kallikrein inhibitor VA999024, or its combination with the plasma kallikrein inhibitor VA999026, inhibited oedema formation to the same extent ($P<0.01$) but did not induce vascular damage. Furthermore, VA999024 inhibited ($P<0.05$), rather than augmented tissue kallikrein activity. When icatibant was combined with VA999024 and VA999026, progression from oedematous to haemorrhagic pancreatitis was prevented completely. In conclusion, reduced oedema formation by B₂ antagonists prevents influx of endogenous kallikrein inhibitors and thus causes an excessive activity of kallikrein in the pancreas leading to vascular damage. This can be prevented by a combined inhibition of both tissue-type and plasma-type kallikrein

P219**Effects of the nimesulide on clinical variables in patients with early rheumatoid arthritis**

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Nonsteroidal anti-inflammatory drugs are commonly used for treatment of the pain associated with rheumatoid arthritis (RA). During the past decade interest is increasing in assessing of the effects of cyclooxygenase-2 selective inhibitor nimesulide in patients with early RA. Our objective was to evaluate the effects of nimesulide in patients with early RA. The study included 21 outpatients (1 male, 20 females) with early RA (mean age 42.90±13.46 years, mean disease duration 5.98±3.71 months, 3 RF+, 18RF-). All patients received nimesulide 200 mg/day during 3-4 weeks. The clinical variables (morning stiffness duration (min), patient and physician global assessments, global pain assessment by visual analogue scales (0-100 mm), disease activity score by DAS28) were collected at baseline and at 4 weeks. Study results showed that morning stiffness duration reduced from 184.05±312.70 to 133.90±277.00 min ($p<0.05$); patient global assessment decreased from 61.56±22.73 to 46.59±24.29 mm, physician global assessment decreased from 55.28±19.29 to 41.60±21.35 mm, global pain assessment weakened from 58.70±28.02 to 48.27±32.39 mm ($p<0.01$). The DAS28 decreased from an initial mean score of 4.81±1.71 to 4.2±1.85 ($p<0.01$). Patient global assessment correlated with physician global assessment at baseline and at 4 weeks (Spearman r was 0,64 to 0,65 accordingly). Nimesulide represents an effective drug for the treatment of joint pain and stiffness, but it does not give remission of early RA. The treatment course should be long by disease-modifying antirheumatic drugs.

P220**Pharmacological evaluation of the anti-inflammatory and cytotoxic activities of crude extracts from the Mediterranean marine algae and sponge**

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As part of our search for new anti-inflammatory or anticancer potential drugs, aqueous extracts of macro-algae and invertebrates collected from Mediterranean Tunisia coasts were evaluated for their anti-inflammatory and cytotoxic activities. The present study has established that the aqueous extract from the brown algae of the genus *Zonaria* tested at different doses (50, 100, 200 mg/ kg) for their anti-inflammatory activity, using the carrageenan paw oedema test (Winter *et al*, 1962) in male albinos Wistar rats (150-170 g) and in comparison to reference drugs: Dexamethasone (1 mg/kg) and aspirin (300 mg/kg), exhibited, in a dose dependent manner, a significant inhibitory effect on rat paw oedema; the % inhibition of oedema, 3h after carrageenan injection ranged from 66% to 86%. We also established that the aqueous extract of the marine sponge of the genus *Spongia*, showed a strong cytotoxic activity against three human tumour cell lines (A-549, MCF-7, HCT-15), using a MTT cytotoxicity assay (Bissery *et al*, 1991). At concentration of 0.50 to 1.25 mg/ml, this extract suppressed, dose dependently, the proliferation of the three cell lines by more than 75%. The IC₅₀ values ranged from 0.80 to 0.90 mg/ml. The pharmacological activities of these active extracts are discussed in accordance with the secondary metabolites present in the brown algae, *Zonaria* (Puntip *et al*, 2003) and *Spongia* (Grassia, *et al*, 1994). Purification these extracts are under investigation.

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Grassia, *et al*, 1994. Tetrahedron 57 (29), pp 6257-6260;

Puntip *et al*, 2003. Journal of applied psychology, 15(2-3):225-228;

Winter *et al*, 1962. Proc. Soc. Exp. Biol. Med. 111: 544 – 547.

P221**Pharmacological evaluation of the anti-inflammatory and analgesic activities of the aqueous extract from the Mediterranean sponge, *Petrosia ficiformis***

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As part of our search for anti-inflammatory substances from marine sponges, the anti-inflammatory and analgesic activities of the aqueous extract from the mediterranean sponge, *Petrosia ficiformis* were investigated in animal models using the carrageenan-induced paw oedema assay (Winter *et al*, 1962) in male albinos wistar rats (150-170 g) and acetic acid writhing test in mice (Koster *et al*, 1959). The aqueous extract (50, 100 and 200 mg/kg) administered intraperitoneally, and in comparison to reference drugs: dexamethasone (1 mg/kg) and acetylsalicylic acid (300 mg/kg), exhibited, in a dose dependent manner, a significant inhibitory effect on the rats paw oedema ($p < 0.001$). The % inhibition of oedema, 3h after 0.05 ml of 1% carrageenan injection were higher than 50% and ranged from 51% to 64%. In addition, this aqueous extract (200 mg/kg), S/C administered in mice and in comparaiso to a reference drug : acetylsalicylic acid (200 mg/kg), significantly reduced the nociception induced by the 1% acetic acid intraperitoneal injection ($p < 0.01$). The % inhibition of writhing, 30 min after acetic acid injection was 53 %. The pharmacological activities of this active extract are discussed in accordance with the secondary metabolites present in the sponge of the genus *Petrosia* (Giner, 1999; Shin *et al*, 1998; Lim *et al*, 1999). Purification and determination of chemical structures of compound(s) in this extract are under investigation.

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Koster *et al*, 1959. Fed Proc 18: 412-416. ;

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P222**The effects of an NMDA receptor antagonist in cutaneous, visceral pain and behavioral models**Liliana TARTAU*University of Medicine and Pharmacy Gr. T. Popa, Iasi, Romania*

Aim: Experimental research on the effects of an NMDA receptor antagonist 3-((+)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP potent and selective), in cutaneous, visceral pain and behavior models. Material and method: The experiments were carried out, with white mice (20-25g), divided into 3 groups of 7 animals each, treated intraperitoneally with the same volume of solution, as follows: Group I: saline solution 0,1ml (Control); Group II (MOR): morphine 0,2mg/kbw; Group III (CPP): CPP 0,7mg/kbw. Tail flick was used to assess CPP induced cutaneous antinociception. The model of visceral pain used consists of chemical stimulation of the colon with capsaicin. CPP psycho-motor abilities were tested in the Activity Cage device, in order to investigate the both global motor behaviour and the number of escape attempts. Data were statistically analyzed with SPSS for Windows version 10.0 and ANOVA method. Results and conclusions: Intraperitoneal administration of the NMDA receptor antagonist CPP (0,7mg/kbw) resulted in antinociceptive effects in tail flick test. In the colon hyperalgesia test, CPP exhibited antinociception which was less intense than that of morphine, whose analgesic effect is demonstrated in this experimental model. In our experiment CPP administration do not influence the global motor behavior in mice.

P223**Anti-inflammatory action of salvianic acid A through the inhibition of nuclear factor- κ B activation in peritoneal macrophages**Jun Yuan, Jihong Yao, Xiaofeng Tian*Dalian Medical University, Dalian, China*

Our aim was to investigate the anti-inflammatory effect and possible mechanism of salvianic acid A (SAA) in mouse peritoneal macrophages. Peritoneal macrophages were obtained from BALB/c mice. LPS induced nitric oxide (NO), tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in supernatant, protein expression of inducible nitric oxide synthase (iNOS), matrix metalloproteinase-9 (MMP-9) and activation of nuclear factor-kappa B (NF- κ B) in the extract were measured. SAA strongly inhibited the excessive production of NO, TNF- α and IL-6 in LPS-induced peritoneal macrophages in a concentration-dependent manner and blocked the expression of iNOS and MMP-9. Treatment with LPS alone increased the translocation of NF- κ B (p65) from cytosol to the nucleus, but the SAA inhibited the translocation of NF- κ B (p65). The results showed that SAA had strong anti-inflammatory effects in LPS-stimulated peritoneal macrophages. The important mechanism is due to its inhibition of NF- κ B activation.

P224**The role of hydrogen sulphide in hepatic and renal injury in an experimental model of scald burn in the rat**Patricia Marques¹, João Rocha¹, Pedro Baracho¹, Rui Pinto¹, Bruno Sepodes¹, Christoph Thiemeermann², Helder Mota-Filipe¹¹*Unit of Pharmacology and Pharmacotoxicology - Faculty of Pharmacy of the University of Lisbon, Lisbon, Portugal,* ²*The William Harvey Research Institute, London, UK*

Evidence available so far supports a pro-inflammatory role for the gaseous mediator hydrogen sulphide (H₂S). The present study aimed to investigate the role of endogenous and exogenous H₂S in an experimental model of burn injury. Sixty-seven anesthetized and shaved male Wistar rats (280-450g) were randomly allocated into 6 experimental groups and subjected to a tracheotomy, after which they received a 30% total body surface area third-degree burn by immersion in 99°C water for 10 seconds, with exception of the sham group which was immersed in water at room temperature. Six hours after burn injury, blood samples were collected for determination of biochemical markers of liver injury and kidney dysfunction. Pre-

treatment with propargylglycine (PAG, an irreversible inhibitor of cystathionine- γ -lyase (CSE), an enzyme responsible for H₂S formation in liver and kidney) significantly reduced the burn-induced hepatic injury (ALT: 27 \pm 6 vs. 256 \pm 60 IU/L). Treatment with sodium hydrosulphide (NaHS, a donor of H₂S) significantly increased hepatic injury (ALT: 690 \pm 164 vs. 256 \pm 60 IU/L; AST: 1569 \pm 331 vs. 631 \pm 55 IU/L). In what concerns the kidney function, neither PAG (urea: 110 \pm 2 vs. 114 \pm 4 mg/dL; creatinine: 2,20 \pm 0,14 vs. 1,58 \pm 0,16 mg/dL) nor NaHS (urea: 116 \pm 5 vs. 114 \pm 4 mg/dL; creatinine: 2,30 \pm 0,30 vs. 1,58 \pm 0,16 mg/dL) were able to improve the burn-induced renal dysfunction. In conclusion, with this study we demonstrate that: *i*) H₂S is involved in the pathogenesis of burn injury and, *ii*) inhibition of CSE (and subsequently H₂S production) seems to protect the liver from burn-induced injury in the rat, while in the kidney the functional role of this gaseous mediator is still not very clear.

P225

Effect of hydrocortisone on ATP-induced inhibition of neuromuscular transmission in frog skeletal muscle

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The inhibitory effects of ATP, a P2 receptor agonist, on the contractile responses of isolated sartorius muscle of *Rana latibunda* frogs evoked by electrical field stimulation were studied using pharmacological organ-bath technique. The effects of hydrocortisone applied *in vitro* and *in vivo* on contractility of sartorius muscle were examined. ATP (100 μ M) significantly reduced the amplitude of contraction of sartorius muscle, while PPADS (10 μ M), a P2 receptor antagonist, abolished this effect. Hydrocortisone (10 μ M) added directly into the bath solution caused slight, but significant decrease of sartorius muscle contraction. After incubation of muscle preparations together with hydrocortisone (10 μ M) and ATP (100 μ M) no inhibition of muscle contractility was registered. A single injection of hydrocortisone (100 mg/kg) to frogs did not significantly change the contractility of isolated sartorius muscle induced by electrical field stimulation; however, after treatment of frogs with hydrocortisone for 14 days (100 mg/kg/day) ATP (100 μ M) retained their inhibitory action on contractility of the muscle, and its effects were antagonized by PPADS (10 μ M). It is concluded that hydrocortisone has some antagonistic action at P2 receptors in the frog neuromuscular junction, and its effect is lost after long treatment with this glucocorticoid.

P226

Quinoline derivatives as memory enhancers: synthesis and pharmacological evaluation

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Memory or cognition dysfunction is a neurodegenerative disorder of brain, in which selective apoptosis of cholinergic neurons, in a specific region of the brain, leads to paucity of acetylcholine resulting to the loss of memory and learning functions (Cacabelos *et al.*, 2000). The present study aims to develop some newer 6-aminoquinoline derivatives, as drug candidates, as potential acetylcholinesterase inhibitors. Acetanilide after nitration was subjected to Skraup's synthesis to yield 6-aminoquinoline (1) which was further treated with different carboxaldehydes e.g., pyridine-2-carboxaldehyde (1a), pyridine-3-carboxaldehyde (1b) and pyridine-4-carboxaldehyde (1c) to obtain the target compounds. These synthesized compounds were evaluated for their pharmacological activity of employing elevated plus-maze model (Sharma *et al.*, 1992). Albino mice (LAKA strain) weighing 20-25 g of either sex, were used in evaluation. Finally, the biochemical study was carried out by Ellman's method to elucidate the mechanism of action of these compounds. Results clearly showed that compound (1c) has significantly higher anti-amnesic activity. While, biochemical study confirm its anti-cholinesterase action. With the above results it can be concluded that that new compound (1c) synthesised, was found to be a potent cognitive enhancer

Cacabelos, R. *et al.*, *Drugs Today* 2000, 35 (7), 415-495.

Sharma, A.C. *et al.*, *Prog. Neuropsychopharmacol. Biol. Psychiat.* 1992, 16, 117-125

P227**The protective effect of vitamin E on locus coeruleus in early model of Parkinson's disease in rat: immunoreactivity**

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Free radicals derived from molecular oxygen have been reported to be responsible for changes in motility and mucosal damage observed in intestinal ischemia-reperfusion injury. Melatonin has been considered as an antioxidant that prevents injuries resulted from I/R in various tissues. The present study was designed to determine the effect of melatonin on the contractile responses of acetylcholine (ACh) and KCl, on malondialdehyde (MDA), a product of lipid peroxidation, and reduced glutathione (GSH) levels and to assess histopathological changes in the smooth muscle of terminal ileum subjected to ischemia-reperfusion. The intestinal ischemia-reperfusion was induced by occlusion of superior mesenteric artery of rat for 30 min, followed by a period of reperfusion for 3 h. Melatonin at doses of 10 or 50 mg/kg was administered via the tail vein in 5 min prior to reperfusion. Following reperfusion, segments of terminal ileum were rapidly taken and transferred into isolated organ bath and responses to ACh and KCl were recorded. Samples of terminal ileum were also taken for measuring the MDA and GSH levels. EC₅₀ values of these contracting substances were seriously reduced in the ischemia-reperfusion group compared to that of the sham-operated control group. The decreased contraction response to ACh and KCl was significantly ameliorated by a dosage of 50 mg/kg of melatonin, while not by a dosage of 10 mg/kg. Similar pattern of the effect was observed in the tissue levels of MDA and GSH as well as in histological improvement. Melatonin appeared to be restoring the amounts of tissue MDA and GSH back to about control levels. These results suggest that the high dose of melatonin not only physiologically but also biochemically and morphologically could be useful to normalize contractility injured by oxidative stress in intestinal ischemia-reperfusion.

P228**Establishment of the model of oxygen-glucose deprivation *in vitro* rat cortex and hippocampal neurons**

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Our objective was to establish a model of oxygen-glucose deprivation *in vitro* rat cortex and hippocampal neurons. Cortex and hippocampal neurons cultured for 10~12 days were exposed to Earle's solution with sodium dithionite(Na₂S₂O₄)1mmol/l and free glucose in stand of original culture medium containing blood serum for 2, 4,8,12,24 and 36h respectively. 3-(4,5 - dimethylthiazol-2yl)—2,5—diphenylterazolium bromide(MTT) assay was applied to evaluate the neuronal vigor. A biochemical method was used to determined lactate dehydrogenase (LDH) activity. The rat cortex and hippocampal neurons vigor significantly decreased in a time-dependent manner after the original culture medium was replaced by Earle's solution with sodium dithionite 1mmol/l and free glucose for 2~36h,and the neuron survival rate decreased to 25.4% and 24.2% respectively after oxygen-glucose deprivation for 36h. However, LDH release gradually increased in a time-dependent manner. There showed a strongly negative correlation for cortex or hippocampal neurons between the changes of LDH activity and neuronal survival rate ($r = - 0.983, - 0.992, P < 0.01$). The model of oxygen-glucose deprivation *in vitro* rat cortex and hippocampal neurons was established successfully by applying Earle's solution with sodium dithionite 1mmol/l and free glucose.

P229**Carboxyamidotriazole inhibits proliferation of human breast cancer cells via G2/M cell cycle arrest and apoptosis**

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Carboxyamidotriazole (CAI) has been shown to be able to induce growth inhibition and apoptosis in cancer cells. We demonstrate that CAI significantly inhibits proliferation of cultured MCF-7 human breast cancer cells in a dose-dependent manner with an IC_{50} of 26 μ M. Reduced proliferation of MCF-7 cells in the presence of CAI correlated with accumulation of cells in G2/M phase and induction of apoptosis. A treatment of MCF-7 cells with 30 μ M CAI caused a time-dependent decrease in the levels of proteins that regulate G2/M progression, including Cdk1, Cyclin B1, and Cdc25C. A simultaneous increase in the expression of p21 protein was observed. We also demonstrated a concurrent decrease of the mitochondrial membrane potential ($\Delta\Psi_m$), and down-regulation of anti-apoptotic protein Bcl-2. In conclusion, it seems reasonable to hypothesize that the anti-tumor effect of CAI in MCF-7 cells is based on G2/M cell cycle arrest and inducing apoptosis.

P230**The relationship between blood cells and drugs**

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The ability of blood cells in the upper layer is mainly dependent upon drugs during suffering diseases. Therefore, it is most important to prescribe proper drugs based on the relationship between drug and blood cell. For that purpose, I have developed New- Matsumoto Method. The New Matsumoto Method is used. Assuming that the entire amount of daily dosage of drug is absorbed in 5l of blood, the absorbed amount of drug in 3ml of blood is calculated.

$$5,000\text{ml} : \text{Drug Amount (amg)} = 3\text{ml} : X$$

$$X = 3\text{amg}/5,000\text{ml}$$

The daily dosage of drug that a patient is administered is added to the upper and lower layers of blood cells collected using MH Method. After twice of filtering, 1ml of the solution and 9ml of physiological saline are mixed, obtaining 10ml of solution. Then this diluting procedure is repeated: i.e. 1ml of the solution and 9ml of physiological saline are mixed, obtaining 10mL of solution once again. Using a syringe for tuberculin, 0.6ml of the solution is poured into a flask containing 3ml of RPMI-1640 (tissue culture medium). Then it is cultured in an incubator under the condition of 5% CO_2 at 37°C ° and diachronically monitored with an inverted phase-contrast microscope. When the daily dosage is large in quantity such as Chinese medicine, one-tenth of daily dosage is dissolved in 10mls of physiological saline. The efficacy of the following drugs and therapies can be examined by the above-mentioned methods.

- 1) The prescribed drugs with side effects
- 2) The drugs that doctors do not prescribe but is supposed to be effective
- 3) a) The relationship between the upper layer of blood cells governing natural healing power and supplements, Chinese medicine, massage, acupuncture, moxa cauterly, Qigong, exercise, balneotherapy, etc.
b) Each therapy should be tested separately, and the upper (U) and lower (L) layers of blood cells should be examined before and after each therapy. If the conditions of U and L are normalized or relieved, the therapy can be considered as effective; otherwise, it should not be considered as effective. When a therapy appears to be effective despite that no effects are detected in the test, it might be caused by mental factors: "illness comes from the mind".

P231**Thrombin-induced connective tissue growth factor expression in human lung fibroblasts requires ASK1/JNK/AP-1 pathway**

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In this study, we investigated the role of apoptosis signal-regulating kinase 1 (ASK1) in thrombin-induced AP-1 activation and connective tissue growth factor (CTGF) expression in human lung fibroblasts. The thrombin-mediated CTGF expression and CTGF-luciferase activity were inhibited by the dominant-negative mutants (DNs) of ASK1, JNK1, JNK2, an AP-1 inhibitor (curcumin), and a PAR1 receptor antagonist (SCH 79797), but not by a PAR4 antagonist (tcY-NH₂). Thrombin caused ASK1 Ser967 dephosphorylation, dissociation of ASK1 and 14-3-3, and a subsequent increase in ASK1 activity. Thrombin-induced JNK activation was inhibited by ASK1DN. Thrombin caused increases in c-Jun phosphorylation, the formation of an AP-1-specific DNA-protein complex, and the recruitment of c-Jun to response elements on the CTGF promoter. The thrombin-induced CTGF-luciferase activity was major controlled by the -747 to -184 bp upstream of transcription start site on human CTGF promoter. Furthermore, the thrombin-induced CTGF-luciferase activity was attenuated by transfection with the deleted AP-1 binding site construct. Furthermore, thrombin-mediated AP-1 activation was inhibited by ASK1DN and SP600125. These results suggest for the first time that thrombin, acting through PAR1, activates the ASK1/JNK signaling pathway, which in turn initiates c-Jun/AP-1 activation and recruitment to CTGF promoter, and finally induces CTGF expression in human lung fibroblasts.

P232**ASK1 in peptidoglycan-induced COX-2 expression in RAW 264.7 macrophages: Involvement of C/EBP β**

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In this study, we investigated the role of ASK1 in PGN-induced C/EBP β activation and COX-2 expression in macrophages. The PGN-induced COX-2 expression was attenuated by ASK1DN, JNK1 DN, JNK2DN, a JNK inhibitor (SP600125), and an AP-1 inhibitor (curcumin). PGN caused ASK1 Ser967 dephosphorylation, dissociation of ASK1 and 14-3-3, and a subsequent increase in ASK1 activity in time-dependent manners. In addition, PGN increased the activity of protein phosphatase 2A (PP2A). Suppression of PP2A activity by the specific inhibitor, okadaic acid, markedly inhibited PGN-induced ASK1 Ser967 dephosphorylation and COX-2 expression. Treatment of cells with PGN caused the activation of JNK; this effect was inhibited by ASK1DN. PGN caused increases in c-jun phosphorylation, the formation of an AP-1-specific DNA-protein complex, and AP-1-luciferase activity. The PGN-mediated increase in AP-1-luciferase activity was inhibited by SP600125 and curcumin. Treatment of macrophages with PGN caused the increases in C/EBP β expression and the binding activity of C/EBP β to the C/EBP binding sequence of COX-2 promoter; these effects were inhibited by ASK1DN, SP600125, and curcumin. Furthermore, PGN-induced COX-2-luciferase activity was attenuated in cells transfected with the COX-2 reporter construct possessing the C/EBP binding site mutation. Our data demonstrate for the first time that PGN might activate ASK1 through PP2A activation to cause JNK/AP-1 activation, which in turn induces C/EBP β expression and subsequent activation, and ultimately results in COX-2 expression in RAW 264.7 macrophages.

P233

New RNAi system for analysis of pharmacological relevant genes

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The RNAi technology has opened new insights in pharmacology by elucidation of the mode of action of known drugs or of drug candidates. In cellular assays the low delivery rate of siRNA can limit the applicability of conventional RNAi approaches. We have bypassed this limitation by using Q-tech- ready to use cells which already comprise a preset knock down mediated by an adenoviral shRNA vector. We have used Q-tech.shp53 (knock down of p53 tumorsuppressor) and analyzed the responsiveness to 5-fluorouracil (5-FU) compared to a control (Q-tech.control). The results indicate an enhanced resistance to 5-FU in Q-tech.shp53 compared to Q-tech.control. These results are in line with clinical data that show poor prognosis in cancer patients with inactive p53. The high data quality with Q-tech was confirmed by high content analysis which showed almost complete knock down of p53 and excellent reproducibility between batches. The new concept of Q-tech allows for unraveling the mode of action of drugs even in cellular systems that are resistant to conventional transfection.

P234

Hepatoprotective activity of extracts of *Sapindus mukorossi* and *Rheum emodi* in CCl₄ induced liver damage: *In vitro* and *in vivo* studies

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Our aim was to study the hepatoprotective activity of *S.mukorossi* and *R.emodi* extracts in CCl₄ treated male rats. The dried powder of *S. mukorossi* and *R. emodi* was extracted successively with petroleum ether, benzene, chloroform and ethanol and concentrated in vacuum. Rat hepatocytes in primary monolayer culture methods were used for *in vitro* study. In *in vivo* studies the hepatoprotective activity of the extract of the fruit pericarp of *S. mukorossi* and rhizomes of *R. emodi* was studied on liver damaged (CCl₄ induced) male rats. *In vitro* study: CCl₄ damaged primary monolayer culture of the hepatocytes was treated with the extracts of *S. mukorossi* and *R. emodi*. Hepatoprotective activity was observed in the CCL₄ damaged primary monolayer culture. *In vivo* study: Extracts of the fruit pericarp of *S. mukorossi* (2.5mg/ml) and rhizomes of *R. emodi* (3.0mg/ml) were found to have reparative properties in CCL₄ induced liver damaged rats as judged from serum marker enzyme activities. We can conclude from this study, that the extracts of *S. mukorossi* and *R. emodi* are hepatoprotective both *in vitro* and *in vivo*.

P235

Hepatoprotective activity of *Melia azedarach* leaf extract against paracetamol induced hepatic damage in rats

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Our aim was to study the hepatoprotective activity of the methanolic leaf extract of *Melia azedarach* against paracetamol induced treated male rats. The dried powder of leaf extract of *Melia azedarach* was extracted successively with methanol and concentrated in vacuum. In this study the hepatoprotective activity of the methanolic leaf extract of *Melia azedarach* was studied on liver damaged (paracetamol induced) male rats. In this study: Over dosing of paracetamol to

rats is reported to decrease the activity of anti-oxidative enzymes (GPx, GST, SOD and CAT) in liver. Simultaneous administration of crude extract (500mg/kg of body weight) and paracetamol (2g/kg of body weight) to rats for 7 days protect the loss of functional integrity. *Melia azedarach* methanolic leaf extract inhibit the oxidation and maintained the activity of antioxidant enzymes to the normal level. We can conclude from this study, that the methanolic leaf extract of *Melia azedarach* possesses significant hepatoprotective activity due to its antioxidant property.

P236

The Anti spasmodic effect of supermint extract in rat ileum in an *in vitro* model

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Previous studies indicated that supermint 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 supermint extract in rat ileum Male Wistar rats (220-250 g) were used. Pieces of ileum (20-30 mm) 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 supermint extract was added in five concentration and change in contraction calculated. It has been shown that 0.1 Hz stimulation of rat ileum induced contractions which are depressed by atropine. Addition of aqueous extract of supermint extract to the organ bath during 0.1Hz stimulation decreased contraction in a dose dependent manner (EC₅₀=0.7mg/ml). It seems that supermint extract shows its effect by inhibition of the cholinergic system because the contraction known to be due to release of acetylcholine at 0.1Hz was decreased by addition of supermint extract.

P237

A study on hypolipidemic effect of *Luffa aegyptiaca* mill fruits

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The methanolic extract of *Luffa aegyptiaca* mill fruits were investigated for their possible hypolipidemic effect on hypercholesterolemia induced in New Zealand white rabbits by feeding the animals with normal diet supplemented with 1% cholesterol and 10% ground nut oil for 8 weeks. Rabbits with normal diet and hypercholesterolemic diets throughout the experiment were used as negative and positive control respectively. There was a significant increase in the weight of hypercholesterolemic rabbits when compared to normal control. It was observed that hypercholesterolemic rabbits treated with normal diet elicited a significant reduction in fasting serum cholesterol (10.5%) triglycerides (15.5%) and LDL cholesterol (40.11%), but a significant increase in HDL cholesterol (16.4%) and also in the HDL/LDL ratio (1.10). Methanolic extract of *Luffa aegyptiaca* mill fruits significantly reduced serum total cholesterol by 61.2 % and 51.5% respectively, triglycerides by 45.6 % and 26 % and LDL cholesterol by 85 and 83 % respectively. They also increased serum HDL by 24.2 and 25 % respectively leading to increased HDL / LDL Cholesterol ratio (3.42 and 3.35 respectively). This trend was similar with aorta stained with eosine and haematoxylin showed fewer lesions in the hypercholesterolemic rabbit treated with methanolic extract of *Luffa aegyptiaca* mill fruits when compared with control hypercholesterolemic rabbits. These observations demonstrated that *Luffa aegyptiaca* mill fruits have strong hypolipidemic effect which compared with improved HDL / LDL ratio is an indication of the possible use of this fruits in the treatment of the diseases associated with hyperlipidemia such as ischaemic heart disease and arteriosclerosis.

P238**Determination of celecoxib in rat plasma by high-performance liquid chromatography with mass spectrometry detection and its application to pharmacokinetic studies**Rodica Cuciureanu¹, Laurian Vlase², Dana Muntean², Magda Cuciureanu¹, Mihai Nechifor¹¹University of Medicine and Pharmacy, Iasi, Romania, ²University of Medicine and Pharmacy, Cluj-Napoca, Romania

A new sensitive and selective liquid chromatography coupled with mass spectrometry (LC/MS/MS) method for quantification of celecoxib (CLX) in rat plasma was developed and validated, in order to use it for the study of pharmacokinetic interactions between celecoxib and grapefruit juice. The CLX was separated on a reversed phase column (Zorbax SB-C18, 100 mm x 3.0 mm I.D., 3.5 μ m) under isocratic conditions using a mobile phase of a 55:45 mixture of acetonitrile and 0.1% formic acid in water. The flow rate was 1 ml/min at the column temperature 40 °C. The retention time of CLX was 1.8 min. The detection of CLX was in MRM mode using an ion trap mass spectrometer with electrospray negative ionisation. The ion transitions monitored were m/z 380 to 316. Sample preparation: the plasma samples (0.2 ml) were precipitated using methanol. Calibration curves were generated over the range of 0.1-6.4 microg/ml. The values of precision and accuracy for CLX at quantification limit were 4.1% and 9.3% for intra-day determinations and 4.6% and 11.2% for inter-day determinations respectively. This is the first high-throughput reported method for analysis of CLX in rat plasma that uses protein precipitation as sample processing procedure and has a run-time less than 2 minutes. Being rapid, simple and selective, the method allows analysis of a great number of plasma samples from clinical study in short time. The validated LC/MS/MS method has been successfully applied to a pharmacokinetic interaction study between CLX and grapefruit juice in rats

P239**Pharmacokinetic interaction between grapefruit juice and diclofenac**Rodica Cuciureanu¹, Magdalena Cuciureanu¹, Laurian Vlase², Dana Muntean²¹University Of Medicine and Pharmacy, Iasi, Romania, ²University of Medicine and Pharmacy, Cluj-Napoca, Romania

Grapefruit juice has been reported to increase the oral availability of many cytochrome P450 substrates, mainly by inhibiting the first-pass metabolism of the drugs metabolized by cytochrome P450 3A in the gut wall rather than in liver. Diclofenac, a nonsteroidal anti-inflammatory drug bearing a carboxylic functional group, undergoes metabolism by acyl glucuronidation and phenyl hydroxylation, with the latter reaction being catalyzed by cytochrome P450 2C9 and 3A4. This study investigates the pharmacokinetic interaction between diclofenac and grapefruit juice in adult Wistar rats. Mature male Wistar rats weighing 180-200 g were randomized into two groups of 10 animals each. Group I received diclofenac 2.5 mg/kg/day per os for 3 days and to group II was given grapefruit juice 2ml/day for 10 days, followed by a concomitant administration of 2.5 mg diclofenac/kg/day and grapefruit juice 2ml/day for three days. Blood samples were taken at 0,33, 0,66, 1, 2, 3, 6, 8, 12, 24, 36 hours after last drug administration and submitted to the liquid chromatographic analysis of diclofenac in plasma. Statistical analysis was performed using analysis of variance (ANOVA one way) followed by Tukey test. Our results showed that group II presented a statistically significant increased AUC_{tot} (20.206 ± 0.000231 versus 0.0496 ± 0.00017 μ g/ml x h, $p < 0.05$) and C_{max} (2.23 ± 0.78 versus 0.575 ± 0.22 μ g/ml, $p < 0.05$) compared to group I. Diclofenac serum levels at 0,33, 0,66 and 1 hour after drug last administration in group II were statistically significant elevated compared to group I (2.23 ± 0.3 versus 1.12 ± 0.02 μ g/ml, 1.34 ± 0.2 versus 0.32 ± 0.01 μ g/ml and 1.22 ± 0.22 versus 0.55 ± 0.03 μ g/ml, $p < 0.05$). The present study demonstrates that grapefruit juice significantly influences the pharmacokinetics of diclofenac.

P240**Endothelial nitric oxide synthase gene 894G>T, Intron 4a/b, -786C>T polymorphisms in gastric cancer**

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Nitric oxide, a labile compound synthesized by NOS, is a major regulator not only of physiological vascular tonus but also of the abnormal vascularity associated with tumors. Endothelial production of NO regulates blood flow and angiogenesis, reduces tumor cell adhesion to endothelium. High concentration of NO and its metabolites cause DNA damage during nitration, nitrosation and deamination. Both positive and negative effects on carcinogenesis and tumor growth, apoptosis, cytotoxic mechanisms may be explained by differential susceptibility of tumor cells to NO-mediated reactions. Methods: In this study three major polymorphisms (786T>C, the 27 base pair variable number of tandem repeats in intron 4, and 894G>T) of eNOS gene was investigated in gastric cancer and normal tissues of 50 patients with gastric cancer and in peripheral blood of 98 healthy subjects. Results: We found no significant differences in intron 4a/b and 894G>T (Glu298Asp) allele and genotype frequencies between controls and patient specimens. Nevertheless, the genotype and allele frequencies of 786T>C polymorphism were found to be significantly different between the healthy controls and tumor tissues. Conclusion: The results suggest that eNOS 786T>C polymorphism may play role in the development of gastric cancer.

P241**Sorafenib (BAY 43-9006) downregulates survivin protein level by inhibition of translation in human NSCLC cells**

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Sorafenib (BAY 43-9006, Nexavar®) has a broad spectrum activity against tyrosin kinase including c-Raf, vascular endothelial growth factor receptor, and platelet-derived growth factor receptor. However, the mechanism(s) of action sorafenib remains incompletely defined. In the present study, the effect of sorafenib on the antiapoptotic protein survivin, an inhibitor of apoptosis protein family, were examined in various NSCLC cell lines. Sorafenib markedly caused a down-regulation of survivin protein expression level in lung cancer cells. Sorafenib treatment did not significantly affect the levels of other IAP family, including XIAP, cIAP1 and cIAP2. To determine whether survivin was downregulated at the transcriptional level, we measured mRNA in lung cancer cells after treated with sorafenib assessed by RT-PCR. The result shows that sorafenib did not alter the survivin mRNA expression level. To further examine the effects of sorafenib on survivin downregulation, 26S proteasome in lung cancer cells was blocked by MG132; and the protein levels of survivin were examined after 24hr in the absence or presence of sorafenib. MG132 did not recover the sorafenib-induced downregulation of survivin. Therefore, survivin downregulation by sorafenib occurred in a 26S proteasome-independent manner. These results suggest that sorafenib-induced survivin downregulation is mediated by inhibition of translation initiation in human NSCLC cells.

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