Posters
Thursday 17 July
Cell signalling

P155

Polypeptide from *Chlamys farreri* prevents UVA-induced HaCaT cells apoptosis partly through inhibition of caspase-8 pathway and mitochondrial pathway

B Li, B Ding, C Wang *Medical College, Qingdao University, Qingdao, China*

Polypeptide from *Chlamys farreri* (PCF) is a novel marine active product isolated from the gonochoric Chinese scallop *Chlamys farreri* which has been recently found to be an effective antioxidant. In this study, we assessed the effect of PCF on UVA-induced intracellular signaling of apoptosis in HaCaT cells. Pretreatment with PCF significantly inhibited UVA-induced apoptosis in HaCaT cells. Pretreatment with the ROS scavenger *N*-acetylcysteine (NAC) and the caspase-8 inhibitor z-IETD-fmk and siRNA were found to effectively prevent UVA-induced apoptosis, suggesting that UVA-induced HaCaT cell apoptosis was partially due to generation of ROS and activation of the caspase-8 pathway. PCF strongly reduced the intracellular reactive oxygen species (ROS) level followed by inhibition the release of cytochrome c. The expression of CD95 and Fas-associating protein with death domain (FADD) was eliminated in a dose-dependent by PCF pretreatment in UVA-irradiated HaCaT cells, followed by inhibition of cleavage of procaspase-8 and procaspase-3, whose activation induced cell apoptosis. Consequently, the protective effect of PCF against UVA irradiation in HaCaT cells is exerted by suppression of generation of ROS followed by inhibition cytochrome c release and inactivation of Fas-FADD- caspase-8- caspase-3 pathway, resulting in blockage of UVA-induced apoptosis.

P156

Role of Ca^{2+}-activated K^+ channels in adenosine A_1 receptor-mediated protection against hypoxia-induced cell death in myocardiac H9c2 cells

L Fretwell, J Dickenson *Nottingham Trent University, Nottingham, UK*

The aim of this study was to determine whether K_{Ca} channels are involved in A_1R-induced cardioprotection in the rat embryonic cardiomyoblast-derived cell line H9c2. To induce cardioprotection H9c2 cells were pre-treated with the selective A_1R agonist N^6-cyclopentyladenosine (CPA; 100 nM) for 30 min prior to 6 h hypoxia (5% CO_2/0.5% O_2 at 37°C). Cells were also treated for 15 min before the addition of CPA with paxilline (1 μM; K_{Ca} inhibitor), iberiotoxin (10 nM; K_{Ca} inhibitor) or DPCPX (10 μM; A_1R antagonist). After 6 h hypoxia cell viability was determined by measuring the activity of lactate dehydrogenase (LDH) released into the culture media. Data are expressed as a percentage of hypoxic-induced LDH release (=100%). Hypoxia-induced release of LDH was reduced by CPA (66 ± 2% of hypoxic control; $p<0.001$; n=6). Furthermore, the A_1R antagonist DPCPX reversed CPA-induced protection (95 ± 3% of hypoxic control; n=5) confirming the involvement of the A_1R. The K_{Ca} inhibitor paxilline alone (1 μM) had no effect on hypoxia-induced LDH release (105 ± 6% of hypoxic control; n=5) but significantly reduced CPA-induced protection (91 ± 5% of hypoxic control; n=5). Similarly, iberiotoxin alone (10 nM) did not significantly reduce LDH release during hypoxia (94 ± 7% of hypoxic control; n=5), but did moderate the protection provided by CPA (91 ± 7% of hypoxic control; n=5). In summary, these data have shown that the K_{Ca} channel plays a role in A_1R-induced cell survival in H9c2 cells.

References:

Cao CM *et al*. Br J. Pharmacol. 2005. 145: 984–991.

Fretwell L and Dickenson JM. Proc Life Sciences. 2007: PC289.

Kakinuma Y *et al*. FEBS. Lett. 2005; 579: 2111–2118.

Xu W *et al*. Science. 2002; 298: 1029–1033.

P158

Protein kinase C- and reactive oxygen species-dependent stimulation of intracellular cAMP in human eosinophils: role of extracellular signal-regulated protein kinases

C Ezeamuzie, N Taslim *Department of Pharmacology, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait*

We have previously reported the existence of a novel protein kinase C (PKC)- and reactive oxygen species (ROS)-dependent pathway for the stimulation of intracellular adenosine 3',5'-cyclic monophosphate (cAMP) production in human eosinophils (Ezeamuzie and Taslim, 2006). The objective of the current study was to investigate the role of the extracellular signal-regulated protein kinases 1/2 (ERK1/2) in the signaling pathway of this response. Immunomagnetically purified human eosinophils from normal or mildly atopic subjects were stimulated *in vitro* with the PKC activator, phorbol myristate acetate (PMA) and the cAMP response in the presence of the phosphodiesterase inhibitor, rolipram, was determined by enzyme immunoassay. The role of ERK1/2 was investigated using specific inhibitors and by Western blot analysis. PMA-stimulated eosinophils generated high levels of cAMP (EC_{50} = 40 nM; E_{max} = 6.4 pmoles/10^6 cells) that was dependent on both PKC and ROS as confirmed by the use of the specific inhibitors – Ro 31-8220 and DPI, respectively, for PKC and the ROS-generating enzyme NADPH oxidase. Pretreatment of cells with the specific ERK1/2 inhibitor PD 98059 (30 μM), but not the p38-MAPK inhibitor SB203580 (1–10 μM), nor the PI3 kinase inhibitor, wortmannin (0.1–1 μM), almost abolished the response. PMA treatment induced the phosphorylation of ERK1/2 with a half-time of about 60 s, consistent with a role in the cAMP response. The ERK1/2 phosphorylation was abolished by both the ERK1/2 inhibitor PD 98059 and the PKC inhibitor Ro 31-8220, but not the NADPH oxidase inhibitor DPI. These results reveal the involvement of ERK1/2 in the signaling mechanism of PMA-stimulated, PKC- and ROS-dependent stimulation of cAMP production in human eosinophils, and show that ERK1/2 phosphorylation is upstream of ROS production in the signaling pathway.

Reference:

Ezeamuzie CI and Taslim N. R Eur J Pharmacol. 2006; 543: 174–180.

P159

Polypeptides from *Chlamys farreri* protect murine thymocytes from ultraviolet ray damaged through mitochondrium

C Yan, C Wang, B Ding, Y Han *Medical College of Qingdao University, Qingdao, China*

In order to identify the protective effects and mechanism of PCF (polypeptides from *Chlamys farreri*) on thymocytes damaged by UV irradiation. We established the irradiation damage models of thymocytes *in vitro* (6.16 mJ/cm^2), The cells were randomly divided into six groups: control group, ultraviolet ray groups (model, 0.5% PCF, 0.25% PCF, 0.125% PCF and 0.1% VitC). The rate of apoptosis and the mitochondria membrane potential on thymocytes were tested using flow cytometry (FCM); the expressions of cytochrome C, Bax and Bcl-2 proteins were examined by Western blot; flow cytometry was used in examining caspase-3 production. The results indicated that PCF could significantly decrease the rate of thymocyte apoptosis induced by UV irradiation ($P < 0.01$); PCF could maintain the stability of mitochondrial transmembrane potential ($P < 0.05$); It also raised the expression of Bcl-2 gene, the expression of cytochrome C and Bax were decreased in groups pretreatment with PCF ($P < 0.05$), The result of flow cytometry revealed that (0.125–0.5%) PCF dose-dependent depressed thymocytes caspase-3 contents ($P < 0.05$). These results suggest that PCF has a the protective effect against damage of thymocytes caused by the irradiation of UV. The mechanisms of these effects are related to the regulation of mitochondrium apoptosis signal transduction.

P160

TRAIL in the production of apoptosis of HaCaT cells after UVA radiation and the influence of Polypeptide from *Chlamys farreri* on it

J Li[a], B Ding[b], W Xu[b], S Guo[b], X Wang[b], P Li[b], C Wang[b] [a]*Binzhou Medical College, Yantai, Shandong, China;* [b]*Medical College of Qingdao University, Qingdao, Shandong, China*

Ultraviolet irradiation (UV) could induce the apoptosis of HaCaT keratinocytes. TRAIL (tumour necrosis factor related apoptosis inducing ligand), a member of the tumour necrosis factor ligand family, initiated apoptosis in a variety of neoplastic cells. There have been few reports about its expression after ultraviolet irradiation and its role in UV-induced apoptosis. The aim of this study was to investigate the role of TRAIL in UVA-induced apoptosis of HaCaT cells and the influence of Polypeptide from *Chlamys farreri* (PCF) on it. TRAIL mRNA expression in HaCaT cells was identified by the gene chip screening as being up-regulated after UVA radiation. Real-time PCR analysis confirmed the increased expression of TRAIL mRNA and Western blotting demonstrated the same change of protein expression. Using agarose gel electrophoresis, the inhibitory effect of TRAIL neutralization antibody on UVA-induced apoptosis was demonstrated. Our data showed that TRAIL was directly involved in the production of apoptosis of HaCaT cells after UVA radiation. PCF has been demonstrated as an antioxidant and has inhibitory effect on UVA-induced apoptosis (Li et al., 2007). In this study, the influence of PCF on TRAIL apoptotic pathway induced by UVA was investigated. Cells were divided into five groups: control group, UVA model group, UVA + 5.69 mM PCF group, UVA + 2.84 mM PCF group, UVA + 1.42 mM PCF group. Our data showed that 1.42–5.69 mM PCF inhibited UVA-induced mRNA and protein expression of TRAIL in HaCaT cells dose-dependently. FADD and caspase-8 are involved in apoptosis signalling by TRAIL (Kischkel *et al*., 2000), so we investigated the impact of PCF on FADD and caspase-8. Our results showed that PCF attenuated UVA-induced FADD protein expression and caspase-8 activation in a dose-dependent manner. These data demonstrated that PCF could attenuate UVA-induced TRAIL expression and block TRAIL apoptotic pathway. It has been suggested that ROS participated on TRAIL-induced apoptosis (Perez-Cruz et al., 2007), so the inhibitory effect of PCF on TRAIL apoptosis signalling may partly be attributable to its anti-oxidative ability.

References:

Kischkel FC *et al*. Immunity. 2000; 12: 611–620.

Li JL *et al*. Radiat Environ Biophys. 2007; 46: 263–268.

Perez-Cruz I *et al*. Apoptosis. 2007; 12: 225–234.

P161

Major contribution of Gs to CRF receptor-mediated calcium signalling in recombinant HEK293 cells

E Gutknecht[a], T Steckler[a], G Vauquelin[b], FM Dautzenberg[a] [a]*CNS Discovery, Johnson & Johnson Research and Development, Beerse, Belgium;* [b]*MBFA, Vrije Universiteit Brussels, Brussels, Belgium*

Corticotropin releasing factor (CRF) is the major regulator of the hypothalamo–pituitary–adrenal (HPA) axis and plays an important role in the regulation of stress and anxiety. CRF activates two different class B family G-protein coupled receptors, CRF_1 and CRF_2, resulting in Gs activation and production of cAMP. In our study, we used HEK293 cells stably expressing human CRF_1 and $CRF_{2(a)}$ receptors; i.e. the most abundant ones in the brain. Stimulation of both receptors induced a rapid, transient increase in the cytosolic calcium concentration (measured with the FDSS, Hamamatsu). These calcium transients were not affected by extracellular calcium depletion but inhibited by blockade of intracellular IP_3 receptors. Various approaches were used to further analyse the signalling pathways involved, including G-protein overexpression, specific inhibitors and siRNA technologies. Transfection of the $hCRF_1$-HEK and $hCRF_{2(a)}$-HEK cells with G-alpha q and G-alpha 16 increased agonist efficacy and potency compared with mock transfection. When the cells were incubated with pertussis toxin (PTX) to block the Gi mediated pathways in $hCRF_1$-HEK and $hCRF_{2(a)}$-HEK cells, the calcium transients were reduced by ~30% and ~20% of the response in control cells, respectively. G-alpha s

© 2008 The Authors

knockdown by siRNA decreased the calcium transients by 60–70% compare to the response in cells transfected with a control siRNA, indicating that Gs plays a major role in this response. Combination of G-alpha s siRNA and PTX did not decrease the calcium transients any further, pointing to a sequential connection between Gs- and Gi-activation in HEK cells. The cellular cAMP sensor Epac has been reported to link cAMP and Gs activation to intracellular calcium mobilization in those cells (Schmidt *et al.*, 2001). We are currently studying the effect of the Epac knockdown with siRNA on the $hCRF_1$- and $hCRF_{2(a)}$ receptor-induced calcium signalling. Supported by Johnson & Johnson.

Reference:

Schmidt M *et al.*, Nat Cell Biol. 2001; 3(11): 1020–4.

P162

Effect of oxidative stress on carbachol-evoked secretory responses and caspase3 activity in the isolated rat parotid gland

A Mata[a], D Marques[a], MA Martínez-Burgos[b], J Silveira[a], J Marques[a], MF Mesquita[c], JA Pariente[b], GM Salido[b], J Singh[d] [a]*ITB, Biomedical Technology Institute, Faculdade de Medicina Dentária da Universidade de Lisboa, Lisboa, Portugal;* [b]*Department of Physiology, University of Extremadura, Cáceres, Spain;* [c]*Oral Biology Research Group, Instituto Superior de Ciências da Saúde – Egas Moniz, Monte de Caparica, Portugal;* [d]*School of Forensic and Investigative Science, University of Central Lancashire, Preston, France*

The parotid glands are highly active secretory systems subjected to continuous stress resulting in several pathophysiological conditions. In numerous situations damage to the glands is caused by reactive oxygen species (ROS) deriving from oxygen metabolism. This study investigated the effect of hydrogen peroxide (H_2O_2) on carbachol (CCh)-evoked amylase secretion, cytosolic free calcium levels ($[Ca^{2+}]_i$) and on caspase-3 activity in the isolated rat parotid gland to determine the role of oxidative stress on the function of this gland. Amylase secretion, $[Ca^{2+}]_i$ and caspase 3 activity in parotid gland tissue were measured using established fluorimetric methods. H_2O_2 had little or no effect on amylase secretion compared to basal level. Combining (CCh) with H_2O_2 resulted in an attenuation of the CCh-evoked amylase secretion compared to the response obtained with CCh alone. CCh evoked a large increase in $[Ca^{2+}]_i$ with an initial peak followed by a plateau. In a free extra-cellular Ca^{2+}($[Ca^{2+}]_o$) medium containing 1 mM EGTA, CCh evoked only the initial peak of the Ca^{2+}response. H_2O_2 alone evoked a gradual increase in $[Ca^{2+}]_i$ and this effect was dose dependent. Combining H_2O_2 with CCh resulted in a decrease in $[Ca^{2+}]_i$ compared to the effect of CCh alone. In a nominally free medium containing 1 mM EGTA, H_2O_2 still evoked a small increase in $[Ca^{2+}]_i$ but this response was much less pronounced when compared to the results obtained with H_2O_2 in normal $[Ca^{2+}]_0$. Combining H_2O_2 with CCh resulted in only a small transient increase in $[Ca^{2+}]_i$. Following CCh stimulation, H_2O_2 application resulted in a large increase in $[Ca^{2+}]_0$ in normal $[Ca^{2+}]_i$. This effect of H_2O_2 was abolished in a nominally free $[Ca^{2+}]_0$ medium containing EGTA. H_2O_2 can stimulate caspase-3 activity in parotid gland tissue. A similar response was obtained with betulinic acid, while TPS had no significant effect on caspase-3 activity. The results have indicated that like CCh, H_2O_2 can also mobilize Ca^{2+} from intracellular stores and facilitate its influx into the cell from extra-cellular medium. This effect of H_2O_2 may be due to its activity to induce apoptosis in the parotid gland since H_2O_2 can stimulate the activity of caspase-3, a marker of cellular apoptosis.

P163

Evidence of Ca^{2+} entry pathways in rat white adipocytes

P Pulbutr, SLF Chan, PA Smith *School of Biomedical Sciences, The University of Nottingham, Nottingham, UK*

Abnormal handling of intracellular Ca^{2+} concentration ($[Ca^{2+}]$i) in white adipocytes has been described to be associated with insulin resistance and diabetes mellitus. However, little is known about the Ca^{2+} entry pathways and their related functions in white adipocytes. This study aimed to investigate the possible Ca^{2+} entry pathways in rat white adipocytes. Rat white adipocytes were isolated from epididymal fat pads by collagenase digestion. Changes in $[Ca^{2+}]$i were measured as fluorescence intensity changes using the Ca^{2+}-sensitive dye, Fluo-4 AM. Fluorescence intensity was presented in terms of percent of control, basal fluorescence intensity. Oxytocin (1 μM) elicited a prompt increase in $[Ca^{2+}]$i, with the peak fluorescence intensity of 282 ± 34% of control (mean ± SEM, $n = 21$), followed by fall to a sustained plateau phase (123 ± 5%). Omission of extracellular Ca^{2+} did not affect the peak fluorescence intensity but the mean fluorescence intensity of the plateau was significantly decreased to 83 ± 22% ($P < 0.05$). 2-APB (75 μM), a selective store-operated Ca^{2+} channel blocker, significantly decreased the plateau phase to 104 ± 6% ($P < 0.05$). Isotonic high K^+ (50 mM) buffer solution induced an extracellular Ca^{2+}-dependent increase of $[Ca^{2+}]$i, with a peak fluorescence intensity of 207 ± 17% ($n = 21$). This response was markedly attenuated by the L-type Ca^{2+} channel blockers, nifedipine and verapamil (20 μM: 124 ± 4%, $P < 0.05$; 149 ± 9%, $P < 0.05$, respectively). When adipocytes were perifused with an isotonic low Na^+ (98 mM) buffer solution, $[Ca^{2+}]$i was significantly increased (130 ± 4%, $n = 11$; $P < 0.05$). This peak fluorescence intensity was significantly reduced by the addition of KB-R 7343 (10 μM), a putative Na^+/Ca^{2+} exchange inhibitor (101 ± 3%, $n = 4$; $P < 0.05$). Collectively, oxytocin elicited an increase in $[Ca^{2+}]$i in rat adipocytes via (i) inducing Ca^{2+} release from the internal stores and (ii) triggering extracellular Ca^{2+} entry through store-operated Ca^{2+} channels. The results from this indicate the presence of store-operated Ca^{2+} channels, functional L-type Ca^{2+} channels and a reverse mode of Na^+/Ca^{2+} exchanger in rat white adipocytes. The roles of these proposed Ca^{2+} entry pathways in adipocytes still need further investigation.

P164

Putative palmitoylation sites in human protease-activated receptor-1 ($hPAR_1$) are critical for receptor signalling to calcium

X Guo, A Morice, S Compton *University of Hull, Hull, UK*

Proteinase-activated receptors (PARs) are a novel family of G protein-coupled receptors (GPCRs) that possess a tethered-ligand buried within the receptor N-terminus. Activation of PAR_1, PAR_2 and PAR_4 occurs through the proteolytic exposure of the tethered-ligand which subsequently triggers receptor activation. Palmitoylation is a post-translational modification that results in the addition of fatty acids to cysteine residues in the C-terminus of some GPCRs. Although recent evidence has suggested that palmitoylation can have a profound effect on GPCR function, the role of palmitoylation in regulating PAR function is currently unknown. Thus it is our working hypothesis that putative palmitoylation sites affect $hPAR_1$ receptor function. Wild-type-$hPAR_1$ (wt-$hPAR_1$) and $hPAR_1$ mutants ($hPAR_1$C387A, $hPAR_1$C388A and $hPAR_1$C387AC388A) were generated and permanently expressed in KNRK cells. Flow cytometry was employed to assess cell surface expression and to assess the degree of receptor internalisation following agonist challenge with the PAR_1 agonists thrombin and the selective PAR_1-activating peptide (PAR_1-AP) TFLLR-NH_2. Elevations in intracellular calcium were assessed as a measure of receptor function. Flow cytometry revealed that $hPAR_1$C387A and $hPAR_1$C388A displayed similar cell surface expression (~80%) to that of wt-$hPAR_1$. Whilst $hPAR_1$C387AC388A displayed only ~40% cell surface expression compared to that of wt-$hPAR_1$. $hPAR_1$C387A and $hPAR_1$C388A displayed similar sensitivity to the PAR_1 agonists thrombin and TFLLR-NH_2. Surprisingly, $hPAR_1$C387AC388A failed to respond to either PAR_1 agonists. This difference in signalling was not due to differences in cell surface expression, since a wt-$hPAR_1$ cell line with similar cell surface expression to $hPAR_1$C387AC388A displayed robust responses to both agonists. In agonist triggered internalisation experiments wt and mutant receptors internalized in response to thrombin and TFLLR-NH_2, except for wt-$hPAR_1$ which only internalised in response to thrombin. Thus putative palmitoylation sites within PAR_1 regulate receptor expression and are critical for receptor signalling to calcium.

P165

Inhibition of c-Myc down-regulation by sustained ERK activation prevents methotrexate induced differentiation in A549 human lung adenocarcinoma cells

R Alemany[a], O Vögler[a], A Gutiérrez[a], M Navarro[a], T Ros[a], C Saus[b], J Ginés[b], A Sampol[b], JC Amat[b], J Martín[a], A Galmés[b], J Besalduch[b], JM Serra[a] [a]*University of the Balearic Islands, Palma de Mallorca, Balearic Islands, Spain,* [b]*University Hospital Son Dureta, Palma de Mallorca, Balearic Islands, Spain*

Non-small cell lung cancer (NSCLC) is characterized by severe resistance to chemotherapy. Here, we demonstrate that A549 adenocarcinoma cells permanently differentiate with the antimetabolite methotrexate (MTX) when blocking the antitumoral resistance mechanism normally counteracting this process. MTX (1–10 μM) induced growth arrest accompanied by sustained ERK1/2 phosphorylation and moderate reduction of c-Myc levels after 96 h, while only a low percentage of cells differentiated. Combination with the MEK inhibitor U0126 eliminated MTX-induced ERK1/2 over-phosphorylation and nearly abolished c-Myc expression, while provoking radical morphological changes in all cells. Besides the appearance of multilamellar bodies and intracellular cytokeratine reorganization, modulation of molecular markers occurred in a manner consistent with differentiation (gelsolin +300%; surfactant-protein-A and C-60%). Similar to U0126, c-Myc inactivation with specific siRNA initiated differentiation only in the presence of MTX, demonstrating that inhibition of the MAPK/ERK pathway alone or downregulation of c-Myc are not sufficient to induce this process. Importantly, withdrawal of MTX and U0126 neither reversed differentiation nor reactivated proliferation. Our results reveal that maintenance of a certain threshold of c-Myc expression through sustained ERK1/2 activation represents the molecular mechanism that confers resistance to MTX-induced differentiation in A549 cells, and provide a novel molecular basis for therapeutic strategies based on irreversible differentiation of cancer cells using conventional chemotherapeutic antimetabolites in combination with inhibitors of the MEK/ERK pathway or c-Myc.

P166

Use of RNA interference to investigate the regulation of the putative signalling phosphoinositide PtdIns5*P*

A Wilcox, K Hinchliffe *The University of Manchester, Manchester, UK*

The phosphoinositides are a group of biologically active phospholipids that play multiple essential roles in cellular regulation, through their interactions with specific binding proteins. One member of this family, phosphatidylinositol 5-phosphate (PtdIns5*P*) is found ubiquitously in higher eukaryotes, and has been suggested to play roles in cellular responses to stress, and in cellular activation by the agonists insulin and thrombin. However, the molecular mechanisms governing the synthesis and breakdown of this lipid are not fully understood: in particular, a number of alternative mechanisms of PtdIns5*P* synthesis have been postulated. To obtain more information about PtdIns5*P* regulation, we have used RNA interference (RNAi) to suppress expression of proteins implicated in its turnover, and examined the effects of this knockdown on the production of PtdIns5*P* in cells exposed to the phosphotyrosine phosphatase inhibitor pervanadate, which provokes robust increases in intracellular PtdIns5*P* concentration. We find that knockdown of PIP_2 4-phosphatase, an enzyme that stimulates PtdIns5*P* production in the nucleus in response to pro-apoptotic stress, is without effect on pervanadate-stimulated PtdIns5*P* production. However, knockdown of lipid phosphatases of the myotubularin family markedly attenuates the response. Moreover, the effects of knockdown of PIP4Ks, lipid kinases that remove PtdIns5*P* by phosphorylation, show that the cytoplasmic PIP4K2 alpha isoform is involved in negatively regulating pervanadate-stimulated PtdIns5*P* production, whereas the nuclear PIP4K2 beta is not. Our results are consistent with the ability of pervanadate-stimulated tyrosine kinase signalling to generate PtdIns5*P* outside the nucleus, via

© 2008 The Authors

a mechanism involving myotubularins. Thus, it will be important when investigating the possible roles of PtdIns5*P* in receptor-activated signal transduction to determine the subcellular location of the PtdIns5*P* involved.

P168

Characterisation of PACAP- and VIP-mediated signalling in CHO-hPAC1 cells and mouse neural stem cells

T Werry, E Liu, A Lodge, C Langmead *GlaxoSmithKline, Harlow, Essex, UK*

The Family B G Protein-coupled receptor (GPCR) family contains several receptors that transduce extracellular neuropeptide hormone signals into intracellular responses. The neuropeptides vasoactive intestinal peptide (VIP) and pituitary adenylyl cyclase-activating peptide (PACAP) activate Family B GPCRs known as PAC1, VPAC1 and VPAC2 to variously activate Gq/11, Gs and/or Gi/o-type G proteins. We show that PACAP, but not VIP, modulates proliferation in mouse neural stem cells (mNSCs) that endogenously express PAC1 and (at very low levels) VPAC2 receptors. We subsequently sought to characterise PACAP-38- and VIP-mediated signalling in a CHO cell line expressing recombinant PAC1 receptors and compare these pharmacological profiles with the corresponding response characteristics of these ligands in mNSCs. In CHO cells, the PAC1 receptor couples to both calcium (Ca^{2+}) release and activation of extracellular signal-regulated kinase (ERK). PACAP-38 shows slightly higher potency in both assays than VIP, although the potency of both is in line with their respective binding affinities. In addition, the duration of acute ERK activation by PACAP-38 was more sustained than that in response to VIP. In contrast, neither neuropeptide ligand stimulated Ca^{2+} release in mNSCs, although ERK activation was observed in response to PACAP-38 and, with much lower potency, VIP. We investigate various hypotheses that may underlie the differences in signalling in the two cell lines in response to VIP and PACAP-38, including cell-specific ERK signalling mechanisms and the presence of receptor-activity modifying proteins (RAMPs) in mNSCs.

P169

Polypeptide from *Chlamys farreri* inhibit UVB-radiation-induced activation of NF-κB signaling pathway and apoptosis in HaCaT cells

X Liu, B Ding, C Wang *Medical College, Qingdao University, Qingdao, China*

An increasing incidence of human skin cancer and other adverse effects of solar ultraviolet radiation enhance the need for novel chemoprevention strategies. Polypeptide from *Chlamys farreri* (PCF) has been identified as a potent antioxidant and photoprotective agent. Our previous study has preliminarily demonstrated that PCF could reduce the intracellular reactive oxygen species (ROS) production and inhibit UVB-radiation-induced HaCaT cells apoptosis. The goals of this study were to evaluate whether the NF-κB signaling pathway can be activated by UVB-radiation at the dose of 20 mJ/cm^2 and determine its role in PCF protecting UVB-induced HaCaT Cells apoptosis. Our immunofluorescent staining and Western blot analysis results show that UVB irradiation could promote the translocation of NF-κB/p65 so its expression in nucleus increased; the expression of p-IκBα was increased while IκBα was decreased detecting by Western blot analysis and RT-PCR. Pre-treatment with PCF and ROS scavenger NAC markedly suppressed IκBα degradation so as to inhibit UVB-induced activation of NF-κB/p65 in a dose-dependent manner. Furthermore, we found that the PCF and NF-κB inhibitor sulfasalazine significantly encouraged the proliferation of UVB-induced HaCaT cells and protected against UVB-induced apoptosis using the MTT method and DNA fragments respectively, NAC also effectively inhibited UVB-induced apoptosis. We concluded that UVB-radiation-induced the activation of NF-κB signaling pathway played an important role in UVB-induced apoptosis. PCF obviously protects HaCaT cells from apoptosis induced by UVB and part of the antiapoptotic effect of PCF might be mediated by its ability to decrease intracellular ROS level and modulate the NF-κB signaling pathway. Our data suggest that PCF is an effective agent for ameliorating UVB-mediated damage by modulating cellular pathways and merits further evaluation as a photochemopreventive agent.

© 2008 The Authors

P170

Changing trend in the use of antibiotics over 10 years in a tertiary care hospital

MP Singh, V Bansal, V Jose, P Pandhi *PGIMER, Chandigarh, India*

Antibiotics are amongst the most commonly prescribed drugs. Over the past decade a large number of new antibiotics with more beneficial pharmacokinetic and pharmacodynamic properties have entered the market. This study was planned to see the changing trend of antibiotic use in a tertiary care hospital in northern India. One thousand inpatient prescriptions each, in the year 1995 and 2005 were screened with regard to the pattern of antibiotic use. A drastic increase in the use of antibiotics was observed over a span of ten years. This increase was significantly noted for antibiotics like amoxicillin, amoxicillin–clavulanic acid, third and fourth generation cephalosporins, fluoroquinolones, amikacin and metronidazole. However a decline in the use of crystalline penicillin, ampicillin, cloxacillin, cotrimoxazole, chloramphenicol and gentamicin was observed. There was a clear evidence of change in prescription trend in antibiotics probably due to altered drug resistance patterns of microorganisms and resurgence of apt antibiotics which more suits the need of the situation.

P171

Integration of e-learning into drug disposition teaching

M Hollingsworth, K Breakey, S Clarke, T Speake *Manchester University, Manchester, UK*

Drug disposition is a core unit for BSc Pharmacology students and an optional unit for other BSc students. The unit has consisted of nine lectures and 15 e-learning programs (Drug Disposition Tutorials, http://www.sheffbp.co.uk/; Pharma-CAL-ogy, http://www.pharmacalogy.com) for several years. The impact of introducing electronic problem based learning (ePBL) questions was investigated. In the 2005–2006 session students had ePBLs consisting of 5 topics, each being a separate node. The ePBLs were formative only and a mark of 70% had to be achieved in a node before a student could move onto the next node. Assessment of the unit consisted of an end of unit examination, with 60% from MCQs and 40% from an essay. In the 2006–2007 and 2007–2008 sessions there were 10 nodes on 5 topics of which the first of a pair was formative and second one of the pair was summative. The marks from the ePBLs contributed 5% to the unit mark with the examination contributing 95%. The % of nodes completed was determined. In 2005–2006 only four out of 40 students completed all 5 ePBL nodes and the overall completion was 40 ± 40 (median ± inter-quartile range, $n = 39$). The exam mark was 50 ± 18%. The completion rate of the ePBLs was significantly increased in 2006–2007 (100 ± 10%, $n = 50$; $P = 0$, Mann–Whitney *U*-test) and in 2007–2008 (93 ± 20%, $n = 62$; $P = 0$) compared with 2005–2006. The exam marks in 2006–2007 (60 ± 20%; $P = 0$) and in 2007–2008 (56 ± 14%; $P < 0.05$) were also increased compared with 2005–2006. It is suggested that fully integrating ePBLs into the unit increased their take up by students and improved examination performance.

© 2008 The Authors

P172

Relaxant effect of *Pycnocycla spinosa* seed extract on rat uterus contraction

H Sadraei, G Asghari, M Andishah *Isfahan University of Medical Sciences, Isfahan, Iran*

Hydroalcoholic extract of *Pycnocycla spinosa* is a relaxant of rat ileum and inhibits diarrhoea in the mice (Sadraei *et al.*, 2003). The objective of this research was to investigate effect of *P. spinosa* seed extract on rat isolated uterus contraction for comparison with terbutaline. Seeds from *P. spinosa* were collected during summer in Isfahan–Iran and hydroalcoholic extract was obtained by percolation using 70% ethanol. Female Wistar rats (200–250 g), pretreated a day before with estrogen (100 μg/kg, s.c.), were killed and their uteruses were removed and secured in Tyrode's solution in an organ bath at 37°C and gassed with O_2. Isotonic contractions induced by oxytocin (0.002 IU/ml) and KCl (80 mM), were recorded before and after cumulative addition of the extract or terbutaline. All experiments were conducted in parallel with time-matched controls adding an equivalent volume of vehicle. Tissue contraction was measure 10 min after addition of each concentration of the extract or terbutaline and expressed as percentage of initial response for each tissue. Mean (SEM values were calculated for each group of results and significance of differences between the means were calculated by two-tailed paired Student's *t*-test. Seed extract of *P. spinosa* concentration-dependently (10–160 μg/ml, $n = 6$) inhibited the uterus contractions induced by KCl. With 160 μg/ml *P. spinosa* bath concentration, response to KCl was abolished. Relaxant effect of the extract was further examined on contraction induced by oxytocin. Seed extract of *P. spinosa* also reduced the tissue response to oxytocin in a concentration-dependent manner (2.5–160 μg/ml, $n = 6$), completely inhibited tissue response at 160 g/ml bath concentration. Terbutaline (25–100 mM) concentration-dependently inhibited response to oxytocin while only partially (24 ± 7%) inhibited uterus contraction induced by KCl ($n = 6$). From this study it was concluded that seed extract of *P. spinosa* is a potent relaxant of rat uterus contraction induced by KCl or oxytocin.

Reference:

Sadraei H *et al.* Phytother. Res. 2003; 17(6): 645–649.

P173

The protective effect of *Nigella sativa* oil in the brain of the biliary obstructed rats

H Toklu, O Sehirli, T Inac, G Sener *Marmara University School of Pharmacy, Istanbul, Turkey*

Oxidative stress is one of the important mechanisms of jaundice induced encephalopathy. The aim of this study was to examine the possible protective effect of *Nigella sativa* (NS) against the oxidative stress of brain tissue induced by experimental obstructive jaundice in rats. Biliary obstruction was performed in male Wistar albino rats by bile duct ligation and scission (BDL). Intragastric NS oil or saline was administered for 28 days. At the end of the experiment, in half of the rats blood–brain barrier (BBB) permeability was evaluated by Evans blue (EB) extravasation. Other rats were decapitated and brain tissue samples were obtained for the measurement of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) and Na^+-K^+-ATPase activities. Chronic biliary obstruction caused a significant increase in the BBB permeability which was verified by EB extravastion while this effect was attenuated by NS oil treatment. On the other hand, brain GSH level and Na^+-K^+-ATPase activity, depressed by BDL, was elevated back to control level in NS oil-treated BDL group. Increase in tissue MDA level, and MPO activity due to BDL were also attenuated by NS oil treatment. Our results suggest that NS oil treatment protects the brain from oxidative damage following bile duct ligation in rats. This effect possibly involves the inhibition of neutrophil infiltration and lipid peroxidation; thus, restoration of oxidant and antioxidant status in the tissue. Accordingly, supplementing cirrhotic patients with adjuvant therapy of NS oil may have some benefit against hepatic encephalopathy.

P174

The effect of norbuprenorphine on gastrointestinal transit in mice lacking mu opioid receptors

A Cowan, S Inan *Temple University School of Medicine, Philadelphia, PA, USA*

Norbuprenorphine (norBUP) is the N-dealkylated metabolite of buprenorphine (BUP). We are comparing the pharmacological properties of both compounds and in this study we investigated the importance of mu opioid receptors (MORs) in norBUP-induced slowing of gastrointestinal transit in male, MOR knockout (KO) mice (C57BL6 background; 20–25 g) and their wild-type (WT) littermates. Groups of 6–8 mice were deprived of food for 18 h. The animals received (s.c.) saline, BUP or antinociceptive doses of norBUP (0.50–4 mg/kg) immediately before a charcoal meal. Each mouse was decapitated 20 min later. The intestine was excised and the distance travelled by the meal from the pyloric sphincter was measured and calculated as a percentage of the total length. Transit (mean % distance travelled) was similar in saline-injected KO and WT animals (60% and 61%, respectively). In agreement with Roy *et al.* who tested morphine, we report that BUP (1 mg/kg)-mediated antagonism of transit in mice is a MOR-mediated function (19 ± 1% in WTs and 47 ± 2% in KOs). In WTs, norBUP was efficacious in arresting transit (only 13–16%) with doses (0.50–2 mg/kg) close to the antinociceptive ED_{50} value (0.68 mg/kg, s.c.) in these animals. Doses of norBUP that were inactive in the writhing test with KO mice, provided a consistent yet unexpected result on transit in these animals. Irrespective of dose (0.50–4 mg/kg), transit was constant at only ~30%. Thus, in mice that lack MORs, norBUP can nonetheless slow transit but only to a limited extent. NorBUP therefore influences transit in at least two different ways – one via MORs and one by an as yet to be determined mechanism. In either case, norBUP likely contributes to the 'constipating' action of BUP, the parent compound, in mice. (NIDA, DA13429).

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

P175

Differential activation of G proteins and extracellular signal-regulated kinase 1/2 phosphorylation via human dopamine $D_{4.4}$ receptors

P Heusler, SL Bruins, I Rauly-Lestienne, C Palmier, D Cussac *Pierre Fabre Research Center, Castres, France*

[^{35}S]GTPγS binding and Extracellular signal-Regulated Kinase phosphorylation (pERK) experiments were performed to differentiate agonist activity at the human dopamine $D_{4.4}$ receptor stably expressed in CHO cells. For [^{35}S]GTPγS binding, membrane preparations were preincubated with ligands (30 min), and [^{35}S]GTPγS was added for an additional 30 min. Radioactivity incorporated was determined by liquid scintillation counting. In pERK experiments, cells were stimulated for 5 min and cell lysates were assayed for content of phosho-ERK 1/2 using an enzyme immunometric assay kit (Biosource, Camarillo, CA, USA). In both functional assays, we tested a series of agonists with varying intrinsic activities, including dopamine and the catecholamines noradrenaline and adrenaline, several non-specific dopaminergic agonists or selective D_4 receptor ligands as well as current or candidate antipsychotics. Both tests allowed differentiation of agonist activity over a wide range. In the pERK assay, agonists were generally about twofold more efficacious than for [^{35}S]GTPγS binding, suggesting signal amplification for the former pathway. Interestingly, no correlation was found between agonist potencies obtained in both assays. To test for the influence of assay duration on this parameter, short-duration [^{35}S]GTPγS binding was performed (i.e. coincubation of membranes, ligands and [^{35}S]GTPγS for 10 min). In these experiments, potencies were generally reduced. However, agonists exhibiting comparably high potency in the pERK assay seemed to be less affected by this assay duration-dependent potency shift. We conclude that it is useful to perform different functional assays to characterize agonist activity at $D_{4.4}$ receptors. Results should be interpreted with respect to the particular properties of the experimental system in terms of signal amplification and response kinetics.

P176

Pharmacological profile of the bradykinin B_2 receptor antagonist MEN16132 in cultured rat knee chondrocytes

F Bellucci, P Cucchi, C Catalani, S Giuliani, CA Maggi, S Meini *Menarini Ricerche S.p.A., Florence, Italy*

Chondrocytes (CCK) play a key role in the pathophysiology of knee osteoarthritis (Wieland *et al.*, 2005), and no evidence for the participation of bradykinin (BK) has been shown so far. In order to characterize, in the rat CCK, the pharmacological profile of MEN16132, a high affinity and selective non-peptide B_2 receptor antagonist (Cucchi *et al.*, 2005), radioligand and functional studies were performed. In binding experiments ([^{3}H]BK, K_d 1.13 nM, B_{max} 2800 sites/cell, respectively, $n = 3$) MEN16132 totally inhibited the [^{3}H]BK specific binding (K_i 0.65 nM, $n = 3$) and resulted about 7-fold more potent than icatibant (K_i 4.6 nM, $n = 3$). In the inositolphosphates (IP) assay, MEN16132 and icatibant (30 nM) induced a rightward shift of the BK concentration-response curve (pK_B 10.2 and 9.5, respectively, $n = 3$) reducing the agonist maximal effect (E_{max}). Both antagonists concentration-dependently inhibited the PGE_2 output produced by BK (100 nM), and MEN16132 resulted about 7-fold more potent than icatibant (IC_{50} 1.3 and 8.5 nM, respectively, $n = 3$). In cells pretreated with rat recombinant interleukin 1α (IL-1α, 10 ng/mL, 24 h), MEN16132 inhibited the BK (100 nM) induced PGE_2 release with a potency (IC_{50} 1.2 nM, $n = 3$) comparable to that found in untreated CCK. In conclusion, this is the first evidence of the presence of B_2 receptors in rat knee CCK. Present results confirm the high affinity of MEN16132 at the kinin B_2 receptor, and the high potency in blocking BK activated signalling in rat CCK.

References:

Cucchi P. *et al.* Eur. J. Pharmacol. 2005; 528: 7–16.
Wieland H.A. *et al.* Nat. Rev. Drug Discov. 2005; 4: 331–344.

P177

Stimulation with noradrenaline induces a reduction of β-amyloid levels in neuronal cells and in APP/PS1 transgenic mice

M Sastre[a], M Heneka[b], J Walter[b], T Klockgether[b] [a]*Imperial College London, London, UK,* [b]*University Bonn, Bonn, Germany*

Noradrenergic deficits have been described in the cortex and hippocampus of Alzheimer's disease (AD) patients secondary to locus ceruleus (LC) degeneration, probably contributing to the emergence of cognitive impairments in behavior. In addition, it has been suggested that norepinephrine (NE) may play a role in modulating inflammatory responses in AD. We sought to study the molecular mechanisms by which NE could be protective against AD. For that purpose, we determined the effect of NE on amyloid precursor protein (APP) processing in N2a cells permanently transfected with APP cDNA. 10 μM NE decreased the levels of Aβ in control cells as well as in immunostimulated cells. The levels of soluble APP (APPs) and carboxy-terminus fragments (CTFs) were increased in cells transfected with noradrenergic receptors compared with untransfected cells. Moreover, noradrenergic stimulation resulted in an increased phosphorylation of glycogen synthase kinase-3β (GSK3-β), while the total levels of GSK3-β remained unchanged. Additionally, *in vivo* evidence of the effect of NE was observed in 3 months old APP/PS1 transgenic mice acutely treated with DL-threo-Dihydroxyphenylserine (DL-threo-DOPS) 1 mg/g i.p., which is a non-physiological precursor for NE, showing a decrease in cortical Aβ levels. These results indicate a contribution of the noradrenergic system in the regulation of Aβ generation by reducing GSK3-β activity and suggest a beneficial effect of a NE precursor as treatment for AD.

P178

Identification of novel CAR agonists by using two-step virtual screening together with *in vitro* assays

J Vanamo[a], B Windshügel[b], J Jyrkkärinne[a], A Poso[b], W Sippl[c], P Honkakoski[a] [a]*Dept of Pharmaceutics, University of Kuopio, Kuopio, Finland;* [b]*Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland;* [c]*Department of Pharmaceutical Chemistry, Martin Luther Universität Halle-Wittenberg, Halle, Germany*

Constitutive androstane receptor (CAR; NR1I3) recognize several endo- and exogenous compounds and are involved in the metabolism of these substances by regulating the expression of CYP and other metabolic enzymes and transport proteins. Human CAR has a relatively large and flexible ligand binding pocket (LBP) which is the cause of the wide ligand selectivity of the receptor. Despite of the wide spectrum of ligands, only a few agonists that activate CAR via binding to the LBP are currently known. During the last few years virtual screening techniques have become an important method in drug discovery when identifying novel bioactive molecules. These procedures have been used to identify new ligands or substrates for many proteins, but so far they have not been widely used to find nuclear receptor ligands. In this study we report the discovery of novel human CAR agonists based on a two-step virtual screening approach in combination of cell-based activity assays. We screened a drug-like compound collection for potential CAR agonists using two molecular modelling approaches: A structure-based pharmacophore was used to screen the database after which, the matching compounds were docked in the CAR crystal structure. 30 compounds were purchased and tested *in vitro* in a CAR activation assay as well as with mammalian 2-hybrid system (M2H) in hepatoma cells. 17 out of the 30 chemicals increased the CAR activity by at least two-fold and were considered as CAR agonists. These results were supported by mammalian two-hybrid system. All the new agonists discovered in this study can be grouped into two different chemotypes: substituted sulfonamides and thiazolidin-4-one derivatives. Four chemicals giving a high activation in these assays were tested in primary human hepatocytes for their ability to induce the expression of CYP2B6 mRNA. This study demonstrates the potential of combining virtual screening with biological methods for identifying novel potential nuclear receptor ligands for drug discovery.

P179

B cell receptor-induced apoptosis in WEHI-231 cells is dependent on cAMP and Epac

M Grandoch[a], M López de Jesús[a], KH Jakobs[a], AA Weber[a], M Schmidt[b] [a]*Institut für Pharmakologie, Universität Duisburg-Essen, 45122 Essen, Germany;* [b]*Department of Molecular Pharmacology, University of Groningen, 9713 AV Groningen, the Netherlands*

Signaling by the B cell antigen receptor (BCR) is essential for B lymphocytes homeostasis and immune function. BCR ligation promotes either activation and survival or growth arrest and apoptosis. Thus, dysfunctioning of BCR-induced apoptosis in B cells may lead to immunological diseases. Here we report on novel mechanisms involved in BCR-induced growth arrest and apoptosis in the immature B lymphoma cell line WEHI-231. Stimulation of the BCR activates both, extracellular signal-regulated kinases ERK1/2 and the phosphoinositid 3-kinase (PI 3-kinase) effector protein kinase B (PKB)/Akt, the latter even important for the ERK response. Activation of ERK1/2 and PKB/Akt by the BCR was cyclic AMP dependent, but largely protein kinase A (PKA) independent. Activation of endogenously expressed Epac proteins by a specific cyclic AMP analog mimicked the BCR-induced ERK1/2 and PKB/Akt activation, a process being accompanied by PKA independent Rap1 activation. Epac-specific cyclic AMP increased the activation of growth arrest and apoptosis by the BCR. In particular, Epac activation enhanced BCR-induced cleavage of caspase-3 and poly-ADP-ribose polymerase, cellular responses being further increased by PI 3-kinase inhibitors. Our data indicate that BCR-induced growth arrest and apoptosis in WEHI-231 cells involve cyclic AMP-dependent Epac proteins leading to the suggestion that Epac signaling to ERK1/2 and PKB/Akt may define the balance between apoptotic and anti-apoptotic signaling properties of the BCR.

P180

The free fatty acid G protein coupled receptor GPR40 undergoes agonist-mediated internalisation, prevented by R258A mutation.

N Holliday *University of Nottingham, Nottingham, UK*

GPR40 is stimulated by long chain fatty acids (such as linoleic acid, LNA), and contributes to β-cell insulin secretion (Briscoe *et al.*, 2006). Here we show that LNA stimulates GPR40 endocytosis, and that this is inhibited by Arg258 mutation, a basic transmembrane VII amino acid implicated in agonist activation (Sum *et al.*, 2007). Ca^{2+} responses were measured on a Flexstation (1.5 μM Fluo-4) in HEK293T cells transiently transfected with FLAG-tagged GPR40 cDNAs, and incubated in HEPES buffered saline/0.1% fatty acid free BSA. pEC_{50}s from pooled response curves were calculated with Graphpad Prism 5.01. Internalization was assessed by confocal microscopy in stable 293TR cell lines expressing pcDNA4TO GPR40-venus(v)YFP or GPR40 R258AvYFP, after 18 h induction by tetracycline (Tet, 1 μg/mL). GPR40 Ca^{2+} increases were observed after LNA (pEC_{50} 4.84 ± 0.09; 100 μM: 1.73 ± 0.03 fold over basal; $n = 5$), or GW9508 (Briscoe *et al.*, 2006; pEC_{50} 6.56 ± 0.10; 3 μM: 1.42 ± 0.04 fold; $n = 5$). Responses in cells expressing GPR40 R258A were markedly attenuated (100 μM LNA: 1.30 ± 0.07 fold, and 3 μM GW9508: 1.14 ± 0.03 fold; both $P < 0.001$, Student's *t*-test). After Tet induction, GPR40vYFP and GPR40R258AvYFP were expressed at the plasma membrane, but only GPR40vYFP internalized after 100 μM LNA treatment (15–60 min; Figure 1, scale 10 μM; $n = 3$). Thus GPR40 undergoes endocytosis dependent on an agonist-activated conformation, despite the absence of a conventional C terminal regulatory domain. Dr Birgitte Holst and Prof Thue Schwartz (University of Copenhagen) kindly provided the FLAG GPR40 cDNA. This work was supported by a research grant from the Royal Society.

References:

Briscoe *et al.* Br J Pharmacol. 2006; 148: 619.
Sum *et al.* J Biol Chem. 2007; 282: 292–248.

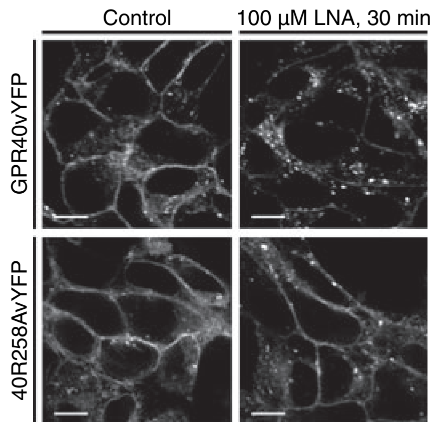
© 2008 The Authors

P181

Acute and chronic imipramine increases 5-HT$_{1A}$ receptors maximal density in heart atria of the rats

I Bellido[a], A Gomez[b] [a]*Department Pharmacology and Clinical Therapeutics. School of Medicine. University of Malaga, Malaga, Spain;* [b]*Service of Anaesthesia. Virgen de la Victoria University Hospital, Malaga, Spain*

The antidepressant drugs modulate central and peripheral serotonin 5-HT$_{1A}$ receptors function. The most common cardiovascular side effects of the antidepressant drugs are cardiac arrhythmias and orthostatic hypotension (Park, 1999; Nykamp *et al.*, 2005). It has been reported that 8OH-DPAT, increases cardiac output in rats (Tianiakow *et al.*, 2007), and that the positive effect of 8OH-DPAT on heart frequency is blocked by NAN-190, thus indicating a specific 5-HT$_{1A}$ receptor mediated mechanism (Lehr *et al.*, 2004; Ngampramuan, *et al.*, 2007). We study the 5-HT$_{1A}$ receptors of the heart atria characteristics after acute and chronic treatment with imipramine. Male Wistar rats (n = 32, weighted 234 ± 15 g) were intra-peritoneally injected with saline (SAL) (0.5 ml/kg/day), acute imipramine (IMIa) 10.9 mg/kg/x three time/1 day, or chronic IMI (IMIc) 10.9 mg/kg/x one time/x 20 days. The forced swimming test (FST) was done to measure the antidepre-ssant effect; and the open-field test and the Rota-Rod test were done to measure the locomotor activity. The 5-HT$_{1A}$ receptors were characterized with ^{3}H-8OH-DPAT (0.01–10 nM) saturation experiment in heart atria membrane homogenates using 5-HT 1 µM to measure the non specific binding. Only IMIc showed antidepressant effects in the FST and diminish the motor activity in the open-field test. Both imipramine treatments increases the 5-HT$_{1A}$ receptors maximal receptor density in the heart atria with respect to SAL (IMIa B$_{max}$ 6.44 ± 2.09 fmol/mg of protein, and IMIc B$_{max}$ 4.24 ± 0.90 fmol/mg of protein versus SAL 2.02 ± 0.62 fmol/mg of protein, $P < 0.05$). There were no changes in the 5-HT$_{1A}$ receptors affinity linked to acute or chronic imipramine treatments. The increased 5-HT$_{1A}$ receptors maximal receptor density induced by imipramine acute and chronic treatments may contribute to the arrhythmias and other cardiovascular complications observed with this treatment.



References:

Lehr T *et al.*, Comp Biochem Physiol C Toxicol Pharmacol. 2004; 138(2): 213.

Ngampramuan S *et al.* Am J Physiol Regul Integr Comp Physiol. 2008; 294(1): 132.

Nykamp *et al.* Ann Pharmacother. 2005; 39(3): 543.

Park KS *et al.* Yonsei Med J. 1999; 40(2): 144.

Tiniakov R *et al.* J Pharmacol Exp Ther. 2007; 320(2): 811.

P182

Inhibitory effects of ATP on pannexin-1 currents

W Ma, A Surprenant *Faculty of Life Sciences, University of Manchester, Manchester, UK*

Pannexin-1 (panx1) has been identified as the 'large pore' activated by the ATP-gated P2X7 receptor. There is currently little known about panx1 as an ion channel. We have asked whether ATP has direct effects on panx1 currents in the absence of P2X7 receptors. Whole-cell patch clamp recordings were performed on HEK cells transiently expressing human or mouse pannexin-1. Panx1 currents were distinguished by their characteristic current-voltage (IV) relationship: they showed depolarization-evoked outward rectification and were rapidly and reversibly blocked by 30 µM carbenoxolone (CBX). The current was not affected by lanthanum (2 mM), which blocks endogenous Trp-like currents in HEK cells. Extracellular ATP inhibited the outward panx1 currents following in a dose-dependent manner with an IC$_{50}$ of 630 µM. Similar inhibitory effects on panx1 currents were also observed with UTP and GTP, the IC50s of which were 1200 µM and 1122 µM, respectively. Pyrophosphate (PPi) did not inhibit panx1 currents at either 0.1 or 5 mM while ADP and AMP only minimally inhibited panx1 currents. Nucleoside triphosphates, but not their phosphate groups, directly inhibit panx1 currents when panx1 is expressed in cells lacking the P2X7 receptor.

P183

Experimental research on the effects of different dopaminergic receptor antagonists in cutaneous and visceral pain models

A Hristov, I Sacara, L Tartau *University of Medicine and Pharmacy 'Gr T. Popa', Lasi, Romania*

D$_1$-like receptors show high affinity for SCH 23390 and SKF 83566 which are selective antagonists for these subtypes and also moderate affinities for typical dopamine agonists such as SKF 82958, SKF 38393, SKF82526 and dihydrexidine. D$_1$ receptors are found at high levels in the typical dopamine rich regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercle, whereas the distribution of the D$_5$ receptors is more restricted. The aim of our study was to investigate the effects of different dopaminergic receptor antagonists on cutaneous and on an accepted visceral pain model. The experiment was carried out, with white mice (20–25 g), divided into 5 groups of seven animals each, treated intraperitoneally with the same volume of solution, as follows: Group I (DW): distilled water 0.1 ml; Group II (coded M): metamyzole 10 mg/kgbw; Group III (coded SCH): SCH-23390 0.3 mg/kgbw; Group IV (coded PMZ): pimozid 0.7 mg/kgbw; Group V (coded MCP): metoclopramide 10 mg/kgbw. The nociceptive cutaneous testing was performed using hot plate assay. The model of visceral pain used consists of chemical stimulation of the colon with capsaicin. Intracolonic administration of capsaicin triggered visceral pain-related nociceptive behaviour. The behaviour modifications (licking abdomen, stretching, contractions of the abdomen) were observed, scored and data were statistically analyzed by Fisher–Tackey tests. SCH-23390 0.3 mg/kgbw and pimozid 0.7 mg/kgbw do not manifest antinociceptive effect in both cutaneous and visceral pain models. Metoclopramide 10 mg/kbw displayed significant antinociceptive effect in both cutaneous and visceral pain models. The antinociceptive response of selective opioid agonists was also studied in combination with selective dopamine receptor agonists and antagonists. It is concluded that D-2 receptor agonists not only have intrinsic antinociceptive activity, but can also potentiate opioid-induced antinociception. Similarly, dopamine D-2 receptor antagonists appear to potentiate opioid-induced antinociception in the mouse tail immersion test.

P184

Radioligand binding affinity and *in vivo* occupancy of inhibitors of the noradrenaline reuptake transporter (NET) in the rat

H Root, L Fish, S Tunboonheng, C Napier *Pfizer Global Research and Development, Sandwich, Kent, UK*

Inhibitors of NET are known to demonstrate efficacy in a variety of CNS disorders including depression, anxiety, ADHD, stress urinary incontinence and neuropathic pain, yet the occupancy level required to produce efficacy in these different conditions has not been determined. Understanding this relationship will facilitate discovery of more efficacious drugs. The aim of this study was to develop *in vitro* and *in vivo* binding assays for rat NET, using [^{3}H]-methylreboxetine (MRB), a potent and selective NET inhibitor (Ding *et al.*, 2003). Saturation and kinetic experiments were performed to define the conditions for [^{3}H]-MRB binding to rat (male, Sprague Dawley) cortical membranes. Binding affinities (Ki) of test compounds (reboxetine (RBX), nisoxetine (NSX), MRB, atomoxetine (ATX), desipramine (DMI), and iodoreboxetine (INER) were determined in competition assays with [^{3}H]-MRB (4 nM, 90 min incubation). For *in vivo* occupancy, rats were dosed subcutaneously with either: vehicle or test compound and 30 min later, dosed with [^{3}H]-MRB (20 µCi/rat) via a tail vein and after a further 30 min killed by decapitation. Terminal blood was collected for analysis of drug plasma concentration and the cortex rapidly dissected and homogenised in buffer. Aliquots of homogenate were filtered and filter bound radioactivity quantified by scintillation counting. A separate group of rats were dosed with DMI to define non specific binding. Concentration response curves were analysed by non-linear regression performed to derive Ki and occupancy EC$_{50}$ (free plasma, nM) values. As expected, the test compounds displayed high affinity for rat NET with Ki values for MRB, INER, NSX, ATX, DMI and RBX of 3.13, 7.47, 9.19, 9.69, 9.89 and 12.55 nM, respectively. Furthermore, they all inhibited *in vivo* [^{3}H]-MRB binding in a concentration dependent manner but with a different rank order of potency with EC$_{50}$ values for INER, MRB, RBX, ATX, DMI and NSX of 0.18, 0.68, 1.40, 5.93, 29.3 and 30.5 nM, respectively. For INER and RBX there was >10-fold difference between Ki and occupancy EC$_{50}$. These findings demonstrate that [^{3}H]-MRB is a useful radioligand for determining *in vitro* binding affinity and *in vivo* occupancy of NET inhibitors in rat brain and confirm that *in vitro* potency alone cannot be used to predict *in vivo* target site occupancy.

Reference:

Ding *et al.* 2003; 15: 345–352.

P185

Epidermal growth factor receptors in the nucleus. Agonist-stimulated appearance of phosphorylated receptor in the hepatocyte nucleus

Y Luo, Z Cheng, N Rahbi, J Hall, M Boarder *De Montfort University, Leicester, UK*

Epidermal growth factor receptors (EGFR) can be found in the nucleus of hepatocytes; this nuclear localisation increases following partial hepatectomy, perhaps functioning as a transcription factor promoting cell proliferation (Marti and Hug, 1995; Lin *et al.*, 2001). We have shown (Luo *et al.*, this meeting) that incubation of hepatocytes with EGF stimulates EGFR internalisation independent of receptor tyrosine phosphorylation. Here we investigate the relationship between agonist-stimulated receptor phosphorylation, cell cycle progression and appearance of receptors in the nucleus. Hepatocytes were prepared from Wistar male rats (250–350 g). EGF (3 nM) was added after 24 h in culture and [^{3}H]thymidine incorporation into DNA measured for the last 4 h of 48 h. For Western blots EGF was added after 2 days in culture and blots developed for tyrosine-phosphorylated EGFR (P-EGFR). To establish the relationship between phosphorylation of the EGFR (ErbB1) and EGF-stimulated cell cycle progression assessed by [^{3}H]thymidine

© 2008 The Authors

incorporation into DNA we stimulated cells in the presence of either AG 1478, AG 875 or gefitinib (ErbB1, ErbB2 or non-selective tyrosine kinase inhibitors respectively). We show that the IC_{50} for AG 1478 was 153 nM (inhibition significant: $P < 0.05$ by 2-way ANOVA), for gefitinib was 208 nM ($P < 0.05$), and for AG875 inhibition was non-significant (1-way ANOVA). Furthermore, inhibition of EGF-stimulated P-EGFR by AG 1478 was shown by western blot using an anti-P-EGFR antibody. This establishes a requirement for EGFR1 (ErbB1) tyrosine phosphorylation in EGF-stimulated cell cycle progression. Incubation of cells with Alexa 488-EGF and confocal visualisation with the EGFR shows that while EGF and EGFR internalise, they cannot be seen within the nucleus. However, when imaging was with the anti-P-EGFR the receptor was visualised within the nucleus. This EGF-stimulated appearance of P-EGFR within the nucleus was blocked by AG 1478. Western blot of nuclear extracts also established the appearance of P-EGFR in the nucleus. These results show EGF can stimulate appearance of P-EGFR uncoupled from EGF ligand in the nucleus of rat hepatocytes.

References:
Lin SY. *et al.* Nat Cell Biol. 2001; 3: 802–808.
Marti U and Hug, M. J Hepatol. 1995; 23: 318–327.

P186

Endosomal signalling from internalised epidermal growth factor receptors in hepatocytes

Y Luo, M Boarder *De Montford University, Leicester, UK*

Agonist-induced internalisation of receptors has been considered as part of the down regulation cycle, but recently there has been interest in signaling from endosomal epidermal growth factor (EGF) receptors (EGFR). Here we study extracellular signal-related kinase (ERK) signalling from endosomal EGF-EGFR complexes in hepatocytes. [3H]thymidine incorporation into DNA of rat (male Wistar ~300 g) hepatocytes was with a 2-pulse stimulation procedure: EGF (3 nM) was added after 24 h in culture for 1 h, and then again for the last 14 h of a 48 h incubation. For Western blots EGF was added after 2 days in culture and blots developed for phospho-EGFR or phospho-ERK. To demonstrate signalling from internalised receptors cells were stimulated with EGF in the presence of EGFR-tyrosine kinase inhibitor AG-1478 (300 nM) and measurements taken after both agonist and inhibitor were removed by extensive washing (including an acid wash in some cases). Receptor-ligand internalisation was demonstrated with confocal imaging. Anti-EGFR immonohistochemistry illuminated the cell periphery in unstimulated cells. On incubation with EGF punctuate fluorescence appeared within the cell. When combined with incubation with Alexa 488-EGF the receptor and ligand internalised into largely the same compartments. EGF-stimulated internalisation was not reduced by the presence of AG-1478. When EGF was added for 30 min after incubation with AG 1478 and extensive washing, the ERK-phosphorylation response was reduced to 26.4 ± 10.8% of control EGF stimulation. When EGF and AG 1478 were incubated together and then both washed out followed by a further incubation the phospho -ERK response was 29.6 ± 10.8% of control. Similar experiments showed that EGF stimulation of [3H]thymidine incorporation occurs when extracellular EGF and AG 1478 were simultaneously removed, and the only signalling was from endosomal EGF-EGFR complexes. The stimulation under these circumstances was significantly above a no EGF control ($P < 0.001$, Bonferonni's) and similar to the stimulation in the absence of AG 1478 treatment. These results show that in primary rat hepatocytes EGF stimulation leads to clathrin-internalisation of EGF-EGFR complexes which continue to signal from within the cell activating ERK and leading to cell cycle progression through S-phase

P187

DRD4 gene expression after long-term lithium treatment

L Carbonell, ML Cuffí *Universitat de Barcelona, L'Hospitalet de Llobregat, Spain*

Several lines of evidence of a potential role of dopamine in bipolar disorder have been suggested (Mitchell *et al.*, 1992). D4 dopamine receptors are codified by DRD4 gene; this gene is localized in the chromosome 11, which has great interest in the search for genes for bipolar disorder (Hayden and Nurnberger, 2006). Lithium, a mood stabilizer, modifies the gene expression of some receptors coupled to adenylyl cyclase (AC) system, such as mu-opioid receptor (de Gandarias *et al.*, 2000) and α_{2D}-adrenoceptor (Cuffí *et al.*, 2006). The aim of this study is to observe if long-term lithium treatment alters the expression levels of D4 dopamine receptors, also coupled to AC system in rat cerebral cortex. Male SD rats were treated with LiCl (120 mg/kg, i.p.) or saline once a day for 10 days. After the last administration, rats were left for 2 days before starting the experiments in order to avoid the interference of circulating lithium and were killed at 2, 6 and 16 days after the last injection. The cerebral cortex was quickly removed and stored at −80°C. Total RNA was extracted and cDNA was synthesized using the TaqMan Reverse Transcription kit. DRD4 expression levels were measured by Quantitative RT-PCR. ANOVA analyse showed a difference of D4 dopamine receptor mRNA levels among days ($F_{2,33} = 3.290$; $P = 0.052$) but no changes were detected between treatments ($F_{1,33} = 1.309$; $P = 0.262$). DRD4 gene expression was significantly decreased at the first time-point measured (2 days); the difference in mRNA levels was 0.459 (CI 95% 0.94–0.02). Moreover, the presence of an interaction between treatments and days ($F_{2,33} = 3.397$; $P = 0.048$) indicated that the effect of treatments was not the same across all the days. DRD4 gene expression was diminished by long-term lithium treatment. This decrease was only observed immediately after finishing the treatment and DRD4 expression levels quickly increased until basal expression. This would indicate that DRD4 gene expression is only altered when lithium is present. This study was supported by ACESB07/02. Universitat de Barcelona. Spain.

References:
Cuffí ML *et al.* FASEB J. 2006; 20: LB113.
de Gandarias JM *et al.* Neurosci Lett. 2000; 279(1): 9–12.
Hayden EP and Nurnberger JI Jr Genes Brain Behav. 2006; 5: 85–95.
Mitchell P *et al.* J Affect Disord. 25(1): 1–11.

P188

Exposure-response relationship for the efficacy of fesoterodine

A El-Tahtawy, B Malhotra *Pfizer Inc, New York, NY, USA*

Antimuscarinic (AM) drugs are widely used for the treatment of overactive bladder (OAB); activity at the M2 and M3 muscarinic receptor subtypes is considered relevant for their efficacy. Some AM drugs have balanced M2/M3 selectivity (tolterodine, fesoterodine, and oxybutynin), whereas others are M3-selective (darifenacin, solifenacin). Exposure-response relationship (E-R) of a drug's effect(s) may depend on the affinity/capacity of receptor subtypes. This analysis was performed to characterize E-R for the efficacy of fesoterodine. Efficacy data from a 12-week double-blind Phase 3 study of placebo and 4 and 8 mg fesoterodine were analyzed for their relationship with plasma concentrations of 5-hydroxymethyl tolterodine (5-HMT), a metabolite and the principal active moiety of fesoterodine. Efficacy parameters included changes from baseline (BL) in the number of urgency urinary incontinence episodes, micturition frequency, and mean volume voided per micturition. Age, body weight, gender, and BL symptom levels of the subjects and 5-HMT concentrations were analyzed as possible predictors of efficacy. 5-HMT concentrations and subject BL symptom levels were found to be the strongest predictors of fesoterodine efficacy. For each efficacy endpoint, the E-R was found to be steep across 5-HMT concentrations up to 5 ng/mL (representing >95% patients), with distinct separation by dose level. Similarly, the effects strongly correlated with BL symptom levels. Age, gender, and body weight did not appear to be strong predictors of efficacy. While therapeutic doses of solifenacin (5, 10 mg) and darifenacin (7.5, 15 mg) do not show appreciable increments in efficacy with increasing dose, a dose response for several OAB endpoints is apparent for oxybutynin (5, 10, 15 mg) and fesoterodine (4, 8 mg), which have balanced M2/M3 selectivity. These analyses highlight the steep E-R as the principal contributor to the observed dose response of fesoterodine efficacy.

P189

Noncompetitive interactions at muscarinic receptors within a simple ternary complex model

J Proska *Department Physical Electronics, Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague, Prague, Czech Republic*

Fifteen years ago, it was shown that the effects of alcuronium on rates of [3H]NMS association and dissociation could be explained only by a reaction mechanism in which [3H]NMS binds only to free receptors (not occupied by alcuronium), whereas alcuronium binds both to free receptors and to receptors occupied by [3H]NMS. It has been proposed that alcuronium blocks entry to the pocket containing the [3H]NMS binding site (Proska *et al.*, 1994; Tucek *et al.*, 1995). Subsequently, within a ternary complex model (TCM), it has been documented that positive (alcuronium and strychnine, respectively) and negative 'allosteric' modulators compete for the common binding site on muscarinic receptors (Proska *et al.*, 1995). It has recently been found, that alcuronium and strychnine, respectively, enhance the binding of adiphenine, 4-DAMP, diphenylhydramine, diphenylpyraline, hyosciamine, N-piperidinyl benzilate, tropacine, scopolamine and some tricyclic antidepressants into orthosteric binding site at rat atrial (M2) receptors. Also, comparison of the kinetic data from studies with alcuronium, strychnine, gallamine, anatruxonium, truxillonium and diadonium, respectively, reveals different mechanisms of interaction with M2 receptors, though all ligands interact within a simple TCM. Multimodal binding and steric effects have been rather undervalued thus far in comparison with peculiarly intuitive concepts of multiple 'allosteric' binding sites and ligand induced conformation changes. In this work, a physical and terminological inconsistency of rather marketing term 'neutral cooperativity' is documented. All programs for modelling and data analysis were written in Mathematica language (Wolfram Research, Inc.). Wolfram Research, Inc., Mathematica, Version 6.0.2, Champaign, IL (2007). Work was partially supported by Research Project MSM6840770022.

References:
Proska *et al.* Mol Pharmacol. 1994; 45: 709–717.
Proska *et al.* Mol Pharmacol. 1995; 48: 696–702.
Tucek *et al.* Trends Pharmacol Sci. 1995; 16: 205–212.

© 2008 The Authors

P190

Experimentally-induced diabetes alters the levels of aromatase both in peripheral and central nervous systems

N Burul-Bozkurt, C Pekiner, P Kelicen *Hacettepe University, Ankara, Turkey*

Diabetic neuropathy is one of the most common and important complications of diabetes mellitus. Diabetes mellitus has also been associated with an increased risk of Alzheimer's disease (Peila *et al.*, 2002). Estrogens reduced the risk of Alzheimer's disease and prevent neuronal loss in several experimental models of neurodegeneration (Garcia-Segura *et al.*, 2001). Aromatase catalyzes the conversion of androgens to estrogens and expressed in a variety of tissues including neurons. Insulin is known to stimulate the activity of aromatase (Garzo *et al.*, 1984). This study was designed to investigate the effects of experimental diabetes on aromatase expression in nervous system. Gender-based differences were also investigated. Male and female Sprague-Dawley rats (300–320 g) were injected with streptozotocin (35 mg/kg) to induce diabetes. At the end of 4- and 12-weeks sciatic nerve and hippocampal tissue homogenates were prepared and evaluated for aromatase proteins by western blot. Student's t test was used for statistical analysis and $P < 0.05$ was considered significant. Aromatase expression in sciatic nerves of both genders were decreased in 4-weeks of diabetes ($P < 0.05$, $n = 4$), but in 12-weeks the enzyme levels were increased in female rats ($P < 0.05$, $n = 4$) and reached to control levels in male animals. Aromatase levels were not altered in hippocampal brain extracts at 4-weeks but increased at 12-weeks in female diabetic rats ($P < 0.05$, $n = 4$). No significant differences were observed at aromatase levels of hippocampus in male diabetic rats. In conclusion, these results indicated for the first time that, diabetes mellitus altered the expression of aromatase both in central and peripheral nervous systems. The peripheral nervous system is more vulnerable to damage than the central nervous system in diabetes. These effects of diabetes differs with gender and compensatory neuroprotective mechanisms is more efficient in female rats. This study is supported by Hacettepe University Research Foundation. No:06D05301001.

References:

Garcia-Segura *et al.* Prog Neurobiol. 2001; 63: 29–60.
Garzo *et al.* Am J Obstet Gynecol. 1984; 148: 657–662.
Peila *et al.* Diabetes. 2002; 51: 1256–1262.

P191

Enzymatic activities of monoamine oxidase (type A and B) and semicarbazide-sensitive amine oxidase (SSAO) in colonic arteries of type 2 diabetic patients

SF Nunes[a], IV Figueiredo[a], JS Pereira[b], ET Lemos[c], S Baptista[c], F Teixeira[c], MM Caramona[a] [a]*Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal;* [b]*Portuguese Oncology Institute of Coimbra, Coimbra, Portugal;* [c]*Therapeutics Unit, Institute of Pharmacology and Experimental Therapeutics, Medicine Faculty, Coimbra, Portugal*

The aim of this work is to study the enzymatic activities of monoamine oxidase (Type A and B) and semicarbazide-sensitive amine oxidase (SSAO) in the colonic arteries of type 2 diabetic patients in order to know if monoamines metabolism and their liberation is different from the human control tissues. A radiochemical method was used, the specific substrates were: ^{3}H-5-hydroxytryptamine creatinine sulphate [^{3}H-5HT] (32–1000 μM) for MAO A; ^{14}C-β-phenylethylamine hydrochloride [^{14}C-PEA] (5–160 μM) for MAO B; and, ^{14}C-benzylamine hydrochloride [^{14}C-BZ] (50–1600 μM) as specific substrate for SSAO. Selective inhibitors for the enzymes were also used (clorgyline 10^{-4}M as MAO A inhibitor and selegiline 10^{-4}M as MAO B inhibitor). The protein content in homogenates was determined by the method of Lowry, using human serum albumin as a standard. The assays were performed to obtain information about the K_m and V_{max} using a linear regression analysis of the data following the method described by Woolf Hanes Plot. The assay of noradrenaline content in the arteries was performed by means of high-pressure liquid chromatography with electrochemical detection. MAO A activities from arteries of non-diabetic patients have more activity for the substrate than the ones from diabetic patients. The affinity of MAO B in diabetic patients is statistically higher when compared to the non-diabetic. Tissue-bond SSAO shows a statistically greater activity in non-diabetic patients (Table 1). The endogenous NA content is also statistically less in the diabetic arteries 0.038 ± 0.023 μg/g tissue when compared to the control group 0.252 ± 0.09 μg/g tissue (mean ± SD, n = 8/10, respectively).

In conclusion, arteries homogenates from type 2 diabetes presented decreased MAO A and SSAO activity when compared to non-diabetic homogenates. Thus, our results suggest that in diabetes the enzymes activities have a possible role in this pathogenesis. Supported by FCT – Portugal.

Table 1. Kinetic parameters (K_m and V_{max}) for MAO (type A and B) and SSAO in type 2 diabetic patients and human control tissues. Values are means ± SD.

		Control (n = 10)	Type 2 diabetic patients (n = 8)
MAO A	V_{max} (mM/mg/protein/h)	55.56 ± 9.28	29.79 ± 14.48***
	K_m (μM)	312.9 ± 131.5	237.6 ± 178.2
MAO B	V_{max} (mM/mg/protein/h)	17.18 ± 3.11	13.85 ± 8.07
	K_m (μM)	115 ± 21.18	84.34 ± 34.99*
SSAO	V_{max} (mM/mg/protein/h)	219 ± 42.5	132 ± 50.42**
	K_m (μM)	266.5 ± 90.9	227.9 ± 134.4

*$P < 0.0003$, ** $P < 0.0011$, ***$P < 0.0003$. The significance of the differences between values in the diabetic and in the non-diabetic groups was calculated using the Student's t-test.

P192

Short-term rosiglitazone treatment decreases expression of receptors for advanced glycation end products (RAGE) and proinflammatory cytokines in aortic tissues from AGEs-injected rats

N Belder, M Belder, G Ozansoy, N Ari *Department of Pharmacology, Faculty of Pharmacy, Ankara University, Ankara, Turkey*

The enhanced formation of AGEs and expression of RAGE play an important role in the development of diabetic complications. Administration of AGE-modified proteins to animals produce changes typical of early diabetes-like vascular dysfunction. Rosiglitazone (rosi), is a member of PPAR-gamma ligands, which are used as antidiabetic agent. Rosi has been shown to decrease vascular inflammation and also expression of RAGE, *in vitro* (Marx and Walcher, 2004; Wang *et al.*, 2006). In this study, to determine the effect of short-term rosi treatment on aortic RAGE expression, male Wistar rats (180–200 g, n: 50) were given tail vein injections with either sterile nonglycated BSA (bovine serum albumin solution) or glycated BSA with AGE formation (AGE-BSA solution) or AGE-BSA followed immediately by orally of rosi (8 mg/kg/day), for 10 days (preventive study). At the end of the treatments, expression of RAGE (by semiquantitative RT-PCR) in aortae (E-) and proinflammatory cytokines (TNF-alpha, IL-6, IL-1beta) (by ELISA) in aortae (E+) were investigated. Administration of AGE-BSA solution for 10 days resulted up regulation of RAGE mRNAs in aortic smooth muscle and co-administration of rosi decreased this up regulation. Aortic TNF-alpha and IL-1beta elevated after AGE-BSA and were reduced by 38 ± 2%; 10 ± 1%, respectively ($P < 0.05$; n: 5–7) with co-administeration of rosi. This study provides an *in vivo* evidence for beneficial *pleiotropic* effects of rosi by limiting RAGE expression in AGEs-induced vascular complications. Supported by Turkish Scientific Research Council (TUBITAK, SBAG-HD-183).

References:

Marx N and Walcher Diabetes. 2004; 53: 2662–68.
Wang K *et al.* JPET. 2006; 317: 37–43.

P193

Pharmacological potential of natural and synthetic fatty acids to alter body weight and hepatic lipid metabolism in diet-induced obese mice

O Vögler[a], A López-Bellan[a], R Alemany[a], P Escribá[a], S Tofé[b], J Quevedo[b], S Díaz[b], V Pereg[b] [a]*Department of Biology, Health Sciences Research Institute (IUNICS), University of the Balearic Islands, Palma de Mallorca, Spain;* [b]*Department of Endocrinology and Nutrition, University Hospital Son Dureta, Palma de Mallorca, Spain*

Fatty acids are integral components of lipid metabolism being functionally involved as either substrates, intermediates or end products. Therefore, chemical modification of natural fatty acids is a rational strategy to generate novel compounds with potent antiobese properties. We have recently demonstrated that the monounsaturated fatty acid oleic acid and its non beta-metabolizable synthetic derivative, 2-hydroxyoleic acid, are able to inhibit food intake and to reduce adipose tissue mass in lean Wistar Kyoto rats, albeit with very different efficacy (Vögler *et al.*, 2008). The present work analyses the relationship between chemical structure, antiobese effect and the impact on hepatic key regulators of lipid metabolism of two saturated [stearic acid (SA), 2-hydroxystearic acid (2-OHSA)] and two unsaturated [oleic acid (OA), 2-hydroxyoleic acid (2-OHOA)] C18 fatty acids in an animal model of diet-induced obesity. Male, six week old C57BL/6 mice were fed a diet containing 45 kcal% of mainly cocoa fat (Research Diets #D06112701) for 12 weeks and were then treated orally for 7 days with 375 mg/kg/12 h of the indicated fatty acids. Our results show that only 2-OHOA efficiently reduced body weight (−8.2%), food intake (−33.3%) and adipose tissue mass (−23.4%), whereas OA and SA did not alter these parameters. 2-OHSA even slightly increased body weight and adipose tissue mass. In parallel, quantitative real-time PCR revealed that none of the different carnitine palmitoyltransferase isoform levels (CPT1a, 1b, and 2) were significantly altered by any of the compounds in liver tissue. On the other hand, 2-OHOA drastically reduced (−85%) transcription of hepatic stearoyl-CoA-desaturase 1 (SCD1), whereas 2-OHSA increased its mRNA level significantly (+72%). Neither OA nor SA showed any effect on this enzyme. 2-OHOA seems to act directly on hepatic cells, because a dose-dependent SCD1 reduction was also present in cell culture experiments using the hepatocyte cell line Hep G2. We conclude that the antiobese action of 2-OHOA is, at least partially, attributable to hepatic downregulation of SCD1, an important novel molecular target for antiobesity drugs (Ntambi et al., 2002), and that the structural requirements for a fatty acid to trigger this mechanism include a hydroxyl group in position 2 and a delta-9 unsaturation.

References:

Ntambi *et al.* Proc. Natl. Acad. Sci. U S A 99 2002; 11482–6.
Vögler *et al.* Int. J. Obes. 2008; 32: 464–73.

P194

Changes in the diabetic rat brain $Na^{+}K^{+}$-ATPase and Mg^{2+}-ATPase activities: in vivo and *in vitro* modulation by L-cysteine

A Zarros[a], C Liapi[b], P Galanopoulou[b], K Marinou[a], Z Mellios[a], N Skandali[b], H Al-Humadi[b], F Anifantaki[b], E Gkrouzman[b], S Tsakiris[a] [a]*Department of Physiology, Medical School, National & Kapodistrian University of Athens, Athens, Greece;* [b]*Department of Pharmacology, Medical School, National & Kapodistrian University of Athens, Athens, Greece*

Uncontrolled diabetes is known to affect the nervous system. The aim of this study was to investigate the effect of the antioxidant L-cysteine (Cys) on the changes caused by adult-onset streptozotocin (STZ)-induced diabetes on the rat brain Na^{+}, K^{+}-ATPase and Mg^{2+}-ATPase activities. Thirty-eight male Wistar rats were divided into six groups: C1 (8-week-control), C2 (8-week-control + 1-week-saline-treated), C+Cys (8-week-control + 1-week-Cys-treated), D1 (8-week-diabetic), D2 (8-week-diabetic + 1-week-saline-treated) and D+Cys (8-week-diabetic + 1-week-Cys-treated). All diabetic rats were once treated with an i.p. STZ injection (50 mg/kg body

© 2008 The Authors

weight) at the beginning of the experiment, while all Cys-treated groups received i.p. Cys 7 mg/kg body weight (daily, for 1-week, during the 9th-week). Whole rat brain enzyme parameters were measured spectrophotometrically. *In vitro* incubation with 0.83 mM of Cys for 3 h was performed on brain homogenate samples from groups C2 and D2. Diabetic rats exhibited a significant reduction in the activity of Na^+, K^+-ATPase (−36%, D1 vs. C1; −48%, D2 vs. C2) that was not reversed after 1-week Cys administration. However, *in vitro* incubation with Cys partly reversed the diabetes-induced Na^+, K^+-ATPase inhibition. Mg^{2+}-ATPase activity was not affected by STZ-induced diabetes, while Cys caused a significant inhibition of the enzyme, both *in vivo* (−14%, C + Cys vs. C2; −17%, D + Cys vs. C2) and *in vitro* (−16%, D2 + *in vitro* Cys vs. C2). The present data sheds light on the effects of STZ-induced diabetes on two important adenotriphosphatase enzymes. The inhibition of Na^+, K^+-ATPase reflects a possible mean through which untreated diabetes could affect neuronal excitability, metabolic energy production and certain systems of neurotransmission. The use of Cys as a neuroprotective agent against diabetes is not encouraged by our *in vivo* findings. However, our *in vitro* findings could be indicative of a possible protective role of Cys under different *in vivo* experimental conditions.

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

P195

Screening for the presence of the DPYD mutation (IVS14 + 1G>A) in patients with breast and colorectal cancer treated by 5-fluorouracil-based chemotherapy in Slovakia

M Cizmarikova[a], M Wagnerova[b], V Habalova[c], A Linkova[a], A Kohut[a], L Mirossay[a], A Mirossay[a], A Berc[b] [a]*Department of Pharmacology, Faculty of Medicine, P. J. Safarik University, Kosice, Slovakia;* [b]*Department of Radiotherapy and Oncology, East Slovak Oncology Institute a.s., Kosice, Slovakia;* [c]*Department of Biology, Faculty of Medicine, P. J. Safarik University, Kosice, Slovakia*

Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the catabolism of 5-fluorouracil (5-FU), a widely used cytostatic agent for treatment of many malignancies, including breast and colorectal cancer. It is suggested that patients with a partial deficiency of this enzyme are at risk from developing a severe 5-FU-associated toxicity. Also it was investigated that the gene of dihydropyrimidine dehydrogenase (DPYD) is a subject to many genetic polymorphisms or mutations. Splice site mutation IVS14+1G>A is the most common mutation of DPYD in patients with severe toxicity after 5-FU therapy. The goal of this study was to examine the presence of the IVS14+1G>A mutation in patients with breast and colorectal cancer treated by 5-fluorouracil-based chemotherapy. The correlation of the examined mutation with an occurrence of toxicity after 5-FU-based treatment was the second aim. Toxicity was classified according to WHO criteria. One hundred and thirty-six patients were studied (72 patients with breast cancer and 64 patients with colorectal cancer). DNA was extracted from blood lymphocytes using standard methods and screening for the presence of the IVS14+1G>A mutation was performed by restriction fragment length polymorphism. All patients studied for DPYD mutation in patients in our group were homozygous for wild allele though the statistical correlation with toxicity was not possible to realize. Despite absence of the tested mutation an occurrence of toxicity was also determined even grade III-IV of toxicity. The main toxic side effects were anemia, leucopenia, neutropenia, nauzea and vomiting, hepatic toxicity, diarrhoea and mucous toxicity. Our preliminary results suggest, that the IVS14+1G>A mutation is infrequent in our population. This study also demonstrated that genotyping of this mutation is insufficient in predicting therapeutic toxicity to 5-FU-based chemotherapy and it is important to find others appropriate pre-therapeutic tests to identify patients at risk from developing of toxicity. Supported by the grant VEGA SR 1/2282/05 and research project MZ SR 2005/46-VOUKE-01 from Ministry of Health of the Slovak Republic.

P196

The role of IRAP in the effect of angiotensin IV on cognition in the mouse

B Golding, A Overall, P Gard *University of Brighton, Brighton, UK*

Mouse strain differences have been observed in the behavioural effects of angiotensin IV (AIV). Administration of AIV improves cognitive function in DBA_2, C57 and BKW mice, but not in CD mice. The improved cognitive function is thought to be mediated through the recently discovered 'AT4' receptor, characterised as insulin regulated amino-peptidase (IRAP). Inhibition of IRAP by AIV preserves IRAP substrates: oxytocin and vasopressin, which are both known to have effects on cognition. The aim of this study was to explore the basis of the strain differences by investigating sequence variation within the catalytic domain of IRAP, enzyme activity and expression of the encoding gene. Male mice (20–30 g) of each strain were bred in-house. IRAP activity in whole brain was assessed by catalysis of leucine-p-nitroanilide. RT-PCR was used for expression analysis across strains using SYBR green. Gene sequencing was carried out by MWG biotech. All strains showed an equal inhibition of amino-peptidase activity by AIV. There were no differences in the amino acid sequences in the catalytic and zinc binding domains and gene expression levels were identical. The lack of response in CD mice to AIV cannot be explained through variation within IRAP sequence or expression. This finding suggests that the effects of AIV on cognition are not mediated via the amino-peptidase function of IRAP. There was, however, some evidence of a strain difference in amino-peptidase activity suggesting alternative amino-peptidases (e.g. amino-peptidase N) may influence cognitive function.

P197

The effect of MDR1 C3435T polymorphism on progression-free survival time in patients with breast cancer

M Cizmarikova[a], M Wagnerova[b], V Habalova[a], A Mirossay[a], A Linkova[a], J Petrovicova[a], A Berc[b], I Andrasina[b], A Kohut[a], L Mirossay[a] [a]*Medical Faculty, P.J. Safarik University, Kosice, Slovakia;* [b]*East Oncology Institute, Department of Radiotherapy and Oncology, Kosice, Slovakia*

The MDR1 gene product P-glycoprotein (Pgp) represents the most widely studied membrane protein of the large mammalian ABC transporter family, that increases drug efflux of a number of chemotherapeutics. The main substrates for Pgp include anthracyclines, vinca alkaloids, taxanes, inhibitors of topoisomerases (I, II), mitomycin C and tamoxifen. The C3435T single nucleotide polymorphism (SNP) in exon 26 is the most important MDR1 gene polymorphism. Although it is silent mutation, this polymorphism affects the expression and function of the Pgp in many ways. It was investigated, that C allele is associated with increased Pgp levels and it may play an important role in variability of treatment efficacy. The aim of this study was to examine the MDR1 C3435T polymorphism in patients with breast cancer treated by anthracyclines or taxanes and/or tamoxifen-based adjuvant chemotherapy and investigate the association between this polymorphism and progression-free survival. Sixty one breast cancer patients were studied for presence of the MDR1 C3435T polymorphism. DNA was extracted from peripheral blood lymphocytes using standard extraction method. Polymerase chain reaction-restriction fragment length polymorphism was used for detection of C3435T SNP. Progression-free median survival time of patients in a group CC genotype were 15 months and in CT, TT group were 34 months. This differences between C or T alleles was found to be statistically significant (long rank, Mantel–Cox test, $P = 0.0332$). The results of the present study demonstrated impact of MDR1 genotype on progression-free survival in patients with breast cancer. Supported by the research project MZ SR 2005/46-VOUKE-01 from Ministry of Health of the Slovak Republic, grant VEGA SR 1/2282/05 and VEGA SR 1/3372/06.

P198

Drug utilization and cytochrome P450 2D6 gene polymorphisms in patients with oral lichen planus

O Slanar[a], V Paulusova[b], C Rösch[a], I Drizhal[b], F Perlik[a] [a]*Clinical Pharmacology Unit, 1st Faculty of Medicine, Charles University, Prague, Czech Republic;* [b]*Department of Dentistry, Medical Faculty Hradec Kralove, Charles University, Hradec Kralove, Czech Republic*

Oral lichen planus (OLP) is the second most frequent disease of oral mucosa with unclear ethiology. Drug-induced reactions are, nevertheless, known to trigger OLP. Poor metabolizers of drug metabolizing enzymes may be exposed to substantially higher plasma concentrations of medications, which may subsequently result in higher rate of adverse drug reactions in comparison with patients without liver enzyme deficiency. CYP2D6 is considered to be a major polymorphic enzyme involved in drug biotransformation. Therefore we hypothesized that the *CYP2D6* polymorphism and drug utilization might represent risk factors for OLP. The study population comprised 46 patients with OLP; drug utilization was analyzed in comparison with 60 sex- and age-matched control subjects and historical control of 223 healthy volunteers of Czech nationality was used for evaluation of genotype distribution. Patients and control subjects consumed on average 4.5 and 3.1 prescribed medicaments per day and person, respectively. Approximately 93% of the patients reported daily intake of 1 or more drugs while only 68% of control subjects were regularly treated. OLP patients used hypolipidemics, antidiabetics, NSAIDs and anxiolytics more frequently than control subjects, while the odds ratio for OLP in patients consuming CYP2D6 substrates was 0.68 (95% CI 0.31–1.50) in relation to the control subjects. Distribution of *CYP2D6* genotypes and frequency of poor metabolizers (6.5%) in patients with OLP was similar to historical control group and the data are consistent with the known genotype distributions in other European populations. Our study demonstrates that neither CYP2D6 substrate utilization nor *CYP2D6* polymorphism represent susceptibility factors for OLP. Possible involvement of CYP2C9 deficiency in initiation of drug-induced OLP needs to be further investigated, because the intake of some CYP2C9 substrates was significantly higher among OLP patients than in control subjects. This study was supported by a grant MSM No. 0021620849.

P199

An association study of catalase -262G>T gene polymorphism with Li/Na countertransport activity, blood pressure and their regulation by atorvastatin

A Goulas[a], M Kosmidou[b], D Molyva[a], N Raikos[c], C Savopoulos[b], N Daferera[a], A Hatzitolios[b], V Kokkas[a] [a]*Department of Pharmacology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [b]*First Propedeutic Clinic, AHEPA University Hospital, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [c]*Department of Forensic Toxicology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Atorvastatin was recently reported to significantly reduce blood pressure (BP) as well as red-blood cell sodium–lithium countertransport (Na/Li CT) activity in hypercholesterolaemic patients, independently of its lipid lowering effects (Kosmidou *et al.*, 2008). NA/Li CT activity may be affected by polymorphisms in genes related to antioxidant defense (Goulas *et al.*, 2008) and BP has been associated with production of hydrogen peroxide and catalase activity in experimental systems (Jung *et al.*, 2007). This study examines the effect of a functional catalase gene polymorphism, -262 C>T, on BP, Na/Li CT activity and their regulation by atorvastatin, in a previously characterized group of Greek hypercholesterolaemic patients (Kosmidou *et al.*, 2008). A total of 78 ethnic Greek individuals were genotyped for the catalase -262 C>T gene polymorphism, using a PCR – RFLP method (Goulas *et al.*, 2002). Na/Li CT activity was assayed in isolated red-blood cells with a method based on atomic absorption spectroscopy (Kosmidou *et al.*, 2008). Of the participants, 54 had been treated for primary hypercholesterolaemia, while the remaining 24 had served as normolipidaemic, normotensive controls. Treatment consisted of 20 mg of atorvastatin, once daily, for 12 weeks. Putative associations were examined by running univariate analyses with a general linear model, using age, sex and smoking as covariates (SPSS 14.0). In the hypercholesterolaemic group – but not among normolipidaemic controls – we observed a tendency of -262 CC genotypes to associate with higher Na/Li CT activity and systolic BP, compared to -262 CT/TT genotypes. This tendency was more evident in a subgroup of mildly hypertensive, hypercholesterolaemic patients (for systolic BP, $P = 0.066$). Carriers of the T allele appeared to respond better to the systolic BP lowering effect of atorvastatin. None of these effects reached statistical significance however, indicating the need to extend this investigation to a larger study group.

References:

Goulas A *et al.* Clin Chem Lab Med. 2008; 46: 306–10.
Goulas A *et al.* Neurosci Lett. 2002; 330: 210–2.
Jung O *et al.* Arterioscler Thromb Vasc Biol. 2007; 27: 442–4.
Kosmidou MS *et al.* Am J Hypertens. 2008; 21: 303–9.

© 2008 The Authors

P200

Warfarin binding to human plasma proteins

I Costa[a], G Alves[b], A Fortuna[b], A Falcão[b], M Caramona[a] [a]*Laboratory of Pharmacology, Faculty of Pharmacy, Coimbra University, Coimbra, Portugal;* [b]*Laboratory of Pharmacology, Faculty of Pharmacy and Center for Neurosciences and Cell Biology, Coimbra University, Coimbra, Portugal*

Warfarin is an oral anticoagulant extensively used in clinical practice. The available information relating to warfarin enantiomers binding to plasma proteins is ambiguous. To elucidate this point, an *in vitro* study was conducted to determine whether the binding characteristics of R- and S-warfarin are similar. A pool of human plasma was used in the study. Warfarin solutions were prepared from *rac*-warfarin sodium salt (SmithKline Beecham, Portugal) in Sorensen phosphate buffer, pH 7.4, in a 0.4–22.7×10^{-4} M concentration range. The protein binding of warfarin was characterized using the equilibrium dialysis technique. An HPLC technique (Pais *et al.* 1999) was adopted for warfarin assessment. Figure 1 summarises the results obtained. No statistically significant differences were found between racemic, R- and S-warfarain binding percentages. The binding of warfarin (racemate and enantiomers) proved to be a concentration-dependent nonlinear process, as confirmed by the variation of bound percentages of racemic (99.00 ± 0.06 to 79.74 ± 1.18%), R- (99.00 ± 0.13 to 82.31 ± 0.97%) and S-warfarin (99.33 ± 0.06 to 80.72 ± 1.09%) in the overall concentration range. Racemic warfarin and both isomers revealed high biding percentages, confirming strong affinity to human plasma proteins, as claimed in several studies. Although concentration-dependent, it becomes obvious that the R- and the S-warfarin present a similar degree of binding and their pharmacologically active free fractions are identical (especially considering the usual warfarin therapeutic range).

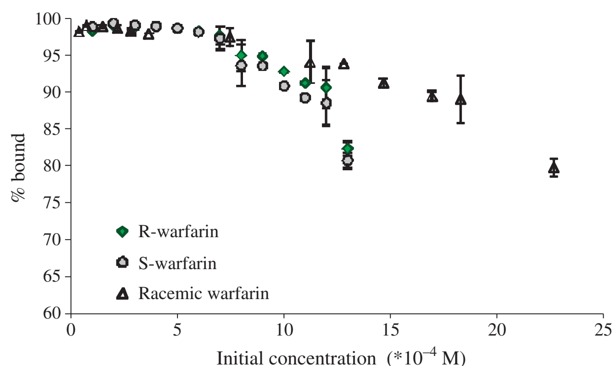


Figure 1. Mean binding percentages of warfarin to human plasma proteins.

Reference:

Pais *et al.* Rev Port Far. XLIX 1999; 53–60.

P201

The suitability of rabbits in pharmacokinetic preclinical testing of rectal suppositories

L Smetanova, V Stetinova, M Nobilis, J Kvetina, J Kopecky *Institute of Experimental Biopharmaceutics, Joint Research Centre of PRO.MED.CS Praha a.s. and Academy of Sciences of the Czech Republic, Hradec Kralove, Czech Republic*

The aim of the study was to verify the suitability of experimental rabbits for comparative pharmacokinetic testing of rectal formulations during preclinical data preparation. Rabbits (breed Chinchilla medium), male (4.0 ± 0.3 kg), kept under standard conditions, fasted 24 h before the experiment, were used. The design of the study: 1. The verification of rabbit rectal blood supply was done under general anaesthesia with retrograde application of Evans blue via vena iliaca interna and vena mesenterica inferior. We concluded that the rabbit rectal blood supply is comparable with that in humans (the upper third of rectum drained via vena mesenterica inferior and the distal two thirds via vena iliaca interna). 2. The pharmacokinetics of four suppository formulations of model drug prepared by different technology) was evaluated in a crossover study with four single-dose administrations (washout periods of 1 week). The anesthetised rabbits were cannulated via major auricular blood vessel, then the suppository was inserted into the rectum. Samples were taken at 0-0.5-1-2-3-4-6 h via the cannulated vessel and detected by HPLC (Nobilis *et al.*, 2006). The results (Table 1) showed the differences in pharmacokinetic parameters of different suppository formulations. The inter-individual variability of model drug was relatively high. The suitability of rabbits for comparative pharmacokinetics of rectal suppositories was confirmed.

Reference:

Nobilis *et al.* J. Chromatogr. A 1119 2006; 299–308.

Table 1. Pharmadiuretic parameters of the model drug.

Formulation	t_{max} (h)	c_{max} (nmol/mL)	AUC (nmol/h/mL)
A (n = 8)	2.25 ± 1.48	39.85 ± 38.92	117.18 ± 104.38
B (n = 9)	1.83 ± 1.66	36.20 ± 21.66	96.75 ± 48.53
C (n = 8)	1.75 ± 2.09	43.18 ± 28.02	122.56 ± 78.31
D (n = 7)	1.36 ± 0.99	36.58 ± 18.41	94.17 ± 49.65

P202

Gender factors in pharmacokinetics of nitrendipine in healthy volunteers

J Zoulova[a], J Kopecky[a], J Pastera[a], J Chladek[a], K Macek[b], J Kvetina[a] [a]*Institute of Experimental Biopharmaceutics, Joint Research Centre of PRO.MED.CS Praha a.s. and Academy of Sciences of the Czech Republic, Hradec Kralove, Czech Republic;* [b]*Teaching Hospital, Hradec Kralove, Czech Republic*

Gender differences in pharmacokinetics have been described in many drugs (Harris *et al.*, 1995; Schwartz, 2003). The aim of our study was to find out the effect of gender on the pharmacokinetics of the dihydropyridine calcium channel blocker nitrendipine. Nitrendipine was given to 20 healthy men (18–39 years, 63–99 kg) and 16 healthy women (20–47 years, 55–75 kg) in a single oral dose of 20 mg (one tablet). Blood samples (9 ml) were drawn 0–36 h after the administration. Plasma nitrendipine concentrations were determined by gas chromatography. Calculated pharmacokinetic parameters are presented in Table 1. Data are given as mean ± standard deviation (SD), except for Tmax data, which are given as median and range. Only a slight tendency to faster absorption was observed in women (higher Cmax and lower Tmax). Statistical analysis of parameters (t-test) revealed no significant differences between men and women (even after adjusting the parameters AUCs and Cmax for 70 kg body weight - data not presented here). High interindividual variability was found both in men and women. It seems that gender has no effect on the pharmacokinetics of nitrendipine.

References:

Harris RZ *et al.*, Drugs 1995; 50: 222–39.

Schwartz JB Clin Pharmacokinet. 2003; 42: 107–21.

Table 1. Pharmacokinetic parameters of nitrendipine in healthy volunteers.

Parameter	AUC(0-t) [ng.h/mL]	AUC(0-inf) [ng.h/mL]	Cmax [ng/mL]	Tmax [h]	ke [h^{-1}]	T1/2 [h]
Men	58.06 ± 41.04	62.16 ± 43.91	16.45 ± 9.58	2.00 (1.00 - 4.00)	0.162 ± 0.090	6.11 ± 4.04
Women	60.18 ± 29.81	63.59 ± 31.78	22.22 ± 14.74	1.25 (0.67 - 6.00)	0.166 ± 0.101	6.70 ± 5.01

P203

Bioequivalence studies: What are the reasons for non-inclusion and withdrawal of healthy volunteers

J Kopecky[a], J Zoulova[a], I Zubata[a], L Vyslouzil[a], K Macek[b], F Perlik[c], O Slanar[c], P Petr[d], H Tesfaye[e], J Sedivy[e], J Kvetina[a] [a]*Institute of Experimental Biopharmaceutics, Joint Research Centre of PRO.MED.CS Praha a.s. and Academy of Sciences of the Czech Republic, Hradec Kralove, Czech Republic;* [b]*Teaching Hospital, Hradec Kralove, Czech Republic;* [c]*1st Faculty of Medicine, Charles University, Prague, Czech Republic;* [d]*Hospital Ceske Budejovice a.s., Ceske Budejovice, Czech Republic;* [e]*Institute of Clinical and Experimental Medicine, Prague, Czech Republic*

Motivation of healthy volunteers for participation in medical research includes purely scientific interest, a wish to contribute to the further development of the drug, knowing somebody with the condition who may ultimately benefit from the drug under research, curiosity, new experiences and indirect seeking of psychiatric help, etc. However, the primary incentive for the majority of volunteers is financial benefit (Price, 2001; Tishler *et al.*, 2002). Financial benefit also entices people into the clinical studies also persons who do not suit inclusion/exclusion criteria. Our aim was to analyse: 1. reasons for non-inclusion of volunteers into the clinical pharmacokinetic bioequivalence (BE) studies; 2. reasons for the withdrawal of already included healthy volunteers from such studies. The last 10 BE studies organised by our institute were analysed retrospectively. The inclusion criteria in these studies were: 18–55 years of age, both sex, body mass index (BMI) 19.0–24.9 kg/m^2, informed consent giving. The standard exclusion criteria were: pregnancy, lactation, liver, renal and/or haematological abnormalities, abnormalities in serum glucose, total cholesterol and/or minerals, enzyme-inductors using, smoking more than 9 cigarettes/day, presence of addicting agents in urine. 365 persons participated in screening visits; 55 of them (15%) were excluded. The reasons for non-inclusion were: abnormalities in liver tests (47.1%), presence of addicting agents in urine (12.9%), hypercholesterolemia (10.0%), abnormalities in haematological tests (7.1%), high BMI (5.7%), abnormalities in renal tests (4.3%) and other reasons (abnormalities in serum minerals, positive pregnancy test, difficult co-operation, smoking, enzyme-inductors using). Of 310 healthy volunteers included into the BE studies, 18 (6%) were withdrawn during studies. The reasons for withdrawal were: intercurrent illness, injury and/or drug use (55.6%), informed consent withdrawal (22.2%), adverse drug reaction (16.7%), non-compliance (5.6%). The results show that the main reason for the volunteers non-inclusion into the BE studies are abnormalities in liver tests and surprisingly also addicting agents in urine (in spite of the fact that volunteers were informed about testing in advance). The number of volunteers withdrawn in the course of the BE studies was neglectable.

References:

Price P. GCP J. 2001; 8(8): 20–25.

Tishler CL *et al.* J Clin Pharmacol. 2002; 42: 365–375.

P204

Effects of iron chelators on acute isoprenaline cardiotoxicity

R Hrdina, P Mladenka, L Zatloukalova, Z Bobrovova *Charles University in Prague, Faculty of Pharmacy, Hradec Kralove, Czech Republic*

Many heart diseases including coronary heart disease are accompanied by elevated levels of catecholamines (Singal *et al.*, 1998). Their oxidative metabolites mediate a production of free radicals, which are postulated to be biochemical initiators of cardiotoxicity (Behonick *et al.*, 2001). We used a model of acute myocardial damage induced by an administration of a synthetic catecholamine – isoprenaline (ISO) – in a dose of 100 mg/kg s.c. to test several iron chelators for their potential

© 2008 The Authors

cardioprotective effects. Total 112 male Wistar:Han rats weighing approximately 300 g were divided into 12 groups receiving iron chelators as follows: deferoxamine (DEF), 2-pyridylcarboxaldehyde-2-thiophenecarboxyl hydrazone (PCTH) and rutin (RU) in equimolar doses (50; 20.4 and 46 mg/kg i.v., respectively) and 50 mg/kg i.v. of lactoferrin (LA). 5 min later, ISO was administered to the half of animals. Control (C) groups received vehicle (saline for comparison of all agents except PCTH, where 20% propylene glycol was administered). 24 h following drug treatment haemodynamic variables, myocardial calcium content and serum cardiac troponin T (cTnT) were determined. ISO alone caused 30% mortality, PCTH prevented it, while DEF or LA had no effect, RU increased mortality to 53%. ISO increased heart rate (HR), mean blood pressure (MBP), myocardial calcium content and cTnT, while decreased stroke volume index (SVI). The mortality data correspond to the observed changes in serum cTnT and partly to the changes in myocardial calcium content caused by tested chelators. SVI was significantly increased by administration of RU and LA alone. The tested drugs did not consistently influenced increase in BP and increase in heart rate caused by ISO.

In conclusion, preventive administration of PCTH at the dose used in this study partly prevented ISO cardiotoxicity and this observation deserves further studies.

This work was supported by a grant of Charles University in Prague 39207/C/FaF.

References:

Behonick *et al*. J Appl Toxicol. 2001; 21: S15–S22.
Singal *et al*. Cardiovasc Res. 1998; 40: 426–32.

© 2008 The Authors