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

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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 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 CP95 and Fas-associated 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.

References:

Polypeptide from Chlamys farreri protect murine thymocytes from ultraviolet ray damage through mitochondrial pathway.

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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 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 CP95 and Fas-associated 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.

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knockdown by siRNA decreased the calcium transient by 66–70% compared to the response of cells transfected with a control siRNA, indicating that Galpha q 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 Go and Galpha q. The cell biology group from University of Extremadura, C面向ceres, Spain; "Department of Physiology, University of Extremadura, C面向ceres, Spain; "Oral Biology Research Group, Institute of Dentistry, University of Lisbon, Lisboa, Portugal; "School of Forensic and Investigative Science, University of Central Lancaster, 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 (H2O2) on carbobol (CCH)-evoked amylase secretion, cytosolic free calcium levels ([Ca2+]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, [Ca2+]i and caspase-3 activity in the isolated rat parotid gland were measured using fluorimetric methods. H2O2 had little or no effect on amylase secretion in the isolated parotid gland tissue. A similar response was obtained with betulinic acid, a substance with antioxidant properties. Flow cytometry was employed to access cell surface expression and to assess the degree of receptor internalisation following agonist challenge with the PAR1 agonists thrombin and the selective PAR1-activating peptide (PAR1-AP) TFFL-R-NH2. Elevations in intracellular calcium were significantly increased when compared to that of wt-hPAR1, hPAR1C387A and hPAR1C388A displayed similar cell surface expression (~80%) to that of wt-hPAR1, whilst hPAR1C387AC388A showed a reduced (~40%) cell surface expression compared to that of wt-hPAR1, hPAR1C387A and hPAR1C388A displayed similar sensitivity to the PAR1 agonists thrombin and TFFL-NH2, except for wt-hPAR1, which only internalised in response to thrombin. Thus, hPAR1C387AC388A, unlike PAR1 agonists, regulate receptor expression and are critical for receptor signalling to calcium.

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

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Non-small cell lung cancer (NSCLC) is characterized by severe resistance to chemotherapy. Here, A549 adenocarcinoma cells permanently differentiate with the antimitobolite methotrexate (MTX) when blocking the antitumoral resistance mechanism normally counteracting this process. We demonstrate that MTX treatment induces a transient increase in 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 multiamphobal bodies and intracellular cytoplasmatic reorganization, modulation of molecular markers occurred in a manner consistent with differentiation (gelosin +100%; surfacetant-protein-A and -C60%). Similar to U0126, c-Myc inactivation with specific siRNA initiated differentiation only in the presence of MTX, demonstrating that K+B, calcium mobilization 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 provides a novel molecular basis for therapeutic strategies based on irreversible differentiation of cancer cells using conventional chemotherapeutic antimitobolites 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 PtdIns5P

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The phosphoinositides are a group of lipids that play multiple essential roles in cellular regulation, through their interactions with specific binding proteins. One member of this family, phosphatidylinositol 5-monophosphate (PtdIns5P), is found ubiquitously in higher eukaryotes, and has been suggested to play roles in cellular responses to stress, and in cellular activation by agonists insulin and thrombin. However, the molecular mechanisms governing the regulation of PtdIns5P synthesis are not fully understood: in particular, a number of alternative mechanisms of PtdIns5P synthesis have been postulated. To obtain more information about PtdIns5P regulation, we have used RNA interference to suppress expression of known PtdIns5P synthesis enzymes, and examined the effects of this knockdown on the production of PtdIns5P in cells exposed to the phosphotyrosine phosphatase inhibitor pervanadate, which provokes robust increases in intracellular PtdIns5P production. We employed a reverse mode of the myotubularin family markedly attenuates the response. Moreover, the effects of knockdown of PIP4Ks, lipid kinases that remove PtdIns5P by phosphorylation, show that the cytoplastic PIP4Kb is required for irreversibly regulating pervanadate-stimulated PtdIns5P production, whereas the nuclear PIP4K2 beta is not. Our results are consistent with the ability of pervanadate-stimulated tyrosine kinase signalling to generate PtdIns5P outside the nucleus, via

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Putative palmitoylation sites in human protease-activated receptor-1 (hPAR1) are critical for receptor signalling to calcium

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Protease-activated receptors (PARs) are novel family of G-protein coupled receptors (GPRs) that possess a tethered-ligand buried within the receptor N-terminus. Activation of PAR1, PAR2, and PAR4 occurs through the proteolytic exposure of the tethered-ligand, activating the GPR function. Palmitoylation is a post-translational modification that results in the addition of fatty acids to cysteine residues in the C-terminus of some GPRs. Although recent evidence has suggested that palmitoylation can have a critical role in the GPR function, the role of palmitoylation in regulating PAR function is currently unknown. Thus in our work we were testing that palmitoylation sites play an role in PAR function. With this knowledge we used siRNA on the hCRF 1- and hCRF2 receptor-induced calcium signalling. Supported by Johnson & Johnson.

Reference

a mechanism involving myotubularins. Thus, it will be important when investigating the possible roles of PtdIns5P in receptor-activated signal transduction to determine the subcellular location of the PtdIns5P involved.

P168

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

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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 adenyl cyclase-activating peptide (PACAP) activate Family B GPCRs known as PAC1, VPAC1 and VPAC2 to variously activate Gq/11, Gs and/or G/i/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 (Ca2+) 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 Ca2+ 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**

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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/cm² 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.
P170
Changing trend in the use of antibiotics over 10 years in a tertiary care hospital
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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
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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.
P172 Relaxant effect of Pycnocycla spinosa seed extract on rat uterus contraction
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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 uteri were removed and secured in Tyrode’s solution in an organ bath at 37°C and gassed with O2. 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 measured 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 µmol) 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:

P173 The protective effect of Nigella sativa oil in the brain of the biliary obstructed rats
H Toklu, O Schiril, 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 extravasation 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 antioxidant 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
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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 WT s and 47 ± 2% in KOs). In WT mice, norBUP was efficacious in arresting transit (only 13–16%) with doses (0.50–2 mg/kg) close to the antinociceptive ED50 value (0.66 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).
Identification of novel CAR agonists by using two-step virtual screening together with in vitro assays

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Constitutive androstanone receptor (CAR; NR1L3) recognizes several endogenous and xenobiotic 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 that activate CAR via binding to the LBP are not 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 with the functional assay. 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 characterized 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 azolones and substituted diazonium salts. Further studies are required to confirm the high activity 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.

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

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Constitutive androstanone receptor (CAR; NR1L3) recognizes several endogenous and xenobiotic 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 that activate CAR via binding to the LBP are not 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 with the functional assay. 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 characterized 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 azolones and substituted diazonium salts. Further studies are required to confirm the high activity 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.
P181
Acute and chronic imipramine increases 5-HT_{1A} receptors maximal density in heart atria of the rats

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The antidepressant drugs modulate central and peripheral serotonin 5-HT_{1A} receptor function. This receptor plays a crucial role in the cardiovascular system, where it participates in the stimulation of stable heart rate. The aim of this work was to study the effect of chronic imipramine (IMIc) treatment on the density of 5-HT_{1A} receptors in heart atria of the rats. 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 antidepressant effect; and the open-field test and the Rotarod test were done to measure the locomotor activity. The 5-HT_{1A} receptors were characterized with [3H]-DPAT (0.01–10 nM) saturation experiment in heart atria membrane homogenates using 5-HT_{1A} μM to measure the non specific binding. Only IMIc showed an increase in heart atria receptors density 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, 6.44 ± 2.09 fmol/mg of protein, and IMIc 6.44 ± 2.09 fmol/mg 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:

P182
Inhibitory effects of ATP on pannexin-1 currents

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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 any purinergic receptor function or ATP receptor function. 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 hemipentameric blocked by 30 μM carbeneoxolone (CBX). The current was not affected by lantathannum (2 μM), 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 6.30 μM. Similar inhibitory effects on panx1 currents were also observed with UTP and GTP, the IC_{50}s of which were 1.20 μM and 11.22 μM, respectively. Diphosphate (DPO) did not inhibit panx1 currents on either 0.1 or 5 mM while ADP and AMP only minimally inhibited panx1 currents. Nucleotide 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

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D_{2}-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 92914, SKF 19-191, SKF83526 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_{2} 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 performed on male Sprague-Dawley rats, divided into 6 groups with 10 animals each, treated intraperitoneally with saline (SAL) (0.5 ml/kg/day), acute imipramine (IMIa) 10 mg/kgbw displayed significant antinociceptive effect in both cutaneous and visceral pain models. Metoclopramide 10 mg/kgbw did not show any antinociceptive effect in both cutaneous and visceral pain models. The antinociceptive response of selective opioid agonists was also studied in combination with sedative dopamine receptor antagonists and antagonists. It is concluded that D_{2} receptor antagonists 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

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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 vivo and in vivo binding assays for rat NET, using [3H]-methylreboxetine (MRB), a potent and selective NET inhibitor (Ding et al. 2001). Saturation and kinetic experiments were performed to define the conditions for [3H]-MRB binding to rat NET (Sprague-Dawley) cortical membranes. Binding affinities (Ki) of test compounds (reboxetine (RBX), aprotinin (APN), metoclopramide (MDI), alomoxetine (ATX), NSX, and idoreboxetine (INER)) were determined in competition assays with [3H]-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 [3H]-MDI (20 μCi/rat) via a tail vein and after a further 30 min kill 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, nsx) values. As expected, the test compounds displayed high affinity for rat NET with Ki values for MRB, INER, NSX, ATX, DMI and NSX of 0.08 ± 0.02, 0.10 ± 0.02, 0.10 ± 0.03, 0.10 ± 0.03, 0.10 ± 0.02 and 0.10 ± 0.02 μM, respectively. Furthermore, they all inhibited in vivo [3H]-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 0.08 ± 0.02, 0.08 ± 0.02, 0.08 ± 0.02, 0.08 ± 0.02, 0.08 ± 0.02 and 0.08 ± 0.02 μM, respectively. For INER and RBX there was >10-fold difference between Ki and occupancy EC_{50}. These findings demonstrate that [3H]-MRB is a useful radioligand for NET and that binding data in vivo can be translated to in vitro occupancy data in the mouse tail immersion test.

References:

P185
Intranasal growth factor receptors in the nucleus. Agonist-stimulated appearance of phosphorylated receptor in the hepatocyte nucleus

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Our previous studies have shown that when different growth factor receptors (IGFR) can be found in the nucleus of hepatocytes: this nuclear localisation increases following partial hepatectomy, perhaps functioning as a transcription factor promoting cell proliferation (Martí and Boulet, 2003; Hug, 1995; Lin et al., 2001). We have shown (Luo et al., 2000; 2001) that phosphorylated IGFRs (pIGFR) can be found in the nucleus of hepatocytes; this nuclear localisation increases following partial hepatectomy, perhaps functioning as a transcription factor promoting cell proliferation (Martí and Boulet, 2003; Hug, 1995; Lin et al., 2001). We have shown (Luo et al., 2000; 2001) that phosphorylated IGFRs (pIGFR) can be found in the nucleus of hepatocytes. Our recent work has shown that following partial hepatectomy (PH), the pIGFR localized in the nucleus increased. In order to determine whether the increase in pIGFR was caused by an increase in the number of receptors in the nucleus. Hepatocytes were prepared from Wistar male rats (250–350 g). BGF (3 μM) was added after 24 h in culture and [3H]hymidamine incorporation (3 μM) was measured for 0.18, 0.36, 1, 3.6 and 12 h. The IC_{50} for PH was 48 h; the IC_{50} for BGF was 48 h. The IC_{50} for BGF was 48 h. The IC_{50} for BGF was 48 h. The IC_{50} for BGF was 48 h. The IC_{50} for BGF was 48 h.

References:
Ding et al., 2003; 15: 345–352.

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incorporation into DNA we stimulated cells in the presence of either AG 1478. AG 1/2 dose was not effective (ErbB3, ErbB2 or non-selective). Fifteen years ago, it was shown that the effects of alcuronium on rates of [3H]NMS binding site (Proska et al., 1995). It has recently 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 simple two state model (TCC) 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 adrenaline, 4-DAMP, diphenylhydramine, diphenylpyraline, hyosciamine, N-piperidyl benzilate, tropocaine, scopoline 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, anatruoxonium, truxil- lonium and dazioxanium, 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 MSM6407702002.

P187 DRD4 gene expression after long-term lithium treatment

L Carbonell, M. Cuffi 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, modulates the gene expression of some receptors coupled to adenyl cyclase (AC) system, such as mu-opioid receptor (de Gandarillas et al., 2000) and 2-adrenoceptor (Cuffi 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 killed and the whole brain was extracted using the SepMan Reverse Transcription kit. DRD4 expression levels were measured by Quantitative RT-PCR. ANOVA analysis showed a difference of D4 dopamine receptor mRNA levels among days (F_1,15 = 5.3; P = 0.032), but no changes were detected between treatments (F_5 = 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_15,15 = 3.197; 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:
P186 Endosomal signalling from internalised epidermal growth factor receptors in hepatocytes

Y Luu, M Boarier 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 the endosomal epidermal growth factor (EGF) receptors (EGFR). Here we study extracellular signal-related kinase (ERK) signalling from endosomal EGF-EGFR complexes in hepatocytes. [3H]tyramine incorporation into DNA of rat (male Wistar ~300 g) hepatocytes was with a 2-pulse stimulation procedure: EGF (3 nM) stimulated appearance of P-EGFR within the nucleus was blocked by AG 1478. When imaging with the anti-P-EGFR the receptor was visualised within the nucleus. This EGF-EGFR internalise, they cannot be seen within the nucleus. However, when imaging with Alexa 488-EGF and confocal visualisation with the EGFR shows that while EGF and EGFR internalisation, these results show EGF can stimulate appearance of P-EGFR uncoupled from EGFR ligand in the nucleus of rat hepatocytes.

References:
P189 Noncompetitive interactions at muscarinic receptors within a simple two state complex model

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 receptors. M2 receptor 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 episode. 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.

References:
Lipnicki 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 killed and the whole brain was extracted using the SepMan Reverse Transcription kit. DRD4 expression levels were measured by Quantitative RT-PCR. ANOVA analysis showed a difference of D4 dopamine receptor mRNA levels among days (F_1,15 = 5.3; P = 0.032), but no changes were detected between treatments (F_5 = 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_15,15 = 3.197; 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.

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P187 DRD4 gene expression after long-term lithium treatment

L Carbonell, M. Cuffi 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, modulates the gene expression of some receptors coupled to adenyl cyclase (AC) system, such as mu-opioid receptor (de Gandarillas et al., 2000) and 2-adrenoceptor (Cuffi 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 killed and the whole brain was extracted using the SepMan Reverse Transcription kit. DRD4 expression levels were measured by Quantitative RT-PCR. ANOVA analysis showed a difference of D4 dopamine receptor mRNA levels among days (F_1,15 = 5.3; P = 0.032), but no changes were detected between treatments (F_5 = 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_15,15 = 3.197; 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:
P190 Experimentally-induced diabetes alters the levels of aromatase both in peripheral and central nervous systems
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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 (Pella et al., 2002). Estradiol reduces the risk of Alzheimer’s disease and prevent neuronal loss in several experimental models of neurodegeneration (Garcia-Segura et al., 2001). Aromatase catalyses the conversion of androgens to estrogens and expressed in a variety of tissues including neurons. Estrogens have anti-inflammatory effects and are thought to protect neurons. Insulin is known to stimulate the activity of aromatase (Garno et al., 1984). This study was designed to investigate the effects of experimentally diabetes on aromatase expression in nervous system. Gender-related 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-week sciatic nerve and hippocampal 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 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 diabetes 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:06D05/301001.


P191 Enzymatic activities of monoamine oxidase (type A and B) and semicarbazide-sensitive amine oxidase (SSAO) in colonic arteries of type 2 diabetic patients
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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 radioactive method was used to measure the enzymatic activities. The study was performed in 20 type 2 diabetic patients: 10 males and 10 females and 20 age- and sex-matched control subjects. MAO activity was studied using [3H-5HT] (32–1000 nM) for MAO A; 14C-benzylamine hydrochloride [14C-BZ] (50–1200 nM) for MAO B and 14C-phenylethylamine hydrochloride [14C-PEA] (5–180 nM) for MAO A; and 14C-semlyemamine hydrochloride [14C-CBS] (50–1618 nM) for SSAO. The substrate concentrations for SSAO were those effective for the enzymes in question. The results of this study showed that type 2 diabetic patients have the greater activity in non-diabetic patients (Table 1). The endogenous NA content is higher when compared to the non-diabetic. Tissue-bond SSAO shows a statistically significant difference in the diabetic patients. The affinity of MAO B in diabetic patients is statistically significantly altered by any of the compounds in liver tissue. On the other hand, 2-OHSA even slightly increased body weight and adipose tissue weight and hepatic lipid metabolism in diet-induced obese mice.


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
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The enhanced formation of AGEs in diabetes play an important role in the development of diabetic complications. Administration of ACE-modified proteins to animals produce changes typical of early diabetes-like vascular dysfunction. Rosiglitazone (ros), is a member of PPAR gamma ligands, which are used as anti diabetic 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 ros treatment on aortic RAGE expression, male Wistar rats (180–200 g, n = 50) were given tail vein injections with either sterile nonmodified BSA (bovine serum albumin solution) or glycated BSA with advanced glycation formation (AGE-BSA) followed immediately by ros (8 mg/kg/day), for 10 days (preventive study). At the end of the treatments, expression of RAGE (by semiquantitative RT-PCR) in aorta (E- and E+Cys) and peripheral cytokines (TNF-alpha and IL-1beta) were measured. 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 for a fatty acid after trigger AGE expression in AGEs-induced vascular complications. Supported by Turkish Scientific Research Council (TUBITAK, SBAG-HD-183).

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. Vigil1, A. Lopez-Billan1, R. Alemany1, P. Escrigli, S. Tofole2, J. Quevedo3, S. Diaza, V. Perez4, 5
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Fatty acids are integral components of lipid metabolism being functionally involved in either substrates, intermediates or end products. Therefore, chemical modification of natural fatty acids is a rational strategy to generate novel compounds with potent antiobesity 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 (Vigor et al., 2008).

The present work analyses the relationship between chemical structure, antiobesity 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 fatty acids (linoleic acid (LA), 2-hydroxyacidic acid (2-OHAA)) fatty acids in an animal model of diet-induced obesity. Male, six week old C57BL/6 mice were fed a diet containing 40% of calories of mainly coconut fat (Research Diets RDO6 112701) for 12 weeks and then treated orally for 7 days with 375 mg/kg/12 h of the indicated fatty acids. Our results show that only 2-OHAA efficiently reduced body weight (~8.2%), food intake (~33.3%) and adipose tissue mass (~21.4%), whereas OA and SA did not alter these parameters. 2-OHAA even slightly increased body weight and adipose tissue mass. In parallel, quantitative real-time PCR revealed that none of the different carnitine palmitoyltransferase isoforms levels (CPT1a, 1b, and 2) were significantly altered by any of the compounds in liver tissue. On the other hand, 2-OHAA drastically reduced (~85%) transcription of hepatic stearoyl-CoA-desaturase (SCD1), whereas 2-OHAA and LA levels significantly altered by any of the compounds in liver tissue. The antiobesity actions are, at least partially, attributable to hepatic downregulation of SCD1, an important novel molecular target for antiobesity drugs.


P194 Changes in the diabetic brain rat NaK-ATPase and Mg2+-ATPase activities: in vivo and in vitro modulation by t-cysine
G. Zhao1,2, L. Haug1,2, M. Metivier3, Z. Sun3, N. Skandali3, H. Al-Humaid4, F. Amantantak5, E. Cikrouman6, T. Tkatsirs6
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Uncontrolled diabetes is known to affect the nervous system. The aim of this study was to investigate the effect of the antioxidant t-cystine (Cys) on the changes caused by adult-onset streptozotocin (STZ)-induced diabetes on the rat brain Na+, K+-ATPase and Mg2+-ATPase activities. Thirty-eight male Wistar rats were divided into 8-groups: C1 (8-week-control), C2 (8-week-saline-treated), C3 (8-week-diabetic), C4 (8-week-diabetic + 1-week-saline-treated), C5 (8-week-diabetic + 1-week-Cys-treated), D1 (8-week-diabetic + 1-week-Cys-treated) and D2 (8-week-diabetic + 1-week-Cys-treated). All diabetic rats were once treated with an ip. L-arginine injection (50 mg/kg body weight)


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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. Mg²⁺-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.

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DPYD is an enzyme that catalyzes the phosphorylation of tegafur (FT) to 5-fluorouracil (5-FU). Inhibition of DPYD activity results in accumulation of FT and increased toxicity. Despite the fact that DPYD activity is crucial for 5-FU metabolism, DPYD inhibitors and their effects on 5-FU-induced toxicity are not well understood.

The aim of this study was to determine the frequency of the DPYD mutation (IVS14 + 1G>A) in patients with breast and colorectal cancer treated with 5-fluorouracil-based chemotherapy. The results of this study will be compared with the results of a previous study performed in our laboratory on a different population of patients.


Department of Radiotherapy and Oncology, Kosice, Slovakia.

Oral lichen planus (OLP) is the second most frequent disease of oral mucosa with unclear etiology. 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 rates of adverse drug reactions. However, there is no evidence of lower enzyme activity in patients with OLP.

The aim of this study was to screen for the presence of the DPYD (IVS14 + 1G>A) mutation in patients with OLP and to compare it with the frequency of this mutation in the general population. The results of this study will be compared with the results of a previous study performed in our laboratory on a different population of patients.


The DPYD gene product P-glycoprotein (Pgp) represents the most widely studied mammalian ABC transporter family, that increases toxic drug efflux of a number of chemotherapeutics. The main substrates for Pgp includes anthracyclines, vincs alkalds, tuxanes, inhibitors of topoisomerases (1, 11), mitomycins, etoposide and doxorubicin. The C343ST polymorphism in exon 26 is the most important DPYD gene polymorphism. Although it is silent mutation, this polymorphism affects the expression and function of the Pgp in many ways. However, only the C allele is associated with increased Pgp expression and may play an important role in variability of treatment efficacy. The aim of this study was to examine the DPYD C343ST 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 DPYD C343ST polymorphism. DNA was extracted from peripheral blood samples and analysed using restriction fragment length polymorphism (RFLP) analysis. The statistical analysis was performed using Kaplan-Meier curves and Cox regression analysis.
Warfarin is an oral anticoagulant extensively used in clinical practice. The available pH 7.4, in a 0.4–22.7 \( \text{nmol/mL} \) or suppository formulations. Differences in pharmacokinetic parameters of different suppository formulations.

Suppositories were inserted into the rectum. Samples were taken at 0-0.5-1-2-3-4-6 h via the cannulated vessel cannulated via major auricular blood vessel, then the suppository was inserted mesenterica inferior and the distal two thirds via vena iliaca interna). 2. The comparable with that in humans (the upper third of rectum drained via vena cava but only 24–60 h after administration). The administration. Plasma concentrations were determined by gas chromatography. The suitability of rabbits in pharmacokinetic preclinical testing of rectal suppositories.

The study: 1. The verification of rabbit rectal blood supply was done under general standard conditions, fasted 24 h before the experiment, were used. The design of comparative pharmacokinetic testing of rectal formulations during preclinical data collection. 2. The 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 racemic racemate and enantiomers) proved to be a concentration-dependent nonlinear process, as confirmed by the binding of racemic warfarin and both isomers revealed high binding percentages, confirming strong affinity to human plasma proteins, as claimed in several studies. Although concentration-dependent, it becomes obvious that the R- and S-warfarin present a similar degree of binding and their pharmacologically active free fractions are identical (especially considering the usual warfarin therapeutic range).

Table 1. Pharmacokinetic parameters of nitrendipine in healthy volunteers.

Parameter AUC(0-5) (ng/h/mL) AUC(0-24h) (ng/h/mL) Cmax (ng/mL) Tmax (h) ke (h⁻¹) T1/2 (h)
Men
58.06 ± 4.10 62.16 ± 4.39 16.45 ± 9.58 2.00 ± 1.00 4.00 ± 1.62 0.095 ± 0.101 6.11 ± 4.04 1.08 ± 0.86
Women
60.18 ± 2.91 63.59 ± 1.78 22.22 ± 1.44 21.25 (0.67 - 6.00) 0.166 ± 0.101 6.70 ± 5.01 6.25 ± 5.01

R-warfarin
S-warfarin
Racemic warfarin

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

Reference:

The aims 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 ilica 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 cava but only 24–60 h after administration). The administration.

The pharmacokinetics of different 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 0.5-1-2-3-4-6 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:

Table 1. Pharmacokinetic parameters of nitrendipine in healthy volunteers.

Table 2. Bioequivalence studies: What are the reasons for non-inclusion of healthy volunteers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC(0-5) (ng/h/mL)</th>
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<tbody>
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<td>22.22 ± 1.44</td>
<td>21.25 (0.67 - 6.00)</td>
<td>0.166 ± 0.101</td>
<td>6.70 ± 5.01</td>
</tr>
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R-warfarin
S-warfarin
Racemic warfarin

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

Reference:

The aims 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 ilica 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 cava but only 24–60 h after administration). The administration.

The pharmacokinetics of different 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 0.5-1-2-3-4-6 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:

Table 1. Pharmacokinetic parameters of nitrendipine in healthy volunteers.

Parameter AUC(0-5) (ng/h/mL) AUC(0-24h) (ng/h/mL) Cmax (ng/mL) Tmax (h) ke (h⁻¹) T1/2 (h)
Men
58.06 ± 4.10 62.16 ± 4.39 16.45 ± 9.58 2.00 ± 1.00 4.00 ± 1.62 0.095 ± 0.101 6.11 ± 4.04 1.08 ± 0.86
Women
60.18 ± 2.91 63.59 ± 1.78 22.22 ± 1.44 21.25 (0.67 - 6.00) 0.166 ± 0.101 6.70 ± 5.01 6.25 ± 5.01

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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-thiophencarboxyl 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.

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References: