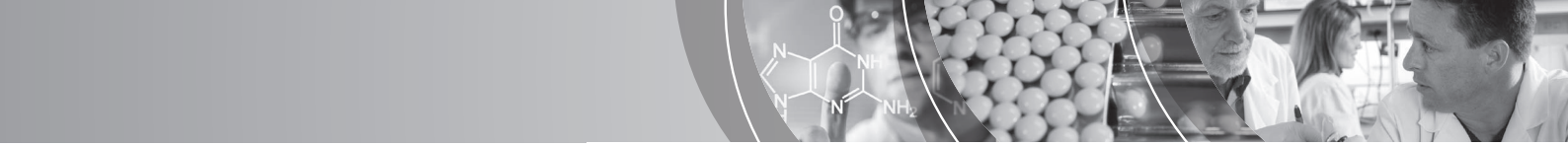

102

We are all in this together! Paths to better collaboration for drug discovery and development

Dr Patrick Smith, Chief Scientific Officer, Dr Craig Rayner, CEO, d3 Medicine

Academia, industry, not-for-profit, government, regulators and the life-science investment community each have critical roles in the discovery, development and approval of new medicines. However, each stakeholders' needs and viewpoints differ and may frustrate collaborations and interactions. By better understanding each other's perspectives, more effective cross-sector collaboration is achievable. This in turn provides great opportunity for more efficient, cost-effective and innovative delivery of medicines to the community.

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Alzheimer's disease and Parkinson's disease: the search for a cure

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Alzheimer's disease (AD) and Parkinson's disease (PD) are incurable and common neurodegenerative diseases that complicate aging. Experimental therapeutic approaches that focus on the protein aggregates that typify these disorders have been disappointing in clinical trials, suggesting that the neurodegeneration is not merely due to proteinopathy. In both diseases, there is a severe dysregulation of metal homeostasis in affected brain tissue, with iron and zinc elevation and copper depletion reported in cortex (AD) and nigra (PD). This is important because iron accumulation in particular causes oxidative damage, which characterizes both diseases. We have determined that the major proteins implicated the disorders have important functions in metal transport, and may be components of a novel metal regulatory system that fails in aging. The amyloid protein precursor facilitates the export of iron from neurons and prevents dietary iron overload from collecting within the brain. Presenilins foster the uptake of copper and zinc, and tau impacts on iron export by trafficking APP to the cell surface. Failure of the metal transport functions of these proteins may explain why metals collect within the protein aggregates that typify these disorders, and may contribute to neuronal dysfunction. Small molecules that correct the abnormalities of metal transport have been effective in animal models of these diseases, and are being tested in clinical trials.

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Neuropeptides and reward-seeking

Andrew J Lawrence, Shaun Y Khoo, Andrezza Kim, Bianca Jupp, Jee H Kim and Robyn M Brown. Florey Institute of Neuroscience & Mental Health, University of Melbourne, VIC.

Introduction. We were the first to demonstrate a role for orexins in both ethanol consumption and cue-induced reinstatement of ethanol-seeking. These effects were specific, with a differential effect of orexin1 receptor (OX1R) antagonism on the motivational strength of ethanol compared to sucrose. Fos studies suggested the prelimbic cortex as a potential locus where ascending orexinergic input could modulate relapse-like behaviour. While a role for OX1R is established in both ethanol reinforcement and ethanol-seeking behaviour, the role of orexin2 receptors (OX2R) in these behaviours is less clear.
Aims. We tested the role of prefrontal cortical OX1R in cue-induced ethanol-seeking and sought to determine whether central OX2R modulate ethanol-seeking behaviour.
Methods. iP rats were trained to self-administer ethanol (10% v/v) or sucrose (0.7-1% w/v) and then implanted with indwelling intracerebral guide cannulae.
Results. Antagonism of prelimbic OX1R with SB-334867 (3µg/side; 300nl/side) significantly attenuated cue-induced reinstatement of ethanol-seeking in an anatomically specific manner, with no effect on sucrose-seeking. Icv injection of the OX2R antagonist TCS-OX2-29 reduced self-administration of ethanol, but not sucrose. Despite reducing ethanol self-administration, TCS-OX2-29 had no impact on cue-induced reinstatement of ethanol-seeking. To determine where in the brain OX2R were acting to modulate ethanol self-administration, TCS-OX2-29 was microinjected into either the shell or core of the nucleus accumbens (NAc). Intra-NAc core, but not shell, infusions of TCS-OX2-29 decreased ethanol responding.
Discussion. OX2R in addition to OX1R may represent a potential therapeutic target for the treatment of alcohol use disorders. However, unlike OX1R, no impact of OX2R antagonism was observed on cue-induced reinstatement, suggesting a more prominent role for OX2R in ethanol consumption compared to relapse.

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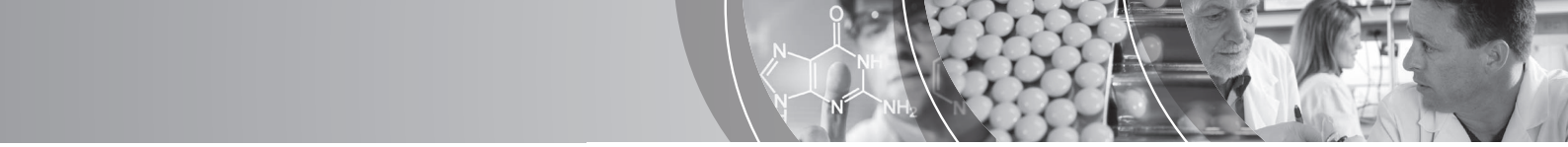
Targeting the P2X7R in the Search for New Antidepressants

Michael Kassiou. School of Chemistry and Brain and Mind Research Institute Faculty of Health Sciences, The University of Sydney, NSW.

Depression is the most common mental illness yet treatment of depression is dominated by drugs that were developed decades ago. Current classes of medication for depression include the serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs) and irreversible monoamine oxidase inhibitors (MAOIs). While use of these drugs has skyrocketed, questions surrounding their efficacy and side effects have intensified. All currently prescribed antidepressant drugs have notable limitations in efficacy and tolerability as well as a slow onset of action and a risk of serious withdrawal symptoms upon discontinuation.
Over the last two decades there has been increasing evidence of a strong relationship between depression and immunological dysfunction in depressed patients that has led to an increasingly influential “cytokine hypothesis” of depression (Felger et al.). Patients with depression have higher than normal levels of inflammatory cytokines including prostaglandin E2 (PGE2), IL-1, IL-2, IL-6, and IFN-gamma. Conversely, patients who are treated with cytokines (e.g. IFN-alpha), such as those with hepatitis, are highly prone to develop depression and are now routinely co-treated with antidepressants to prevent this.
Purinergic P2X7 receptors (P2X7R) are an unusual non-desensitising, cation-selective ion channel directly gated by extracellular ATP. They play an important role in regulating the release of cytokines which makes them an attractive target for antidepressant drug discovery. This presentation will describe our current efforts towards the design of P2X7R ligands as novel antidepressants.

Felger J et al (2013) Neuroscience 246: 199-229.



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Novel approaches for treating anxiety
Dr Deborah Rathgen, Bionomics Ltd

Abstract unavailable at time of printing

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Translating Pharmacogenomic Findings to the Clinic: Challenges and Opportunities
Justin P Rubio, Genetics, GlaxoSmithKline, Stevenage, Hertfordshire, UK

Pharmacogenomics (PGx), the study of variations of DNA and RNA characteristics in relation to drug response, is an established and successful field of research. In recent years, the scope of PGx for explaining variability in medicine response has expanded beyond the study of ADME genes and pharmacokinetics, to also include the identification and characterisation of variation in genes influencing pharmacodynamic endpoints - informing a drug's safety and efficacy.

As of October 2013, the Food and Drug Administration (USA) had recognised 121 PGx biomarkers through their inclusion in drug labels for indications across multiple therapy areas. However, despite the fact that pharmaceutical regulatory agencies cite PGx as an important tool in both drug development and post-marketing pharmacovigilance, PGx testing is not routinely used to inform treatment decisions in the clinic. Here, an industry perspective will be provided of the utility of PGx during drug development and factors that are challenging its clinical translation. Opportunities for enhancing the use of PGx in clinical decision-making will also be discussed.

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Role of basic science and impact of population ethnicity on application of tests

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Variation in rates of drug uptake into cells and drug elimination underlies many individual differences in drug efficacy and toxicity. There is now considerable evidence that pharmacogenomic variation in drug metabolizing enzymes and transporters are major determinants of such differences. The anticancer agent irinotecan is activated to its cytotoxic metabolite SN-38 that is subsequently deactivated by UGT1A1; inactive variant alleles of UGT1A1 are associated with increased incidence of neutropenia and diarrhoea. Similarly, allelic variants of CYP2D6 and CYP2C19 are unable to efficiently activate the prodrugs tamoxifen and clopidogrel, respectively, leading to therapeutic failure. Certain other adverse drug reactions are associated with variant alleles of the Human Leukocyte Antigen (HLA) system. Thus, drug hypersensitivities mediated by abacavir and carbamazepine have been attributed to the HLA-B variants *5701 and *1502, respectively. Because the frequencies of drug disposition and HLA alleles differ between ethnic populations this may contribute to ethnicity-related differences in drug toxicity and therapeutic failure. Furthermore, significant differences within Asian populations in the incidence of certain CYP and transporter gene variants may lead to variable therapeutic outcomes. In recent years the US FDA has made several recommendations based on pharmacogenetic factors as determinants of efficacy and toxicity. Label changes for irinotecan, tamoxifen, warfarin and 6-mercaptopurine have been introduced, which alert clinicians to possible toxicity and impaired efficacy in individuals who carry particular allelic variants. Quite recently, a similar warning has been made that recommends screening new patients of Asian ancestry for the HLA-B*1502 allele before commencing carbamazepine therapy. Further clinical validation is now required for the derivation of dose modification algorithms based on genetic variation. This information would enhance the wider application of pharmacogenetic testing, especially in susceptible ethnic groups.

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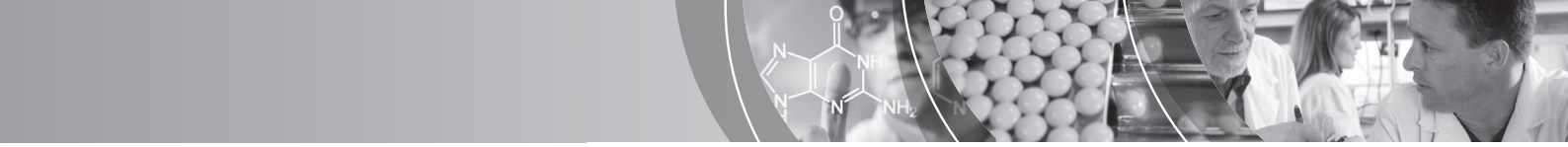
The challenges of making reimbursement decisions about personalised medicine

Prof Robyn L Ward. University of New South Wales and Prince of Wales Hospital

The Medical Services Advisory Committee and Pharmaceutical Benefits Advisory Committee provide advice to the Minister for Health and Ageing on the circumstances under which public funding for drugs, medical technologies or procedures should be supported. This advice is based on consideration of the best available evidence regarding the comparative safety, effectiveness and cost effectiveness of new medical services. This assessment must be coordinated when considering co-dependent technologies such as genetic testing and targeted drugs. For cancer medicines, new molecular tests are often retrospectively identified as potential predictive biomarkers. These markers are used to select individuals with a differential benefit to specific therapies. In the case of high cost therapies such as anti-EGFR antibodies, a predictive biomarker represents an opportunity to limit therapy to the subgroup of patients who are likely to benefit, thus improving the cost effectiveness of the drug. These and other examples (*HER-2* testing for breast and gastric cancer, *BRAF* for melanoma) demonstrate that the optimal clinical and economic performance of a proposed test and drug are often dependent upon each other. There is however a number of challenges associated with assessing co-dependent technologies for reimbursement purposes. These will be discussed in the context of assessing the benefits of personalized medicine.



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Pharmacogenomic testing for Australia: your way or our way or Highway 62: revisited

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Although health care systems differ substantially amongst developed countries, health professionals and regulatory bodies in The Netherlands, France and USA have promoted and instigated pharmacogenomic testing in a strategic and comprehensive manner. Barriers such as evidence base, regulatory framework, knowledge/education, funding, testing standardization/platform, and reporting seem not to be a major hurdle for these progressive countries, but not for Australia. Here, very little pharmacogenomic testing is being done and although with commendable intentions, it is somewhat ad hoc- the "your way" approach, often lacking rigorous evidence-based pharmacology interpretation. TPMT is a good example with genetic testing receiving Medicare rebate in mid 2011, but only 576 tests in the last 12 months have been claimed- although this is a likely underestimate. To date, over 1600 patients have been tested at SA Pathology in Adelaide with specific pharmacology guidance on interpretation provided but whether the prescriber instigated our recommendations or whether the advice was clinically helpful is unknown, due to the inability to readily access medical records. In mid 2011, the Commonwealth Department of Health and Ageing established a Genetics Working Party to, in essence, provide a framework for genetic testing and its funding for the future, which hopefully will include pharmacogenomic testing beyond the cancer arena. Although scheduled to report in early 2013, we await the draft report. It is in this light that ASCEPT met with RCPA in early 2013 to instigate a collaborative partnership to provide formal guidance on specific pharmacogenomic tests- the so called "our way" approach. Progress has been hampered by financial constraints, governance and the enormity of the endeavour- but RCPA/ASCEPT support is strong. So we have come some way since Werner Kalow published his landmark 1962 book "Pharmacogenetics: hereditary and the response to drugs". Pharmacogenomics is rooted in the traditions of the past.

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The effect of sildenafil on spontaneous contractions in human prostate tissue

Brad Wittmer[1,2], Melissa Papargiris[1,3], Mark Frydenberg[4,5], Jeremy Grummet[5], Ross Snow[5], John Pedersen[6], Tim Nottle[6], Andrew Ryan[6], Sam Norden[6], Basu Chakrabarty[2], Gail Risbridger[1], Betty Exintaris[2] Dept of Anatomy and Dev Bio, Monash Univ[1], Melbourne, VIC; DDB, MIPS[2], Melbourne, VIC; APCBR[3], Melbourne, VIC; Dept of Surg, Monash Univ[4], Melbourne, VIC; Aust Urology Associates[5], Melbourne, VIC; TissuPath[6], Melbourne, VIC

Introduction. Phosphodiesterase inhibitors are considered to be a potential new treatment option for benign prostatic hyperplasia (BPH). Of particular interest is sildenafil which has been used in human clinical trials to investigate its efficacy in treating BPH and shown significant improvement in patient international prostate symptom scores; however there is currently no proof of mechanism data.

Aims. To characterise the spontaneous contractions generated by human prostate tissue and investigate the effect of sildenafil on the spontaneous contractions of human prostate tissue.

Methods. This study was conducted on tissue obtained from the transition zone (TZ) of the prostate gland as this is the common site where BPH arises. Transition zone specimens were taken from whole prostates removed from patients undergoing radical prostatectomy. TZ specimens were then further dissected (5mm x 3mm) to be used in conventional tension recording experiments (Dey, et al, 2012) to test the effect of sildenafil.

Results. Spontaneous contractions developed in 100% of TZ prostate tissue preparations (n=4) with an average amplitude of 0.91±0.21 N/g and frequency of 2.80±0.80 contractions per minute.

Initial data has shown that sildenafil 10^{-6}M does not reduce the amplitude and frequency of spontaneous contractions of human tissue preparations. In contrast, sildenafil 10^{-5}M was able to reduce the amplitude and frequency of spontaneous contractions by approximately 30%.

Discussion. These findings are the first to show the effect of sildenafil against spontaneous contractions of the human prostate which is considered to be key in the dynamic component of BPH. Sildenafil 10^{-5}M reduces the amplitude and frequency of the human prostatic spontaneous contractions but not abolish them. This shows that Sildenafil provides an exciting new prospect in the treatment of lower urinary tract symptoms secondary to BPH.

Dey et al (2012) J Urol 187(6):2254-2260

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An aqueous-soluble polar bioactive is responsible for the inhibitory effect of stinging nettle leaf extract on the purinergic component of contractility in the rat prostate gland.

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Introduction. Stinging nettle (*Urtica dioica*) is commercially available for the treatment and symptomatic relief of benign prostatic hyperplasia (BPH). Although the root extract is commonly used to reduce prostate size, the leaf, a complex mixture of water and alcohol-soluble compounds, has been consumed in traditional medicine for the treatment of nocturia (Sezik et al, 2001), and has been shown to have both vasorelaxant properties in pigs (Long et al, 2009), as well as inhibitory effects on the contractility of the intestine of rats (Aviello et al, 2010) .
Aims. The study aimed to investigate the smooth muscle relaxant effects of stinging nettle leaf extract on prostate contractility, to determine the pharmacological mechanism of action, and to identify the responsible bioactive.
Method. Liquid-liquid partitioning, flash reversed-phase column chromatography and preparative HPLC were employed to separate and fractionate the stinging nettle leaf extract (500 mg/ml in 25% ethanol). Analytical HPLC, NMR and LC-MS were used to identify the bioactive.
Isolated organ bath studies were conducted to investigate the effect of the extract and fractions, on αβmethylene adenosine 5'-triphosphate (ATP) (3 nmol/L – 10 μmol/L) induced contraction of the isolated rat prostate gland.
Results. Attenuation of αβmethylene ATP induced contraction (n=6; $P<0.001$) of the isolated rat prostate gland was observed with the leaf extract, aqueous phase, and an isolated fraction. The bioactive was found to be aqueous-soluble and polar.
Discussion. Attenuation of αβmethylene ATP induced contraction implies that the extract either acts as an antagonist at the P2X1-purinoceptor or as inhibitor of the intracellular contractile mechanism activated by this ligand gated ion channel. This effect is due to an aqueous-soluble polar bioactive of the stinging nettle leaf extract.

Aviello G, Scalisi C, et al (2010) Eur J Pharmacol 640:163-167.
Long Y, Han M, et al (2009) Vascul Pharmacol 51:78-83.
Sezik E, Yesilada E, et al (2001) Journal of ethnopharmacology 75:95-115

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The effect of Botulinum toxin type A on release of substance P, ATP and acetylcholine in porcine bladder

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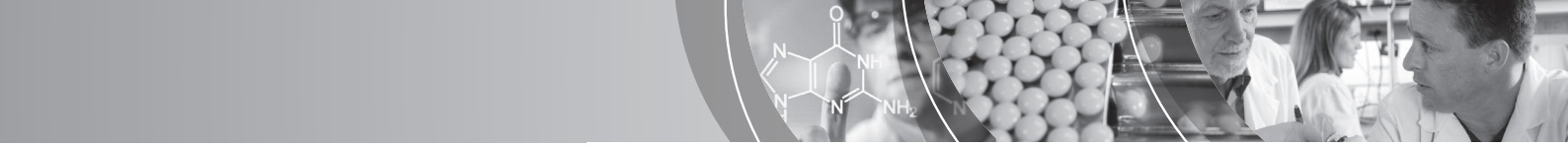
Introduction. Onabotulinum toxin A (formerly known as Botox) has recently been approved by the TGA for treatment of neurogenic overactive bladder. Botox inhibits cholinergic neurotransmission through the cleavage of neuronal SNARE proteins. However, its effect on the release of other neurotransmitters is much less studied.
Aims. To investigate the effect of Botox on electrical field stimulation (EFS)-induced release of SP, ATP and ACh, and to examine expression of the SNARE proteins in porcine bladder.
Methods. Bladder dome segments from female pigs were injected with Botox (1, 5 & 20U/ml) and incubated overnight. After 12h, longitudinal strips of mucosa and detrusor were electrically stimulated at 10Hz, 0.1ms, 100V. Bath fluid was collected for measuring ATP, SP and ACh release. Immunofluorescence staining was performed using anti-synaptosomal-associated protein-25 (SNAP-25) antibody. Gene expression of SNARE proteins was measured by real-time PCR.
Results. EFS-induced release of ATP and SP was higher in mucosa, compared with detrusor. Botox (5 and 20 U/ml) significantly decreased or abolished the release of SP and ACh. ATP release and contractile responses were significantly inhibited by Botox at all concentrations, in both tissue types. There was dense immunostaining of Botox target protein SNAP-25 on nerve endings, and also in urothelium and lamina propria. Botox treatment almost abolished SNAP-25 immunoreactivity. Gene expression for Botox target proteins: SNAP-25, vesicle associated membrane protein (VAMP) and synaptic vesicle proteins (SV2) B and C (but not SV2A), occurred in both mucosa and detrusor.
Discussion. The reduction in EFS-induced release of SP, ATP and ACh, as well as SNAP-25 positive cells, suggests that Botox affects afferent in addition to efferent signalling. Botox acts in the mucosa as well as in the detrusor. Lack of SV2A expression in porcine bladder indicates that Botox exerts its action via SV2B/C, a different mechanism from other species (Dong et al., 2006).

Dong M et al (2006) Science 312: 592-596



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Potential role for pannexin-2 in the enteric nervous system of the human colon

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Introduction. Pannexin-2 (Panx2) is a membrane-spanning protein channel composed of eight subunits, and may form heteromeric channels with Pannexin-1. Panx2 function has yet to be defined, but may have a role in the CNS regulating neuronal differentiation or contributing to ischemic stroke (Penuela et al, 2013). There are no previous studies that look at Panx2 in the human colon, particularly in the enteric nervous system (ENS), which contains chemically coded neuron populations similar to those found in the CNS.
Aims. This study was aimed to characterise and localise Panx2 expression in the human colon.
Methods. Panx2 gene expression was evaluated in the human colon by real-time PCR. Immunohistochemistry was used to localise Panx2 protein and compare this with the localisation of the neuronal-marker class III β-tubulin and the glial cell marker S100.
Results. Panx2 mRNA was expressed in the muscle and mucosa layers of both sigmoid and ascending colon. Expression was 4-fold higher in the muscle layers of ascending colon (n=14) compared to the sigmoid colon (n=17, fold change comparison, P=0.006, Mann-Whitney test). There was no regional difference in the mucosa layer. In ascending colon, Panx2 mRNA was 4-fold higher in muscle (n=14) than in mucosa (n=11, P=0.002). No difference was observed between muscle and mucosa of sigmoid colon. Fluorescent staining showed Panx2 expression on myenteric ganglia, submucosal ganglia and on some nerve fibres of the mucosa. The Avidin-Biotin Complex method of staining showed additional Panx2 expression on blood vessel smooth muscle and on some lamina propria leukocytes. Co-localisation occurred more frequently between Panx2 and β-tubulin compared with Panx2 and S100, indicating limited Panx2 expression on glial cells.
Discussion. Panx2 is expressed in all layers of the human colon. Co-localisation of Panx2 with β-tubulin on colonic ganglia suggests a role for Panx2 within the ENS, possibly in neuronal differentiation.

Penuela S et al (2013) BBA-BIOMEMBRANES 1828:15-22

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Establishing a refined model of the contribution of airway epithelial damage and fibrosis to the pathogenesis of asthma

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Introduction. Asthma develops from injury to the airways/lungs, and stems from airway inflammation (AI) and airway remodelling (AWR), both contributing to airway hyperresponsiveness (AHR). Airway epithelial damage has been identified as a new etiology of asthma but is not targeted by current treatments. Furthermore, it is not incorporated in currently-studied animal models of AI and AWR.
Aim. Therefore, this study aimed to incorporate epithelial damage/repair with the well-established allergic airway disease (AAD) model.
Methods. A 3-day naphthalene (NA) model of epithelial damage/repair was superimposed onto a 9-week ovalbumin (OVA) model of AAD (which undergoes AI, AWR and AHR), using 6-8 week-old female Balb/c mice (n=5-10/group). Mice subjected to the 9-week OVA model, 3-day NA model or respective vehicle-treatments (saline, corn oil) were used as appropriate controls. \
Results. OVA alone significantly increased epithelial thickness, goblet cell metaplasia, subepithelial collagen (assessed by morphometric analyses of various histological stains), total lung collagen (hydroxyproline analysis) and AHR (invasive plethysmography) (all $p<0.05$), compared to that in saline-treated mice. NA alone caused a significant increase in epithelial denudation, subepithelial and total lung collagen (all $p<0.01$) compared to respective measurements from corn oil-treated controls. Strikingly, the OVA+NA model demonstrated a further increase in subepithelial and total lung collagen (both $p<0.05$ vs OVA alone, NA alone), and a trend towards a further increase in AHR compared to OVA alone.
Discussion. We have produced a combined model of airway epithelial damage/repair and AAD, which demonstrates that epithelial damage leads to exacerbated AWR and AHR. Further investigation is ongoing to determine the molecular mechanisms involved.

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Evidence for endosomal ROS production in endothelial cells in response to Influenza A virus infection

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Introduction. Influenza A virus- (IAV) induced acute lung injury is characterised by excessive cytokine and ROS production. IAV enters cells via endocytosis and activates toll-like receptors (TLRs) in endosomes, triggering innate anti-viral cytokine production. Recently, endothelial cells have been implicated in regulating IAV-induced lung inflammation.
Aim. To determine whether IAV and TLR activation causes an increase in extracellular and endosomal ROS production in endothelial cells.
Method. Human microvascular endothelial cells (HMEC) were infected with IAV strain, X-31 (MOI 0.3-10) or treated with the TLR3 agonist, polyinosinic: polycytidylic acid (poly I:C) (1-100 μg/ml), or TLR7 agonist, imiquimod (0.3-10 μg/ml) for 1hr or 24hr. Triple labeling fluorescence microscopy was used to localize IAV (nucleoprotein antibody), early endosomes (EEA1 antibody) and nuclei (DAPI). Extracellular ROS production was measured with L-O12 (100 μM) chemiluminescence and endosomal ROS with fluorescence microscopy using the probe Oxyburst (50 μM). Nox2 and Nox4 mRNA expression was measured with QPCR.
Results. IAV entered HMEC by endocytosis, localized into early endosomes and caused an increase in endosomal ROS production. Neither X-31 infection nor imiquimod treatment influenced extracellular ROS production however, there was a trend for increased extracellular ROS production following poly I:C (100 μg/ml). Nox4 mRNA expression was significantly down-regulated 24 hours post X-31 infection. By contrast, there was a trend for Nox2 mRNA expression to be upregulated following X-31 infection.
Discussion. These findings demonstrate for the first time that IAV internalizes into early endosomes and triggers ROS production within these subcellular compartments. Unravelling endosomal ROS signalling in endothelial cells is likely to improve our understanding of IAV pathology.

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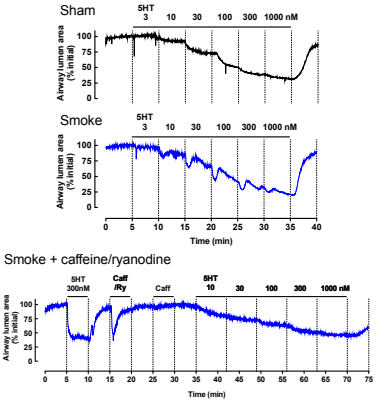
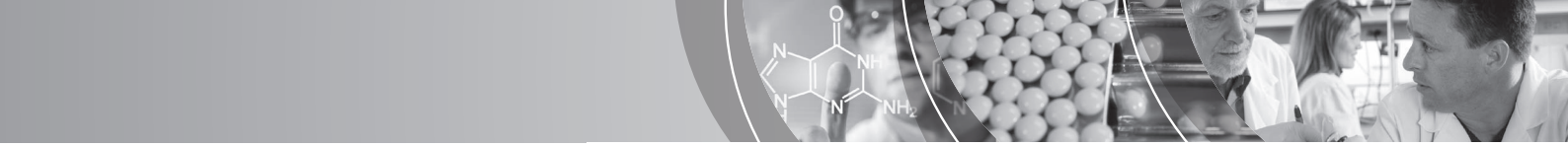
Sub-chronic smoking alters calcium signalling to increase small airway contraction in mice

Chantal Donovan, Huei J. Seow, Ross Vlahos, Jane E. Bourke, Lung Health Research Centre, Dept of Pharmacol and Therapeutics, Univ of Melb, Parkville, VIC.



Introduction: Small airway contraction is an important contributor to symptoms in asthma and COPD. Exposure to cigarette smoke in these diseases may alter contractile signaling pathways, including changes in intracellular Ca^{2+} release and changes in Ca^{2+} sensitivity.
Aim: To assess the effects of sub-chronic cigarette smoke exposure on mouse small airway reactivity to methacholine (MCh), 5HT and bradykinin (BK).
Methods: Balb/C mice were exposed to 3 cigarettes (smoke) or air (sham) 3 times a day for 4 days. On day 5, mice were euthanized with sodium pentobarbitone (i.p.) and lung slices prepared. Small airway contraction was assessed *in situ* using phase-contrast microscopy. The contribution of Ca^{2+} sensitivity pathways to contraction was measured after treatment with caffeine/ryanodine to abolish intracellular Ca^{2+} oscillations.
Results: All constrictors induced stable monophasic contractions in airways from sham mice (n=4-6). After smoke exposure, MCh responses were unchanged but contractions to 5HT were biphasic (Figure 1) and BK elicited transient contractions that were only sustained at high concentrations (n=4-10). Caffeine/ryanodine treatment restored these altered 5HT and BK responses to monophasic contractions of similar magnitude and potency as sham mice (n=4).
Discussion: Smoke exposure caused clear differences in the patterns of airway contraction to 5HT and BK but not MCh, despite all agonists mediating contraction via GPCR signaling. Biphasic and twitchy contractions to 5HT and bradykinin were associated with altered intracellular Ca^{2+} release, as they were absent when this pathway was inhibited. Further exploration of the differences in signalling downstream of MCh, 5HT and BK receptors could identify novel therapeutic targets to minimize the contribution of smoking to altered small airway contraction in chronic lung diseases.



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Extracellular annexin A2 mediates inflammation and fibro-proliferation in models of pulmonary fibrosis

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Introduction. Interstitial lung diseases (ILDs) have in common elements of pulmonary inflammation and fibrosis. Annexin A2 is a participant of such networks by accelerating the conversion of plasminogen into plasmin, and acting as a plasmin-signal transducer (Schuliga *et al* 2013a,b). Annexin A2 also has plasmin(ogen)-independent effects involving interactions with Factor Xa and the toll-like receptor-4 (TLR-4).
Aims. To investigate the roles of annexin A2 in pulmonary inflammation and fibrosis.
Methods. Human parenchymal fibroblasts (PFbs) were incubated with plasminogen (0.5-50 μg/mL), plasmin (0.5-50 mU/mL) or Factor Xa (25 nM). In selected experiments, levels of annexin A2 were reduced by transfection with siRNA. Levels of interleukin-6 (IL-6) mRNA and protein were assessed by PCR (4 h) and ELISA (24 h) respectively, and cell proliferation was assessed by cell enumeration (48 h). Annexin A2 knock-out mice were used in a model of pulmonary fibrosis. The number of inflammatory cells and levels of IL-6 were measured in the bronchial lavage fluid (BALF), and levels of collagen (ie hydroxyproline) were measured in the lung.
Results: Annexin A2 knock-down or antibody-neutralization attenuated plasminogen activation by PFbs, in turn attenuating plasmin-stimulated cytokine production and proliferation. Our evidence suggests that plasmin binds to and cleaves soluble extracellular annexin A2 in the process of plasmin-evoked signalling. We also have evidence of plasmin(ogen) independent roles of annexin A2 in PFb cytokine production and proliferation involving interactions with Factor Xa or TLR-4. In support of a role of annexin A2 in lung pathophysiology, lung inflammation and fibrosis were reduced in annexin A2 knockout mice as compared to wild-type mice after intranasal administration with bleomycin.
Discussion. Our study provides the necessary way forward to understanding the role of extracellular annexin A2 in lung (patho)physiology with a strategic impact on the development of new therapies for ILDs.

Schuliga M *et al* (2013a) *Am J Respir Cell Mol Biol*. [Epub ahead of print]
Schuliga M *et al* (2013b) *Curr Opin in Pharmacology* **13**(3):386-93

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Bitopic modulators of the α_{1A} adrenoceptor.

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Introduction. The development of subtype-selective α_{1A} adrenoceptor (AR) antagonists has been hampered due to the high degree of conservation of the orthosteric binding site amongst biogenic amine receptors. One approach to overcome this is the use of allosteric or bitopic ligands that can interact with the less conserved extracellular loops and transmembrane regions.
Aims. To characterize the allosteric effect of 4-aminoquinoline homologues on the α_{1A}AR.
Methods. The α_{1A}AR was heterologously expressed in COS-1 cells. Membrane preparations were used to determine apparent affinity and modulatory effects of a series of homobivalent 4-aminoquinoline compounds with linkers of 2-12 carbons (C2-C12), as well as their effect on [^{3}H]prazosin dissociation rate. [^{3}H]-phospho-inositol was measured to quantify α_{1A}AR activation. Compounds were docked into an α_{1A}AR homology model based on the dopamine D_3R crystal structure (PDB ID: 3PBL) using GOLD version 5.0.
Results. Docking studies suggested that C2 binds within the orthosteric binding site, while C7-C11 bind within the orthosteric binding site and also interact with a second site in a bitopic manner. 4-aminoquinoline, 10 μM C9, C10, and C11 increased the dissociation rate of [^{3}H] prazosin from the α_{1A}AR 2-2.5-fold, suggesting allosteric modulation. C9 was shown to act as a non-competitive antagonist of norepinephrine at the α_{1A}AR with negative cooperativity ($\alpha\beta$ 0.35; k_{obs}/k_{off} C9 100μM: 1108±222%, n=3). The allosteric site was proposed by docking of 4-aminoquinoline, C9, C10 and C11 to be composed of $S83^{2.61}$, $F86^{2.64}$ and $E87^{2.65}$, of transmembrane helix 2. Further supporting the role of this region in modulating orthosteric ligand affinity, $F86^{2.64}$ was shown to be involved in the process of the dissociation of [^{3}H] prazosin from the α_{1A}AR (k_{off}, wild type 0.07±0.01 min^{-1} n=4; F86A 0.16±0.03 min^{-1} n=3).
Discussion. We have identified a novel negative allosteric modulator of the α_{1A}AR and a region outside the orthosteric site involved in the dissociation process of prazosin from the α_{1A}AR. These findings provide the basis for future ligand and structure based drug development.

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Pathway-selective modulation of CB1 receptor signalling by the allosteric modulator Org27569

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Introduction. The majority of the central nervous system effects of cannabis and other cannabinoid compounds are mediated through CB1 cannabinoid receptors, which are the most abundant GPCRs in the brain (Devane et al, 1988). CB1 ligands can potentially be used to treat a variety of disorders; however their therapeutic applications are limited mainly due to their psychotropic effects. Selective allosterism is one strategy to obtain specific and efficient therapeutics. Recently, a small molecule named Org27569 has been reported to act as an allosteric inhibitor of agonist function, while being an enhancer of agonist binding at CB1 receptors (Price et al, 2005).
Aims. The aim of the present study was to characterise the effects of Org27569 on CB1 receptor signalling pathways activated by various cannabinoid ligands.
Methods. Flp-In CHO cells stably expressing hCB1 receptors were used to determine CB1-mediated signalling using AlphaScreen pERK1/2 phosphorylation and cAMP assays (Perkin Elmer).
Results. In pERK1/2 assays, Org27569 had no efficacy by itself, and either did not significantly alter or only weakly modulated pERK1/2 activation induced by cannabinoid agonists WIN55212, Δ9-THC, methanadamide, anandamide and 2-AG. However Org27569 completely inhibited CP55940- and HU-210-mediated pERK1/2, indicating a probe dependent effect. Strikingly, in contrast to its weak interaction with WIN55212 in pERK1/2 assays, Org27569 significantly inhibited WIN55212-induced inhibition of adenylate cyclase highlighting pathway-specific allosteric effects.
Discussion. Consistent with a previous study (Baillie et al, 2013), using a wide range of endogenous and exogenous ligands we demonstrate that Org27569 displays pathway-selective allosteric modulation and probe-dependence. Compounds such as Org 27569 may therefore be used to gain selective therapeutic effects.

Baillie GL et al (2013) Mol Pharmacol 83(2):322-338.
Devane WA et al (1988) Mol Pharmacol 34(5):605-613
Price MR et al (2005) Mol Pharmacol 68(5):1484-1495

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TRPV4 and Shear Stress Mechanotransduction in Endothelial Cell

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Introduction. Endothelial cells are constantly exposed to blood flow induced shear forces and cellular responses to shear stimuli play a pivotal role in vascular homeostasis and tone. The cell membrane is the major target of external mechanical forces, and mechano-sensitive ion channels are the major player of cellular mechano-transduction.
Aims. The aim of this work was to study the kinetics of endothelial cells mechano-transduction in response to shear stress using a novel microfluidic platform. Further, to investigate the importance of transient receptor potential vaniloid 4 (TRPV4) ion channels on endothelial cell mechano-sensitivity, and the effect of shear stress on TRPV4 channel gating.
Methods. To perform this study we used a novel multi shear microfluidic platform and the changes in intracellular calcium level of endothelial cells in response to mechanical stimulation were measured using Fluo 4 calcium sensitive fluorescent dye.
Results. Endothelial cells responded to shear stress with a rapid increase in intracellular calcium level, which depends on temperature and the applied shear. Elimination of extracellular calcium or blockade of Ca^{2+} entry with a non-specific TRP ion channel pore blocker, Ruthenium Red, or the TRPV4-selective inhibitor HC047067, significantly diminished the $[Ca^{2+}]_i$ response, suggesting that the endothelial cells response to shear stress is mediated by the influx of extracellular calcium through TRPV4. We observed that increasing the shear stress intensified TRPV4 channel opening in response to agonists in a phosphoinositide 3-kinase- dependent manner.
Discussion. We and others have found that TRPV4 is mechano-sensitive. Using a selective TRPV4 antagonist, we found a significant reduction in the response of endothelial cells to shear stress, indicating that TRPV4 is playing a significant role in these cells mechano-sensitivity. This device will be useful for studying shear stress in mechano-sensitive primary cells.

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Molecular Determinants of Allosteric Ligand Binding and Modulation at the M_1 Muscarinic Acetylcholine Receptor.

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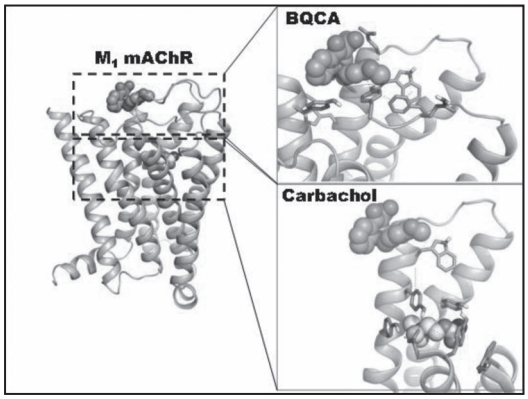
Introduction.The M_1 muscarinic acetylcholine receptor (mAChR) is an attractive therapeutic target for CNS disorders such as Alzheimer's disease and schizophrenia. Several M_1 mAChR selective ligands have been discovered in the past few years. Among those, BQCA, a positive allosteric modulator (PAM) of ACh binding and function at the M_1 mAChR has been well characterized as an unprecedented example of a PAM with very low affinity but a remarkably high cooperativity with ACh. However, the structural basis of binding and function of BQCA remains unknown. A better understanding of the structural determinants of BQCA function is essential for the rational design of improved M_1 mAChRs allosteric ligands, with tailored allosteric properties.



Aims. To resolve key questions surrounding the structural basis of binding, cooperativity and efficacy of BQCA as PAM and agonist at the M_1 mAChR.

Methods. Radioligand binding and Inositol-1-phosphate accumulation assays on Chinese Hamster Ovary (CHO) cells expressing either WT or mutant M_1 mAChR.

Results. We identified residues in the second and third extracellular loops and the top of transmembrane domain (TM) VII as those that form the BQCA binding pocket on the M_1 mAChR and are key contributors to the transmission of cooperativity with the orthosteric agonist carbachol (CCh). Mutation of amino acid residues that form the orthosteric binding pocket and are needed for receptor activation by CCh turned BQCA into an "efficacy only" modulator, whereby the functionally impaired signaling of CCh was 'rescued' by BQCA despite the loss of affinity modulation at these mutants. The residues that contribute to the BQCA binding pocket were confirmed using molecular dynamics simulation models of the M_1 mAChR.

Discussion. BQCA makes a key interaction with W400 in TMVII which suggests that it binds to the "common" allosteric site on mAChRs thereby guiding the design of new ligands that target this site.

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Delineating the dynamics of mu-opioid receptor signalling and regulation

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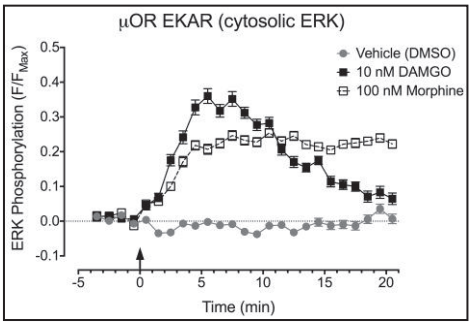
Introduction. Opiates, such as morphine, are the most clinically useful class of analgesic for the treatment of both, acute and chronic pain. Both the analgesic and gastrointestinal effects of morphine are largely mediated by the activation of the mu-opioid receptor subtype. Morphine has also been suggested as a biased agonist, engendering a distinct receptor conformation to that of the endogenous ligands and leading to a different signalling outcome. As such understanding the spatial and temporal dynamics of mu-opioid receptor signalling by different agonists is important for the design of novel drug candidates with effective analgesia but with minimal adverse side effects.



Aims. To delineate the subtleties of mu-opioid receptor signalling, trafficking and regulation by single cell imaging and resonance energy transfer approaches.

Methods. Receptor signalling dynamics were investigated using cytosolic and targeted FRET biosensors for different signalling effectors. β-arrestin and GRK recruitment were assessed using BRET approaches. Receptor internalisation and trafficking were investigated using BRET (REF) and confocal imaging.

Results. Both Morphine and DAMGO induced cytosolic ERK phosphorylation although with different dynamic profiles. DAMGO, but not morphine induced nuclear ERK phosphorylation and this linked to receptor internalisation upon receptor activation. Morphine, but not DAMGO, induced activation of plasma membrane localised PKC. Using a combination of pharmacological inhibitors and mu-opioid receptor mutants we observed differential effects on the signalling of these two agonists.

Discussion. Our results suggest that different cellular components may participate in the signalling of DAMGO and morphine. Whether these differences arise from the differential recruitment of signalling effectors or from differential receptor compartmentalisation is currently being explored.

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Antiproliferative actions of novel ω-3 epoxyfatty acids and isosteric analogues in human breast cancer cells

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Introduction. Unlike other polyunsaturated fatty acid (PUFA) epoxides, the CYP-derived epoxide formed at the ω-3 olefinic bond in ω-3 PUFAs inhibits cell proliferation. This property may be adapted for the development of novel antiproliferative agents for cancer treatment.

Aims. We prepared two series of agents with C20-22 carbon chain lengths – ω-3 epoxyfatty acids and the urea isosteres that have greater metabolic stability – and evaluated their anti-tumour actions in breast cancer cells.

Methods. ω-3 Monounsaturated fatty acids (Cui et al. 2012) were converted to the ω-3 epoxides with *m*-chloroperoxybenzoic acid. The urea isosteres were prepared from protected esters of ω-hydroxy fatty acids by hydroxyl azidation and subsequent Staudinger reduction to the amines. Treatment with ethylisocyanate and saponification yielded the ω-3-urea-substitited fatty acids. Structures and purity were confirmed by NMR, MS and elemental analysis. Agents were tested in MDA-MB 231 (231), MDA-MB 468 (468), MCF-7 and T-47D cells. Proliferation was estimated by mitochondrial reduction of the dye MTT, viability by ATP production, and apoptosis by caspase-3/7 activity and annexin V-FITC staining.

Results. All agents decreased MTT reduction in 231 and 468 breast cancer cells after 24 and 48 hr of treatment (by 20-40% from control at 10 μM), whereas T-47D and MCF-7 cells were less responsive. The C20-epoxide strongly impaired ATP production in 231 cells by 30% and 60% from control after 48 and 120 hr, respectively; ureas were less active. In 231 and 468 cells apoptosis was activated by the C20-epoxide, but not by the analogous C20-urea.

Discussion. Fatty acid epoxides and ureas are novel antiproliferative agents against aggressive breast cancer cells. The epoxides, but not the ureas, decreased cell viability, most likely due to increased apoptosis. Further structural modification is now warranted to enhance the activity of the ω-3 epoxyfatty acids and their urea isosteres.

Cui et al. (2012) J Med Chem 55, 7163-7172

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Development and Validation of an LC-MS/MS Bioanalytical Method for Quantification of Pyridoxal 5´-Phosphate (PLP) in Samples of Human Whole Blood

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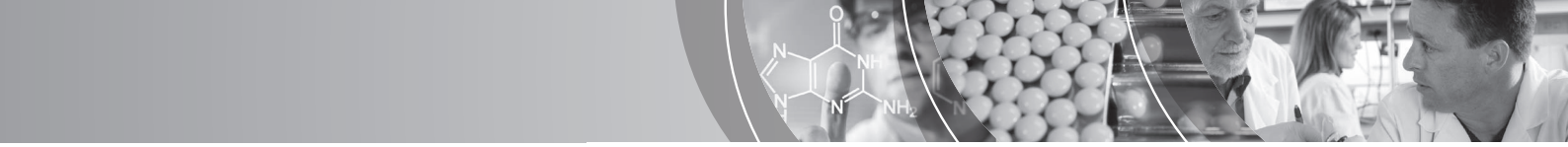
Introduction. PLP is the biologically active form of vitamin B6 which acts as a coenzyme in enzymatic reactions involved in the metabolism of a range of endogenous compounds in the body.

Aims. To develop and validate an LC-MS/MS bioanalytical method for quantification of PLP in samples of human blood collected from patients during a clinical trial evaluating a combination vitamin therapy for reducing the frequency and severity of migraine attacks.

Methods. Aliquots of 10% trichloroacetic acid (400 μl) containing pyridoxal 5´-phosphate (methyl-D3) as the internal standard (50 ng/mL) were added to samples of whole blood (100 μl). Samples were mixed and centrifuged at 14,000 rpm to precipitate lysed cellular material and proteins. The supernatants (10 μl) were injected onto a Symmetry® C18 100 x 2.1 mm, 3.5 μm analytical column with a 5 min run time and the mobile phase comprising 0.2% formic acid in water and 0.1% formic acid in methanol. The MRM transitions were 248.0 →150.0 and 251.0→153.1 for PLP and the internal standard, respectively. The method was validated over the concentration range 2-100 ng/mL based on international guidelines.

Results. This bioanalytical method showed acceptable within-run and between-run precision (precision and accuracy > 85%) for quality control samples spiked at concentrations of 6, 50 and 75 ng/mL and > 80 % at LLOQ. No autosampler carry-over was observed and the recovery was about 70%.

Discussion. Chromatographic methods have superior accuracy for quantification of PLP in biological matrices compared with enzymatic or microbiological assays. MS/MS detection avoids the derivatisation steps required for fluorescent detection, thereby saving time, reducing labour costs and chemical usage, as well as having superior selectivity and sensitivity. The high percentage of PLP in red cells (~ 60%) means that whole blood is a more suitable matrix than serum for quantification of circulating concentrations of PLP.



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Identifying origins of affinity, selectivity and allosterism on the α_1 adrenoceptors.

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Introduction. Bivalent ligands are a conjugation of pharmacophores yielding a single molecule with improved characteristics. Homobivalent 9-aminoacridines show high affinity for adrenoceptors, particularly the α_1 subtype (Adams *et al.*, 1985). The bivalent nature of these molecules suggests that they may be interacting with a secondary binding site on the α_1 adrenoceptors.
Aims. To identify α_1 adrenoceptor residues which will allow subtype-selective and allosteric interactions.
Methods. The three α_1 adrenoceptor subtypes were heterologously expressed in COS-1 cells. Membrane preparations were used to determine apparent affinity and subtype selectivity of a series of bisacridine compounds with linkers of 2-12 carbons (C2-12), as well as any effect on [^{3}H]prazosin dissociation from each receptor.
Results. All tested 9-aminoacridine homologues showed low-, to sub-micromolar affinity at each α_1 adrenoceptor subtype. Notably, C4-bisacridine showed 22 nM affinity at the α_{1A} adrenoceptor (pKi: 7.7±0.1, n=3) 11-fold higher than the monovalent 9-aminoacridine ($p<0.01$) and 16- and 32-fold selective over the $\alpha_{1B,}$ and α_{1D} adrenoceptors ($p<0.01$). Preliminary testing has shown that 100 μM 9-aminoacridine and C4-bisacridine are both able to increase the dissociation rate of [^{3}H]prazosin 1.5-3-fold at the α_{1A} and 8-12-fold at the α_{1B} adrenoceptor.
Discussion. The increased affinity of the bivalent C4-bisacridine suggests that the second acridine unit is interacting with the receptor outside the orthosteric site. The observed increases in dissociation rate of [^{3}H]prazosin are indicative of allosteric interactions. The differences in apparent affinity, and the magnitude of the change in dissociation rates at each receptor are promising for the design of adrenergic ligands with the desirable properties of selectivity and allosterism. The known length of the linker constrains potential allosteric interactions to a limited distance from the allosteric site and allows identification of this allosteric site via *in silico* docking.

Adams A, Jarrott B, Elmes BC, Denny WA, Wakelin LP (1985). Interaction of DNA-intercalating antitumor agents with adrenoceptors. *Mol. Pharmacol.* **27**(4)**:** 480-491.

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Enhancing the blood-brain barrier efflux of β-amyloid: a novel approach for the treatment of Alzheimer's disease?

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Introduction. The brain accumulation of β-amyloid (Aβ) in Alzheimer's disease (AD) has been suggested to result from decreased clearance across the blood-brain barrier (BBB). This hypothesis is supported by decreased BBB expression of the Aβ-transporter proteins low density lipoprotein receptor-related protein 1 (LRP1) and P-glycoprotein (P-gp), the latter of which is under the regulation of Wnt/β-catenin signalling.
Aims. To assess the impact of lithium chloride (LiCl; an activator of β-catenin signalling) on the *in vitro* and *in vivo* BBB expression of LRP1 and P-gp and the subsequent impact on BBB efflux of Aβ.
Methods. Human brain endothelial cells (hCMEC/D3) were treated with increasing concentrations of LiCl (0-10 mM) for 24 h and mRNA and protein expression of LRP1 and P-gp measured by qPCR and Western blotting, respectively. Male Swiss Outbred mice were orally administered NaCl or LiCl (300 mg/kg/day for 21 days), after which, cerebral microvessels were isolated and LRP1 and P-gp expression measured by Western blotting. Finally, BBB efflux of $A\beta_{1\text{-}42}$ was measured following icv injection of ^{125}I-$A\beta_{1\text{-}42}$ to mice and radioactivity in plasma measured over 10 min.
Results. Treatment of hCMEC/D3 cells with LiCl had no impact on the mRNA expression of P-gp, however, LRP1 mRNA expression was increased 4.6-fold with 10 mM LiCl. A 21 day treatment with LiCl resulted in a 1.5 and 3.9-fold increase in mouse brain microvascular protein expression of LRP1 and P-gp, respectively, and a 1.4-fold increase in the brain-to-blood efflux of ^{125}I-$A\beta_{1\text{-}42}$ over 10 min.
Discussion. These studies demonstrate that enhancing the BBB expression of LRP1 and P-gp (likely via β-catenin activation) leads to increased trafficking of Aβ from the brain into the blood. Targeting of these BBB transporters may therefore be a promising therapeutic strategy to reduce brain parenchymal Aβ load, serving as a novel approach for the treatment of AD.

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A combined Mutational Study with Various Antagonists on Serine Residue within the Orthosteric Binding Pocket of ρ1 GABAC Ion Channel Receptors: A Structure-Based Study

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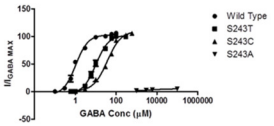
Introduction: As a member of Cys-loop family, ρ1 GABAC receptors are related in structure to other GABAA subunits (Johnston, 2002). ρ1 are homopentameric receptors that have distinct physiological and pharmacological properties, and are believed to be involved in many disorders of memory and sleep (Ng et al, 2011). The extracellular domain of the receptor has GABA binding pockets on the interface between two subunits. When GABA is bound, loop C forms like a cover over GABA. This loop has many residues that are involved in crucial interactions within the protein and/or with ligands. Here we present a study on loop C 'serine243' residue mutants and their interaction with structurally different antagonists.



Aim: To study mutants of a single critical residue and to determine the effects on antagonists such as TPMPA, APPA, THIP and Gabazine.

Methods: Site-directed mutagenesis protocol and two electrode voltage clamp electrophysiology of receptors expressed in Xenopus laevies oocytes.

Results: The loop C S243T and S243C mutants showed a 10 and 60 fold decreased in GABA potency, respectively. THIP and Gabazine showed a similar potency on GABAC S243T receptors while TPMPA and APPA were 4 and 2 times lower in potency compared with wild type receptors. On GABAC S243C receptors Gabazine had a similar potency to wild type; in contrast THIP and TPMPA were decreased in potency by 5 and 60 fold, respectively. Interestingly, APPA weakly potentiated GABA rather than antagonize it on the mutant receptors.

Conclusion: Phosphorus-containing antagonists showed a decrease in potency at mutants. TPMPA and APPA are GABA analogues with the carboxylic acid group bioisoterically substituted with the phosphorus containing acidic moiety. THIP displays lower potency on the S243C mutant that may indicate some interactions between THIP and serine 243. Gabazine potency was not significantly affected at any of mutants, suggesting that it does not interact with the S243 residue.

Johnston (2002) Curr Top Med Chem, 2, 903-913.
Ng et al (2011) Fut Med Chem, 3, 197-209.

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Expression of the small interacting protein CRIP1a modulates cannabinoid CB1 receptor mediated signalling

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Introduction. The endocannabinoid system plays an important role in the pathophysiology of psychiatric disorders like schizophrenia. The cannabinoid CB_1 receptor plays critical roles in regulating many physiological processes from neurogenesis and synaptic plasticity, to learning and memory, through preferentially coupling to $G_{i/o}$ proteins. The cannabinoid receptor interacting protein ($CRIP_{1a}$) is a newly discovered interacting protein of the CB_1 receptor. Thus far, $CRIP_{1a}$ has been shown to reverse the tonic inhibition of voltage-gated Ca^{2+} channels induced by CB_1 receptor activation. We hypothesised that CB_1 receptor function may be positively modulated by knockdown of $CRIP_{1a}$.

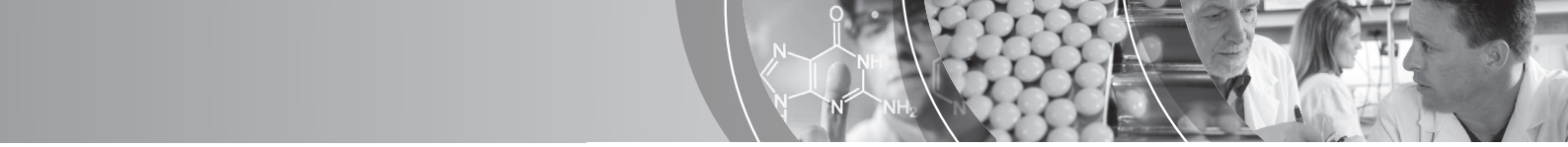
Aims. To determine the effect of $CRIP_{1a}$ knockdown on modulation of CB_1 receptor mediated activation of G-protein coupled inwardly rectifying potassium (GIRK) channels.

Methods. Western blotting was used to measure $CRIP_{1a}$ protein knockdown in AtT20 cells stably expressing CB_1 receptors, following siRNA treatment. Membrane potential studies were conducted using a proprietary membrane potential-sensitive dye and continuous fluorescence readings obtained using a FLEX Station Microplate Reader.

Results. The CB_1 receptor agonist WIN55212-2 produced a maximum change in fluorescence of 30.54±2.31% (-log EC50, 6.80) whilst CP55940 produced a maximum change in fluorescence of 27.09±1.34% (-log EC50, 7.12). Anandamide as expected showed both a decrease in efficacy (22.52±1.00) and potency (-log EC50, 5.68) at the CB_1 receptor. $CRIP_{1a}$ protein knockdown was observed in cells treated with $CRIP_{1a}$-siRNA (20nM), 48 to 72 hours post-transfection ($p < 0.001$). This siRNA-induced $CRIP_{1a}$ knockdown significantly increased anandamide-induced K^+ channel activation ($p < 0.05$) whilst no change was observed in response to WIN55212-2 and CP55940. Pre-treating cells with the CB_1 antagonist AM251 prior to CB_1 receptor agonist administration confirmed these effects to be CB_1 mediated. Effects of $CRIP_{1a}$ knockdown on intracellular ERK1/2 levels and cAMP levels have also been conducted.

Discussion. Together, these findings suggest that $CRIP_{1a}$ modulates CB_1 receptor signalling in the ligand-specific manner.

Niehaus J et al (2007) Mol Pharmacol 72:1557-1566



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Contribution of beta1 adrenoceptors to the development of alcohol dependence.

Paul Klenowski[1], Omkar Laxman Patkar[1], Joan Holgate[1], Peter Molenaar[2], Selena Bartlett[1]
Translational Research Institute, Queensland University of Technology[1], Brisbane, QLD; Institute of Health and Biomedical Innovation, Queensland University of Technology[2], Brisbane, QLD. (introduced by Peter Molenaar, Queensland University of Technology, Brisbane, QLD).

Introduction. Evidence is accumulating to support the hypothesis that stressors contribute to the development of alcohol dependence. Compounds including prasozin and propranolol, which block the effects of noradrenaline at alpha1-adrenoceptors and beta-adrenoceptors (AR) respectively, have shown promise as potential therapeutic agents for the management of stress-induced alcohol consumption and relapse (Walker et al., 2008, Gilpin and Koob, 2010).
Aims. We have extended these studies to characterize a series of selective β_1AR compounds to determine their effect on drinking behavior in mice. We have also performed studies to determine βAR expression in mouse brain.
Methods. Compounds were tested using the drinking in the dark paradigm. Briefly, C57BL/6 mice were housed individually in a reverse light-dark cycle and given access to 1 bottle of 20% ethanol (v/v) and 1 bottle of filtered water for a 2hr period, 5 days a week, 3hrs into the dark cycle. Bottles were weighed 30min and 2hrs after presentation to determine daily ethanol consumption. Compounds or vehicle (0.9% saline) were administered via subcutaneous injection after 4 weeks of ethanol exposure. Radioligand binding and autoradiography studies with (-)-[^{3}H]-CGP12177 were used to label β_1AR binding sites in brains from C57BL/6 mice.
Results. Receptor densities in C57BL/6 mouse brain were (fmol/mg protein) 94 ± 8 for β_{1H}AR, 272.66 ± 38 for β_{1L}AR and 55 ± 2 for β_2AR. Changes in receptor densities caused by ethanol are currently being investigated. The effects of the β_{1L}AR partial agonist and β_{1H}AR antagonist CGP12177 (50 mg/kg), were short lasting, causing a significant decrease in ethanol consumption at 30mins but not at 2hrs. The β_{1H}AR partial agonist Xamoterol had no effect on ethanol intake.
Discussion. We conclude that CGP1277 reduces ethanol consumption via blockade of noradrenaline at β_{1H}AR. Future studies will involve determining whether CGP12177 causes effects on drinking behavior via its actions at β_{1L}AR and/or β_3AR.

Walker BM, Rasmussen DD, Raskind MA, Koob GF (2008) Alcohol 42:91-97.
Gilpin NW, Koob GF (2010) Psychopharmacology (Berl) 212:431-439.

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***In vivo* human dermal nanoparticle toxicology – myth or reality?**

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Introduction. An unprecedented amount of effort is being invested in research into the public perceptions of the risks and benefits of nanotechnology. Most research is exploring public opinion in the general population, investigating perceived risks on the part of consumers to the use of nanotechnology.[1] In Australia, Friends of the Earth have suggested that nano-sized particles within commercial sunscreens may be penetrating into the viable layers of the skin and potentially causing toxicity.[2]
Aims. This paper seeks to examine the extent of penetration and toxicity associated with nano-zinc oxide applied in sunscreen products after application to human skin *in vivo* under various in-use conditions.
Methods. A range of commercial zinc oxide nanoparticles and nano-zinc oxide sunscreens were applied to the forearm of volunteers or to lesions and adjacent skin of psoriatic patients under normal, occlusive, stratum corneum-stripped and occlusive conditions. Nano zinc oxide penetration was assessed directly and non-invasively using multiphoton tomography.
Results. Zinc oxide nanoparticles were predominantly found on the skin surface (stratum corneum) and within the skin furrows adjacent to the viable layers. Massaging and occlusion appeared to cause some zinc oxide nanoparticles to 'pierce' the furrow walls but without causing any morphological or redox state change in the viable epidermis.
Discussion. Zinc oxide nanoparticles do not appear to cause any damage to human skin when applied topically under a range of circumstances. This work confirms our previous studies conducted using excised human skin and suggest that topically applied zinc oxide nanoparticles are not toxic to human skin.

1. https://osha.europa.eu/en/publications/literature_reviews/risk-perception-and-risk-communication-with-regard-to-nanomaterials-in-the-workplace
2. http://nano.foe.org.au/sites/default/files/Nano-ingredients%20in%20sunscreen%202012.pdf

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Accumulation of nanoparticles in the mononuclear phagocyte system: role of albumin binding and unfolding on the surface of nanoparticles

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Introduction. Foreign bodies including nanoparticles that enter the circulation are targeted to the mononuclear phagocyte system (MPS). This system is an important determinant of the biological activity for many nanomedicines because it is responsible for their rapid clearance *in vivo*. Natural and synthetic layered silicate nanoparticles (LSN) are widely used in many industries (e.g. food packaging and cosmetics), and are under development as drug delivery agents and in polymer nanocomposites with industrial and biomedical applications. LSN bind a range of blood proteins forming a surface-associated corona that contributes to its biological identity in the circulation. Which of these proteins may be responsible for the targeting of nanoparticles to the MPS is not well understood.
Aims. To determine whether the proteins in the surface-associated corona of LSN contribute to their biological identity and clearance by the MPS.
Methods. Surface-associated protein corona was assessed by SDS-PAGE. Cell uptake was studied using a combination of flow cytometry and confocal imaging. Secondary structure of LSN-bound proteins was studied by circular dichroism.
Results. Plasma proteins form a hard corona around synthetic LSN. One of the principle proteins is serum albumin. Upon binding, albumin unfolds to reveal a cryptic epitope that specifically recognises Class A scavenger receptors, a major scavenger receptor family associated with the MPS. The epitope(s) appeared to reside in the first or second domain of the protein and could also be exposed by other unfolding processes such as heat denaturation.
Discussion. These findings provide a molecular explanation for the targeting of nanoparticles to the MPS and suggest a novel function for albumin as an opsonin promoting the clearance of foreign particles from the circulation through tissue sequestration via scavenger receptor recognition.

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Uptake and toxicity of nanoparticles used in medicine varies with particle size and protein binding.

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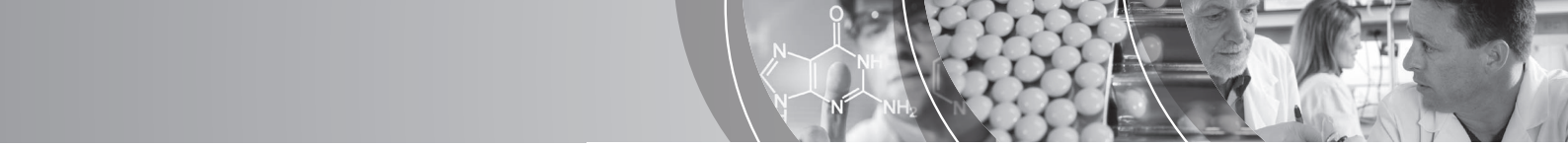
Introduction. Layered silicate nanoparticles are currently under investigation in a number of biomedical applications, such as drug delivery, and as modifiers of polyurethane biomedical devices. However, how these materials interact with proteins and cells remains unknown.
Aims. To determine toxicity and uptake of different sized layered silicate nanoparticles into human cells and to investigate the influence of protein binding to their surface.
Methods. Toxicity was assessed by MTS assay, annexin V staining (apoptosis) and lactate dehydrogenase release (membrane integrity). Cell uptake was studied using a combination of flow cytometry and confocal imaging.
Results. The toxicity of the nanoparticles varied significantly with size. Large particles (>1000 nm) induced cell death faster, and at lower concentrations than small nanoparticles (<100 nm), although both comprised identical core material. In addition, the smaller particles induced apoptosis while the large particles caused rapid membrane disruption. Increasing the serum concentration in the cell culture reduced the toxicity of particles of both sizes. Macrophage like cells did not take up particles of either size in serum-free conditions. However, both small particles in 5% serum and large particles in 80% serum were taken up via the scavenger receptor system. Particles in other serum conditions were either not taken up by cells or taken up by other mechanisms.
Discussion. These findings are in contrast to many other studies that have shown higher toxicity as particles are reduced in size. Different serum conditions affect the interaction between cells and nanoparticles, and the protein corona that forms around the nanoparticles is required for cellular uptake. The various serum conditions which lead to different uptake mechanisms may also contribute to the differences in toxicity observed.



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Macronutrient related hepatotoxic changes provide a link between caloric restriction and inflamm-ageing

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Introduction. Caloric restriction (CR), and more specifically protein restriction, is known to enhance longevity in a variety of taxa. The liver provides a plausible causal link between diet and ageing, as it plays a crucial role in nutritional metabolism and displays age-related hepatotoxic changes such as inflammation, which can be histologically assessed.
Aims. This study investigated the effects of varying protein-to-carbohydrate (P:C) ratios on hepatic steatosis, fibrosis and inflammatory infiltration.
Methods. Three month old C57BL6 mice (n=90) were assigned one of three diets varying in P:C ratio for 8-weeks (P:C of 5%:75%, 34%:64%, and 60%:20%) with a fixed fat ratio of 20%, and allocated to ad-libitum (AL) or 40% CR intake regimes.
Results. H&E staining of the livers showed a positive correlation between P:C ratio and portal inflammation in AL animals ($p=0.0004$), but not in CR animals. Sirius Red staining indicated no effect of P:C ratio on fibrosis, however there was a negative correlation between P:C ratio and steatosis in both the AL and CR animals ($p= 0.0009$ and $p=0.002$ respectively).
Discussion. Together, these findings suggest that high P:C ratios are linked with increased portal inflammation, and low P:C ratios are linked with hepatic fat accumulation. Also, CR animals displayed less inflammation than AL animals, suggesting that protein restriction may be beneficial for healthy liver function. Thus, the results are consistent with the idea that high protein intake is associated with inflammatory liver changes, while low protein intake (either low P:C ratio or CR) is hepato-protective. This study represents a critically important area of research, as even short-term dietary interventions can have profound effects on inflamm-ageing. Understanding this relationship may help form guidelines to promote healthy ageing.

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Bioequivalence of generic mycophenolate mofetil in paediatric renal transplantation

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Introduction. Generic versions of newer transplant immunosuppressive medications have become available in recent years. Bioequivalence is tested in small groups of healthy volunteers, and there is debate amongst the transplant community regarding the adequacy of the current bioequivalence acceptance range, and the ability to extrapolate to the target population for these critical dose narrow therapeutic index drugs (NDITs). Bioequivalence studies do not include children.
Aims. To assess bioequivalence (single crossover) between Cellcept and the generic product Mycophenolate Sandoz, in paediatric renal transplant recipients in maintenance phase of immunosuppression.
Methods. Mycophenolic Acid AUC was estimated using a limited sampling strategy validated in paediatric renal transplant recipients (Filler, 2004). After switching from Cellcept to Mycophenolate Sandoz, repeat Mycophenolic acid AUC was performed after a minimum of 1 week on the new agent. Bioequivalence calculations were performed using Phoenix WinNonLin.
Results. Completed results will be tabulated and ready for presentation at the ASCEPT ASM.
Discussion. Reductions in overall immunosuppression can lead to acute rejection, which can, if not caught early, lead to significant graft damage or even loss. Despite lack of testing in our target population, we seek to show adequate correlation between innovator and generic product, which is felt to be an appropriate assumption following regulatory bioequivalence (Christians et al, 2010). Given the lack of evidence for target ranges for mycophenolic acid in maintenance phase transplant immunosuppression, a switch to a generic brand is best considered as a possible, albeit small, change in overall immunosuppression, which should be adequately monitored with serum creatinine testing.

Christians U, Klawitter J et al (2010) Kidney Int Suppl S1-7
Filler G (2004) Transpl Int 17:120-5

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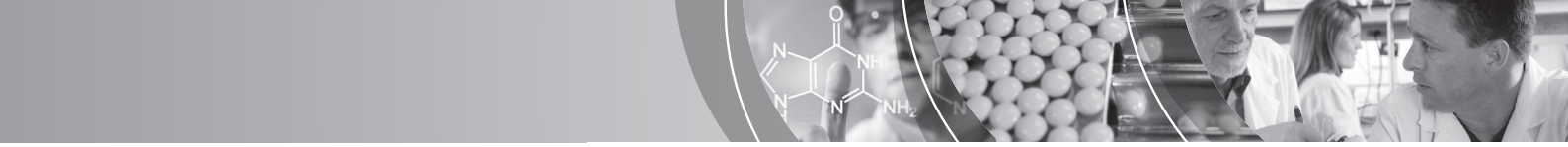
The pharmacokinetics of thiamine in alcohol dependent people: Protocol

Jonathan Brett, Drug Health

Introduction: Thiamine (vitamin B1) is a co-factor for enzymes within the pentose phosphate pathway and krebs cycle and so is essential for glucose dependent metabolism. Alcohol dependent people are at risk of thiamine deficiency for a number of reasons [1]. A recent Cochrane review concluded that there was insufficient evidence to guide thiamine treatment dose, route or duration of therapy [2]. This is largely due to lack of pharmacokinetic-pharmacodynamic (PK-PD) linkage due to a death of pharmacokinetic research in disease states and a lack of PD markers of thiamine utilisation.
Aims: To gain a better understanding of thiamine PK in alcohol dependent patients by developing a population pharmacokinetic model.
Methods: Here we discuss the protocol for this study. We will identify 20 alcohol dependent inpatients and perform rich blood sampling over an intravenous and an oral dosing interval. We will also perform sparse sampling for 20-30 additional patients using blood collected during routine blood collection. We will measure thiamine and its phosphorylated derivatives in whole blood. This data along with other co-variates such as height and weight will be used to develop a population kinetic model within Nonmem. As well as PK parameters and variability we hope to estimate relative bioavailability.

1. Hoyumpa, A., *Mechanisms of thiamin deficiency in chronic alcoholism*. The American journal of clinical nutrition, 1980. **33**(12): p. 2750-2761.
2. Day, E., et al., *Thiamine for Wernicke-Korsakoff Syndrome in people at risk from alcohol abuse*. Cochrane Database Syst Rev, 2004(1): p. CD004033.



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Outcomes of sedation for acute behavioural disturbance in St Vincent's Hospital Emergency Department.

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Introduction. St Vincent's is a large tertiary hospital in a metropolitan area with extensive alcohol and drug use. Given the ongoing controversy regarding the most safe and effective pharmacological sedation for acute behavioural disturbance, particularly in a benzodiazepine tolerant population, a retrospective review of pharmacological sedation for acute behavioural disturbance in the emergency department was undertaken.
Aims. To retrospectively determine the outcomes of administration of pharmacological sedation for acute behavioural disturbance in the emergency department.
Methods. A one month representative patient sample was obtained via a keyword search of primary discharge diagnoses in The Emergency Department Information Systems database. Subsequently, the clinical information system and paper medical records were searched to identify patients who had received sedation for acute behavioural disturbance in the emergency department. Data obtained included patient demographics, comorbidites, aetiology of behavioural disturbance, drug ingestion, sedation administered and adherence to local sedation guidelines, time to sedation, time to onset and duration of sedation, vital signs, ECG parameters, investigations, length of stay and serious adverse effects of sedation.
Results. In a small, preliminary cohort of patients aged 17-95 years, a majority had pre-existing psychiatric illness, including polysubstance abuse. Although drug intoxication was the aetiology of acute behavioural disturbance in ~ 70% of patients, urine drug screens were collected from < 50% of patients. Rate of adherence to local sedation guidelines was ~ 50%. Time to onset of sedation was poorly documented. Approximately 50% of patients required re-sedation, within a timeframe of 25-200 minutes. Emergency department length of stay ranged from 2.88-28.62 hours. No haemodynamic instability, QT prolongation, severe respiratory compromise or extrapyramidal side effects were identified.
Discussion. The low rate of adherence to guidelines and high incidence of re-sedation identified will inform local practice and optimise the outcomes of future patients requiring pharmacological sedation.

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Pilot study of a drug minimisation guide applied to older patients in hospital

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Introduction. Drug-related problems are implicated in 6% to 30% of hospital admissions of older people[1], with patients taking 5 or more medications being 3 times more likely to be taking inappropriate medications compared to those taking fewer than 5[2]. A pilot study was conducted to assess the feasibility of a drug rationalisation intervention in a cohort of older patients admitted acutely to general medicine wards in a tertiary hospital.
Aims. To identify potentially inappropriate drugs suitable targets for discontinuation, patient characteristics associated with higher prevalence of potentially inappropriate medications and constraints in applying and interpreting each of a 9-step guide to patient drug management.
Methods. 100 discharged hospital patients 70 years of age or older, on 7 or more medications that were identified from the interai database had their charts reviewed. A summary of drugs that were eligible for discontinuation and rationale behind the selection was based on patient characteristics: high risk, life expectancy, care goals, diagnosis and drug characteristics: time until benefit, absolute risk reduction, absolute risk of harm and relative utility.
Results. The charts of 67 females and 33 males, with an average age of 86, meeting inclusion criteria were reviewed. The median number of medications was 9, with a potential for 3 medications per person being eligible for discontinuation. The guide provided an easy method for identifying potential medications for discontinuation with mortality score and frailty being strong predictors for medication discontinuation.
Discussion. Most older patients favour taking fewer drugs and avoiding any drug that is likely to cause side effects severe enough to affect functioning, irrespective of its efficacy in preventing future morbid events. We demonstrated that hospitalisation of older patients to general medicine units presents an opportunity for a whole-patient reappraisal of current medication use and the potential for safe deprescribing.

1. Passarelli MC et al Drugs Aging (2005) 22:767-777
2. Beijer HJM et al Pharm World Sci (2002) 24:46-54

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Development of a "design your own experiment" practical class to enhance independent research skills in third year neuropharmacology students.

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Introduction. Throughout their undergraduate training in biomedical sciences, many pharmacology students undertake practical classes where they essentially follow a tried and true recipe, in order to demonstrate a pharmacological action of a drug (whether this be *in vitro, in vivo* or as a computer simulation). While these approaches are valuable, they are not really indicative of what happens in the life of a "real" biomedical researcher.
Aims. To further develop independent research skills in third year neuropharmacology students and give them a "real life" research experience using a combined approach (using information gained from tutorials and lectures) in which students essentially "design their own experiment".
Methods. Science students in our third year Neuropharmacology course (60-73 from 2010-2012) were assigned the task to design an experiment to identify drugs which protect against neuronal injury. They needed to identify the drugs to be tested, concentrations and experimental conditions. Students then performed experiments, analysed data, answered questions related to their results, experimental design, further experimentation and provided a critical analysis of their findings. Course satisfaction surveys were collected from students, with a 54-86% response rate.
Results. Students were generally in agreement with the statements: 'the course was effective for developing my thinking skills e.g. critical analysis, problem solving' (97-100%) and "I have developed my lab skills in this course (91-94%). In the student written comments on the surveys relating to "the best features of the course" many listed the practical classes, including: "Neuronal toxicity prac";"Labs were varied and relevant to lectures"; "Integration of lecture, tutorial & practical component".
Discussion. Overall, the students found this integrated activity extremely engaging and enjoyed the highly relevant nature of this experiment over more traditional "cook book" practical classes. We have developed an innovative new practical class which allows students to advance their independent research skills formulating a testable research hypothesis, and subsequently planning, carrying out and analysing the experiment.

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Medicines education for Junior Medical Officers (JMOs) during hospital orientation.

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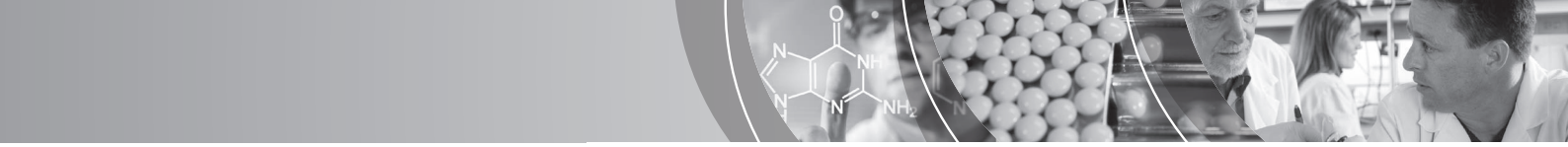
(introduced by Madlen Gazarian, The University of New South Wales, Sydney, NSW).

Introduction. Increasing constraints to medicines education of JMOs with multiple competing demands for JMO time during annual orientation may impact on the quality use of medicines within hospitals.
Aims. To describe the content and format of medicines education conducted in NSW public hospitals during JMO orientation in 2013 and barriers to effective education.
Methods. A link to a SurveyMonkey questionnaire was distributed to all NSW public hospitals pharmacy departments (n=66) in February 2013.
Results. Responses from 28 (42%) were received, with all but 2 providing medicines education. Commonly delivered topics were prescribing on the National Medicines Inpatient Chart (NIMC) (96%), Schedule 8 prescription writing (88%), medication error reduction strategies (88%), hospital formulary (73%) and prescribing high risk medicines (73%). Less common topics were medication reconciliation (46%), medication history taking (35%) and prescribing for vulnerable populations (25%). The majority of hospitals (72%) used didactic lectures of varying length (10-180 minutes). Additional formats included small group tutorials and written material. Seven hospitals used on-line courses. Hospitals, measuring the impact of education (n=11), used quizzes at the time of education (n=6), repeated quizzes throughout the year (n=3), NIMC audits (n=3) and practical completion of tasks such as medication charting (n=3). Barriers included time limitations, high JMO turnover, high volume of education during orientation and lack of co-ordination.
Discussion. There is wide variation in the format and time available for JMO medicines education. There is potential for greater utilization of on-line courses, prior to and during hospital employment, to overcome time constraints during hospital work hours. Further work is required to standardize education, ensuring all JMOs receive a base level of education to an acceptable standard, that the format conforms to effective adult education principles, and that the impact of education is evaluated including linkage with outcomes such as medication errors and adverse outcomes.



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Learning activities to increase student engagement with the results presented in journal articles.

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Introduction. Students when informally surveyed in class indicate that when they 'read' a journal article they look at the abstract and often stop there. They may then read the introduction and discussion (in that order of importance) and actively avoid reading the methods and results sections. In contrast biomedical researchers report that they read the abstract to determine if they want to read the full paper and then focus on the figures and tables presented.
Aims. To give the students confidence in making their own critical analysis of the data presented in journal articles.
Methods. The third level molecular pharmacology course is part of the curriculum for undergraduate students majoring in pharmacology or medicinal chemistry (enrollment of 85 in 2013). Tutorial sessions in this course consist of learning activities led and designed by groups of students on two molecular techniques each fortnight. The following week a 'journal club' is conducted with these techniques being used in the research paper under discussion. A set of questions, which model the approach taken by researchers in critically evaluating research data were provided to help structure the students' engagement with the results. Data was collected via course surveys.
Results. The survey response rate was 78-93% for 2011 to 2013. The majority of the students were in broad agreement with the question 'the course was effective for developing my thinking skills e.g. critical analysis, problem solving' (93-100%). 30-40% of the free text responses on the surveys to the question 'the best features of this course were' listed the tutorial activities. For example, "Although the journal club was very comprehensive and time consuming, I think it was drastically improved my research article reading, understanding and evaluation skills", "Interesting, Challenging journal club increased ability to read and understand papers", "journal club improved my confidence with reading and understanding articles".
Discussion. These learning activities provide students with an understanding of the techniques used and a framework to interpret the data presented in journal articles thus improving confidence in their data analysis skills. Similarly structured reading approaches also resulted in less student anxiety, frustration and improved confidence in regards to data interpretation (Round and Campbell, 2013).

Round JE & Campbell AM (2013) CBE-Life Sci Educ 12:39-46

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Regulation of UDP glucuronosyltransferases 2B15 and 2B17 by microRNAs in cancer cell lines

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Introduction. UDP glucuronosyltransferases (UGTs) inactivate and eliminate cancer-causing chemicals, drugs and steroid hormones. Several studies have shown that UGT expression is down-regulated in cancer cells compared to corresponding normal cells. However, the mechanisms underlying this down-regulation remain poorly characterized, but could include microRNA effects on UGT mRNA stability. In this work, we focus on whether the expression of *UGT2B15* and *UGT2B17*, which help control drug and steroid hormone levels in hormone-dependent cancers, is regulated by microRNAs.
Aim. To determine whether microRNAs can control *UGT2B15* and *UGT2B17* expression in several breast and prostate cancer cell lines.
Methods. Potential regulatory microRNAs were identified by TargetScan, and their effects on UGT expression studied with luciferase reporter assays, site-directed mutagenesis and quantitative real-time polymerase chain reaction (RT-qPCR).
Results. Potential binding sites for 10 microRNAs were identified in the 3'-untranslated regions (3'-UTR) of *UGT2B15* and *UGT2B17*. The expression profiles of the 10 microRNAs in cancer cell lines were examined by Taqman microRNA assays. Transfection of microRNA mimics into the cell lines that express the highest levels of UGTs and the lowest levels of the relevant microRNAs were conducted and RT-qPCR results indicated that microRNAs 376b, 376c, 525, 222 and 331-5p could repress *UGT2B15* and/or *UGT2B17* mRNA levels. To examine whether direct binding of microRNA to UGT mRNA was involved, the 3'-UTRs of *UGT2B15* and *UGT2B17* mRNA were cloned into the pGL3-promoter luciferase reporter vector. The potential microRNA binding sites were mutated and wild-type or mutated reporter constructs were transfected into cells with microRNA mimics. The resultant luciferase activity was down-regulated when miR376c bound to the 3'-UTR of *UGT2B17* and when microRNAs 331-5p, 376b and 376c bound to the 3'-UTR of *UGT2B15*.
Discussion. Taken together, these data provide the first evidence that specific microRNAs can regulate *UGT2B15* and *UGT2B17* expression via alterations in mRNA stability.

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Induction of sulfotransferase 1A3 by dopamine and its role in neuro-protection from dopamine toxicity – role of D1 receptor-NMDA receptor coupling.

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Introduction. Dopamine neurotoxicity is associated with several neurodegenerative diseases and neurons utilize several mechanisms, including uptake and metabolism, to protect them from injury. Metabolism of dopamine involves three enzymes: monoamine oxidase, catechol O-methyltransferase and sulfotransferase. In primates but not lower order animals, a sulfotransferase (SULT1A3) is present that can rapidly metabolize dopamine to dopamine sulphate. In experiments designed to investigate the interaction of the orphan sulfotransferase SULT4A1 with other sulfotransferases, we observed a significant induction in SULT1A3 in the presence of dopamine in human SK-N-MC and SH-SY5Y cells. These observations led us to study the underlying mechanism of induction and to ask whether SULT1A3 had any role in protecting cells from dopamine toxicity.
Aims. To study the underlying mechanism of SULT1A3 induction and to investigate if SULT1A3 has any role in protecting cells from dopamine toxicity
Methods. SUL1A3 induction was assessed by Western analysis of SK-N-MC and SHSY5Y cells. Toxicity was determined by MTT assay.
Results. SULT1A3 and a closely related protein SULT1A1 are highly inducible by dopamine. This involves activation of the D1 and NMDA receptors. Both ERK1/2 phosphorylation and calcineurin activation are required for induction. Pharmacological agents that inhibited induction or siRNA targeting SULT1A3 significantly increased the susceptibility of cells to dopamine toxicity.
Discussion. The results show that dopamine can induce its own metabolism and protect neuron-like cells from damage. A unique feature of the SULT1A3 gene is its presence only in primates. Several researchers have speculated that evolutionary pressure resulting from greater catecholamine demand in humans may be responsible for its emergence. The dysregulation of SULT1A3 expression may be a risk factor for neurodegenerative diseases involving dopamine.

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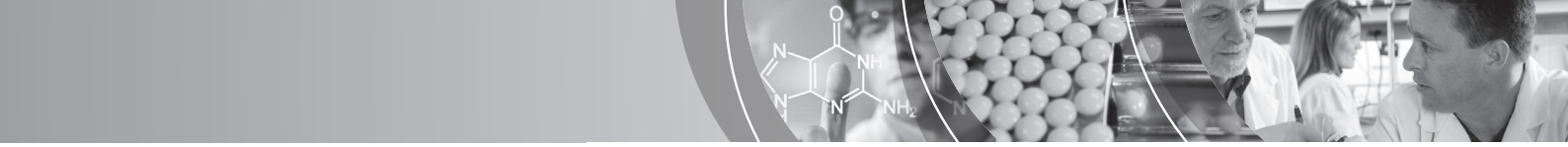
PDZK1 and NHERF1 regulate the function of human organic anion transporting polypeptide 1A2 (OATP1A2) by modulation of cellular trafficking and protein stability

Fanfan Zhou[1], Jian Zheng[1,2], Florence Shin Gee Cheung [1], Ting Chan[1], Ling Zhu[3], Michael Murray[4]. Faculty of Pharmacy, The University of Sydney[1], Camperdown, NSW; Northeast Forestry University[2], Harbin, China; Save Sight Institute, The University of Sydney[3], Sydney, NSW; Discipline of Pharmacology, The University of Sydney[4], Camperdown, NSW.

Introduction. The human organic anion transporting polypeptide 1A2 (OATP1A2) mediates the cellular influx of anionic substances, including a number of clinically important drugs. It has been shown that PDZ domain-containing proteins, especially PDZK1 and NHERF1, regulate the function of certain transporters, but the mechanism is not fully understood.
Aims. We investigated the mechanism by which PDZK1 and NHERF1 regulate OATP1A2 expression and function in an *in vitro* cell model.
Methods. The molecular regulation of OATP1A2 was evaluated in HEK-293 cells that were cotransfected with wild-type and tagged PDZK1 and NHERF1 constructs.
Results. The transport function of OATP1A2 was increased to ~1.6- and ~1.8-fold of control by PDZK1 and NHERF1, respectively, which was impaired by deletion of the putative PDZ-binding domain in the C-terminus of OATP1A2. The increase in OATP1A2 transport was due to increased protein expression at the cell surface. Co-immunoprecipitation experiments indicated that PDZK1 and NHERF1 participated in direct protein-protein interactions with OATP1A2. PDZK1 and NHERF1 modulated OATP1A2 protein expression by altered cellular trafficking, particularly by decreasing transporter internalization, which was found to occur in a clathrin-dependent but caveolin-independent manner. For the first time PDZK1 and NHERF1 were found to stabilise OATP1A2 protein expression in cells.
Discussion. PDZK1 and NHERF1 regulate the transport function of OATP1A2 by dual mechanisms of altered clathrin-dependent internalization and enhanced protein stability. The potential significance of these findings is that PDZ proteins, such as PDZK1 and NHERF1 could be novel molecular targets to modulate the intracellular distribution of drugs that are transported by OATP1A2.



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Casein kinase 2 is a novel regulator of the human organic anion transporting polypeptide 1A2 (OATP1A2)

Florence Shin Gee Cheung [1], Jian Zheng[1,2], Ting Chan[1], Ling Zhu[3], Michael Murray[4], Fanfan Zhou[1]. Faculty of Pharmacy, The University of Sydney[1], Camperdown, NSW; Northeast Forestry University[2], Harbin, China; Save Sight Institute, The University of Sydney[3], Sydney, NSW; Discipline of Pharmacology, The University of Sydney[4], Camperdown, NSW.

Introduction. Human organic anion transporting polypeptide 1A2 (OATP1A2) modulates the influx of anionic substances, including several clinically important drugs such as imatinib (Zhou et al, 2013), into cells. We found recently that a loss of function OATP1A2 gene variant identified in cancer patients carried a defective putative casein kinase 2 (CK2) binding motif.
Aims. This study investigated the role and underlying molecular mechanisms by which CK2 regulates OATP1A2 function in transfected renal cells.
Methods. Transporter uptake assay, biotinylation-based internalization, recycling and targeting assays were used to assess the function, expression and trafficking of OATP1A2 protein in renal cells.
Results. Cellular OATP1A2 function was significantly decreased following treatment with the CK2 specific inhibitor (tetrabromocinnamic acid; TBCA) in a concentration- and time-dependent manner; this was accompanied by impaired transporter expression at the plasma membrane. In further experiments, using a range of biotinylation and stripping protocols, TBCA treatment was found to decrease OATP1A2 internalization, increase transporter recycling and impair membrane targeting. The interplay of CK2 with clathrin- and caveolin-regulated OATP1A2 endocytosis was assessed. In cells that had been pre-treated with clathrin inhibitors, subsequent treatment with TBCA did not produce a further decrease in internalization, which contrasted with the impact of combined treatment with TBCA and caveolin inhibitors on internalization.
Discussion. CK2 is a novel regulator of OATP1A2 function at multiple levels in renal cells. CK2 may regulate OATP1A2 internalization via the clathrin-dependent pathway. The present findings shed light on the mechanisms by which pharmacogenetic variations in OATPs modulate drug performance in patients.

Zhou F et al (2013) AAPS J. 2013 Aug 6. [Epub ahead of print]

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Effect of garlic and gingko on fexofenadine; mechanistic explanation in rats

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Introduction: induction of transporters is an important mechanism of herb-drug interactions. Fexofenadine is transported by P-glycoprotein (P-gp) and organic anion transporting protein (oatp).
Aims: to investigate effect of garlic, gingko and SJW on PK and transport of fexofenadine.
Methods: rats divided into four groups for three experiments, and dosed orally with water (control), garlic (120 mg/kg), gingko (17 mg/kg), or St. John's wort (SJW, 1000 mg/kg) for 14 days. Experiment 1: rats administered fexofenadine iv (10 mg/kg) or orally (100 mg/kg), plasma collected for 24 h and fexofenadine measured by LC-MS. PK parameters were calculated. Experiment 2: livers of rats were perfused with fexofenadine. Concentrations in perfusate were measured by HPLC. CL (total and bilary) and the concentrations in perfusate/liver and liver/bile were calculated. Experiment 3: expression of P-gp and oatp was determined in small intestine by Western blot. Comparisons of PK parameters by ANOVA; those for expression by a t-test. $P < 0.05$ was significant.
Results: garlic and gingko had no effect on PK parameters after iv fexofenadine; SJW increased its CL. Garlic increased AUC and Cmax after oral fexofenadine; garlic and SJW had no effect. Garlic, ginkgo and SJW increased biliary CL from perfusate by 71%, 121% and 234%, respectively. SJW increased biliary CL from liver by 64%. The ratio of liver/perfusate was increased in all treatments. Oatp1a5 in the small intestine was increased by garlic (88%) and SJW (63%). Only SJW increased P-gp (43%). Gingko had no effect.
Discussion: garlic increased absorption of fexofenadine via intestinal oatp. Marginal increase in hepatic CL by gingko was not sufficient to increase CL in vivo and the herb did not affect intestinal transport. In contrast, SJW increased CL in vivo, but its opposing effects on the intestinal transporters meant that it had no overall effect on oral availability.

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Lipid conjugation for drug targetting

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Introduction. The GPCR, Neurokinin 1 receoptor (NK1R), initiates signalling cascades that mediate pain and inflammation. Upon receptor activation by an endogenous ligand such as the neuropeptide Substance P, NK1R is internalised into the cell, which down-regulates the response to pain stimuli. Subsequent to internalisation, SP Is cleaved within an endosome to initiate receptor recycling. Preventing this process may arrest recycling of NK1R; however, drugs need to be targeted to the endosomal network at the site of enzymatic cleavage. Literature suggests that the conjugation of drugs to lipids may improve targeting to specific areas of the cell (Rajendran et al, 2008). The conjugation of drugs to cholestanol may anchor the drug onto the cell surface and during endocytosis of the receptor to target the site of SP degradation.
Aim. To investigate targeted drug delivery towards the endosomal network by the conjugation of cholestanol to drugs.
Method. High resolution confocal microscopy in live HEK293 cells expressing NK1R to observe localisation of cholestanol conjugated fluorophores Cy-5 and Rhodamine B to subcellular structures in comparison to the fluorophores alone.
Results. The Cy 5 lipid conjugate was a more appropriate model for mapping cholestanol directed trafficking than Rhodamine B, as the Rhodamine-cholestanol conjugate was still sequestered in the mitochondria. The Cy5 lipid conjugate localised towards the plasma membrane, before localising into early endosomes at timepoints up to 2 hours. After 2 hours the Cy5 conjugates colocalised with both late endosomes and lysosomes. Internalised NK1R-GFP colocalised with the cy-5 conjugate and early endosomes.
Discussion. We show that drug conjugation to cholestanol can change the cellular trafficking of a fluorophore and could potentially be used for the targeted delivery of drugs to a range of GPCRs and other trafficking proteins in endosomes and lysosomes.

Rajendran L, Schneider A, Schlechtingen G, et al (2008) Science 320:520-523.

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Liver endothelial cell defenestration impairs insulin and glucose uptake in rats

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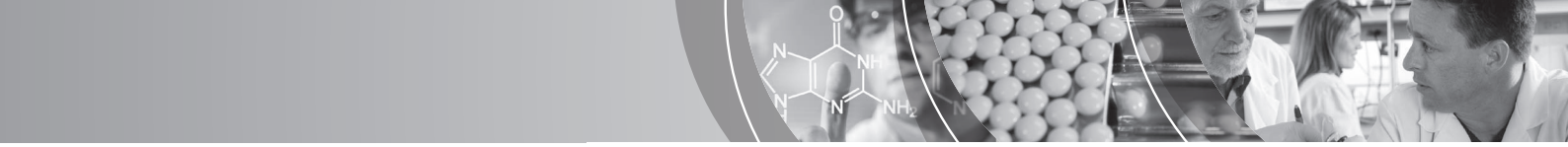
Introduction. The liver is central to insulin signalling and glucose metabolism and changes in liver function have been linked to the pathogenesis of insulin resistance and metabolic syndromes. The liver sinusoidal endothelium is perforated with fenestrations (transcellular pores), which facilitate the transfer of substrates from blood into hepatocytes. We hypothesise loss of endothelial fenestrations (defenestration), such as seen in ageing, will reduce insulin and glucose uptake and subsequent signalling in the liver.
Aims. To investigate insulin and glucose uptake and downstream insulin signalling in normal and defenestrated livers.
Methods. Defenestration is induced in F344 rats with a single i.p. injection of P407 24h prior to experimentation (1g/kg) Multiple indicator dilution method was performed in perfused livers of control (n=9) and treated rats (n=8). The indicators used were Evans Blue (vascular marker), ^{3}H-sucrose (extracellular marker) and either ^{14}C-glucose or ^{14}C-insulin. Absorbance at 620nm and radioactivity of the outflow samples were used to determine the volume of distribution of the indicators. Livers were snap frozen for measurement of protein signalling pathways.
Results. Rats treated with p407 showed a significant reduction in the volumes of distribution as a fraction of the extracellular space for both insulin and glucose, indicating impeded substrate transfer as a result of defenestration (glucose: 1.54±0.06 control vs 1.10±0.10 P407; insulin: 1.08±0.08 control vs 0.81±0.05 P407, $p<0.001$). The limited access of insulin to the hepatocellular membrane was further shown by decreased phosphorylation of Insulin Receptor Substrate-1 protein ($p=0.045$), involved in the early insulin signalling pathway.
Discussion. P407 induced defenestration of the sinusoidal endothelium reduced the volumes of distribution of insulin and glucose in the liver, leading to impaired insulin signalling and sensitivity. This suggests that fenestrations are involved in the transfer of insulin and glucose in the liver and that defenestration may be important hepatic insulin resistance.



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Understanding variability with voriconazole using a population pharmacokinetic approach: Implications for optimal dosing

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Introduction. Voriconazole is a first-line antifungal agent indicated in the treatment of invasive fungal infections. Voriconazole is associated with highly variable, non-linear pharmacokinetics and a narrow therapeutic range.

Aims. This study aimed to investigate voriconazole population pharmacokinetics in adults, including the effect of *CYP2C19* genotype and drug-drug interactions, while also investigating the ability of current dosing recommendations for voriconazole to achieve systemic exposure within the therapeutic range using simulations from the final model.

Methods. Non-linear mixed effects modeling (NONMEM) was undertaken of six voriconazole studies in healthy volunteers and patients. Dosing simulations to examine influential covariate effects and voriconazole target attainment (2-5 mg/L) stratified by CYP2C19 phenotype were performed.

Results. 3352 voriconazole concentrations from 240 participants were analysed. A two-compartment pharmacokinetic model with first-order oral absorption and michaelis-menten elimination best described voriconazole pharmacokinetics. Participants with one or more *CYP2C19* loss-of-function (LoF) alleles had a 41.2% lower V_{max} for voriconazole. Co-administration of phenytoin or rifampicin, St John's wort, predniso(lo)ne, methylprednisolone or dexamethasone significantly increased voriconazole elimination. Among patients receiving voriconazole 200 mg twice daily, trough concentrations on day 7 were predicted to be <2 mg/L for oral and intravenous regimens for 72% and 63% of patients without *CYP2C19* LoF alleles, respectively, with 49% and 35% below this threshold with 300 mg twice daily dosing. Conversely, these regimens resulted in 29%, 39%, 57% and 77% of patients with *CYP2C19* LoF alleles predicted to have voriconazole trough concentrations ≥5 mg/L.

Discussion. Current dosing regimens for voriconazole result in subtherapeutic exposure in many patients who do not carry *CYP2C19* LoF alleles suggesting the need for higher doses, whereas these regimens result in supratherapeutic exposure in a high proportion of patients with reduced CYP2C19 activity. These findings support the essential role of therapeutic drug monitoring in ensuring efficacious and safe voriconazole exposure.

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Acute tocomin treatment improves endothelium-dependent relaxation in aortae from diabetic and western diet fed rats.

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Introduction: Tocotrienols, components of vitamin E with structural similarities to tocopherols, may have beneficial effects on vascular function particularly in pathologies involving oxidant stress eg: diabetes. Tocomin is an extract of palm oil with high tocotrienol content.

Aims: To determine the effect of tocomin (10^{-4}mg/mL, composition: tocotrienol:40%, α-tocopherol:11% and palm olein:38%) on endothelium-dependent relaxation and superoxide production in aortae isolated from diabetic rats and rats fed a standard (SD) or western (WD) diet .

Methods: Wistar rats were treated with vehicle (control) or streptozotocin (50 mg/kg iv) to induce diabetes for 10 weeks. Wistar Hooded rats were fed SD (AIN93G rodent diet, 7% total fat) or WD (SF00-219, 21% total fat). Rats were fed ad lib for 7 weeks after which they were placed on a restricted diet (70% of normal consumption for 10 weeks). Endothelium-dependent relaxation was measured using standard organ bath techniques. Superoxide was measured using lucigenin-enhanced chemiluminessence.

Results: STZ increased blood glucose (control, 5.5±0.3 mmol/L, STZ 27.2±1.9 mmol/L, $p<0.05$) whereas WD had no effect (SD, 5.6±0.3 mmol/L, WD 6.2 ± 0.4 mmol/L). Diabetes impaired endothelium-dependent maximum relaxation (R_{max} control 85±5% vs diabetic 64±4%, $p<0.05$), whereas WD reduced Ach sensitivity (pEC_{50} SD 7.20±0.14 vs WD 6.80±0.09, $p<0.05$). Acute tocomin significantly improved endothelium-dependent relaxation in diabetic rat aortae (R_{max} diabetic+tocomin 87±3%, $p<0.05$) and improved Ach sensitivity in the WD fed rat aortae (pEC_{50} WD+tocomin 7.27±0.12, $p<0.05$). Superoxide was increased in the diabetic and WD aortae (control 573±75 vs diabetic 1053±111 and SD 518±125 vs WD 953±114 superoxide counts/mg dry tissue, $p<0.05$) but was decreased by acute tocomin (diabetic+tocomin 528±58 and WD 953±114 vs WD+tocomin 312±77 superoxide counts/mg dry tissue, $p<0.05$).

Discussion: In diabetic and WD rat aortae the presence of tocomin improves endothelium-dependent relaxation associated with decreased superoxide production.

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Evidence for a role of NOX5 in macrophage pathobiology

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Introduction. Atherosclerosis is an inflammatory disease with macrophages playing a key role in the formation and progression of arterial lesions. Our laboratory has recently shown that the immunomodulatory cytokine, interleukin-4 (IL-4), increases the expression and activity of the reactive oxygen species (ROS) generating enzyme, NOX5, in macrophages. As such we hypothesise that NOX5 contributes to macrophage pathobiology.
Aims. To elucidate the role of NOX5 in macrophage function.
Methods. Human monocytes (THP-1) were differentiated to macrophages with phorbol-12, 13-dibutyrate (PDBu, 10 nM, 24 h). The impact of NOX5 on macrophage function, both in unstimulated and IL-4 (25 ng/ml, 72 h)-stimulated macrophages, was determined via small interfering (si)RNA-mediated gene silencing of NOX5 (50 nM, 72 h). Quantitative real-time PCR was used to measure expression of NOX5 and the scavenger receptor SR-A, while L012-enhanced chemiluminescence and Amplex red were used to measure ionomycin-stimulated superoxide and hydrogen peroxide production, respectively.
Results. IL-4 treatment increased expression of NOX5 2.0-fold (n=4; P<0.05) and ionomycin-stimulated superoxide generation 1.8-fold, (n=5; P<0.05) effects that were abolished by NOX5 siRNA treatment (n=4-5; P<0.05). In addition, in IL-4 treated cells, NOX5 siRNA attenuated hydrogen peroxide production 2.7±0.7 μM (n=6) by 22% (n=6; P<0.05) and reduced the expression of the scavenger receptor, SR-A by 80% (n=3; P<0.05).
Discussion. These findings suggest that NOX5 activity plays a major role in IL-4-dependent increases in macrophage scavenger receptor expression, and may thus be a therapeutic target to limit the damaging effects of macrophage ROS production and lipid uptake in the setting of atherosclerosis.

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Attenuation of BNP effects on neutrophil superoxide release in heart failure patients

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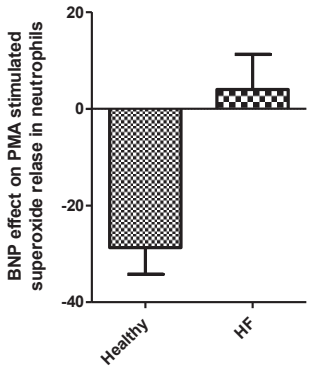
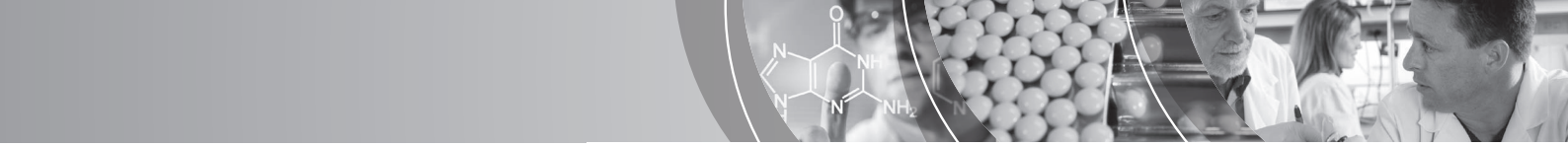
Introduction. Although natriuretic peptides are considered cardioprotective, the net response to endogenous natriuretic peptide is insufficient in heart failure. Equivocal clinical benefit associated with administration of a recombinant B-type natriuretic peptide (BNP), nesiritide, in HF raises the possibility of BNP resistance in such patients.
Aims. To test the hypothesis that BNP responsiveness is impaired in patients with HF.
Methods. HF patients admitted to hospital were studied both at acute presentation and after 3 weeks' therapy. Isolated neutrophils were pre-incubated with either BNP (1μmol/L) or 8-pCPT-cGMP (500μmol/L); superoxide (O_2^-) generated in response to N-Formyl-Methionyl-Leucyl-Phenylalanine (fMLP) (1μmol/L) and phorbol 12-myristate 13-acetate (PMA) (100nmol/L) was quantitated by EPR spectroscopy.



Results. BNP inhibited both PMA and fMLP -associated O_2^- production in healthy subjects by 29±5%(n=21, P<0.01) and 38±7%(n=21, P<0.05) respectively. In acute HF patients, there was significant attenuation of BNP effects versus healthy subjects (P<0.05) for both PMA- and fMLP-induced O_2^- release. Furthermore, the cell-permeable cGMP analog 8-pCPT-cGMP also inhibited neutrophil O_2^- release, with no attenuation of this effect in acute HF patients. Treated HF patients showed no significant sensitization of neutrophils to BNP.
Discussion. BNP inhibits neutrophil O_2^- release. This effect is impaired in both acute and treated HF.



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Evidence for Reciprocal Dysregulation of Asymmetric Dimethylarginine and Myeloperoxidase in Atrial Fibrillation

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Introduction: Atrial fibrillation (AF) has been associated with endothelial dysfunction and inflammatory activation, the latter mediated at least partially by myeloperoxidase (MPO). Previous investigations have suggested that MPO release is inhibited by nitric oxide (NO) and potentiated by the NOS inhibitor asymmetric dimethylarginine (ADMA). MPO also inhibits ADMA metabolism and increases NO catabolism in platelets, contributing to platelet activation. We have reported that the plasma protein thrombospondin-1 (TSP-1) inhibits NO signalling. To date these interactions have only been observed *in vitro*: the current study sought *in vivo* evidence of these interactions in AF.
Methods: Patients hospitalised with AF (n=106) were evaluated. Plasma MPO and TSP-1 concentrations were determined by ELISA, while ADMA was assayed by HPLC. Platelet reactivity to ADP and NO were determined via whole blood impedance aggregometry.
Results: There was a direct correlation between ADMA and MPO ($r=0.220$, $p<0.05$), and between MPO and TSP-1 ($r=0.221$, $p<0.05$). Platelet responsiveness to NO was not significantly correlated with MPO concentrations. Plasma TSP-1 concentrations were directly correlated with extent of ADP-induced aggregation ($r=0.254$, $p<0.01$).
Discussion: These data are consistent with the concept of a nexus between increased concentrations of ADMA (with resultant impairment of NO generation) and release of MPO (with resultant platelet activation and release of TSP-1). The combination of impaired NO effect and inflammatory activation may be critical to pathogenesis and outcomes in AF.

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Omega-3 fatty acid supplementation increases plasma resolvin D1 levels, and decreases aortic dissection, in an apoE$^{-/-}$ mouse model of abdominal aortic dissection

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Introduction. Resolvin D1 (RVD1), a metabolite of the omega-3 fatty acid docosahexaenoic acid (DHA), is a pro-resolving mediator. Since abdominal aortic dissection (AAD) has an inflammatory origin (Saraff et al 2003), we hypothesised that prior supplementation of apolipoprotein E$^{-/-}$ angiotensin II-infused (apoE$^{-/-}$ angII) mice with a high DHA-content diet might increase plasma RVD1 concentration, and attenuate the inflammatory response and AAD.
Aim. To determine the effect of a low- (L-DHA, negligible content) or high-DHA diet (H-DHA, 0.30%) on plasma RVD1 and neutrophil infiltration, in an apoE$^{-/-}$ and C57 angII AAD mouse model.
Methods. Male, 3-4 week apoE$^{-/-}$ and C57 mice received L-DHA or H-DHA for 8 weeks. Mice were infused with angII (1000 ng/kg/min) or 0.9% saline, for 2 days. Plasma RVD1 was determined by enzyme immunoassay, and aorta was embedded, sectioned and stained for neutrophils.
Results. Plasma RVD1 concentration was similar in saline-infused C57 L-DHA (374±41 pg/ml, n=10) and H-DHA mice (366±37 pg/ml, n=9). RVD1 concentration was higher in H-DHA, angII-infused C57 mice (584±77 pg/ml, n=10) than the H-DHA, saline-infused C57 mice ($P<0.05$). In apoE$^{-/-}$, angII-infused mice, RVD1 concentration was higher in H-DHA (743±76 pg/ml, n=9) than L-DHA mice (457±60 pg/ml, n=9; $P<0.05$). Aortic dissection was only observed in angII-infused mice on the L-DHA diet (5/20 mice), with neutrophils localised to the adventitia of these vessels. In angII-infused mice, RVD1 concentration was higher in non-dissected H-DHA (659±56 pg/ml, n=19), than non-dissected L-DHA (469±45 pg/ml, n=15), and dissected L-DHA mice (308±43 pg/ml, n=4; $P<0.05$).
Discussion. The pro-inflammatory stimulus plus high DHA diet was associated with elevated plasma RVD1 levels, fewer dissected aortas, and fewer infiltrating neutrophils. The findings suggest that dietary supplementation with DHA may increase levels of a pro-resolving mediator, and protect against inflammation in a mouse model of AAD.

Saraff K et al (2003) Arterioscler Thromb Vasc Biol 23:1621-1626

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Distinct roles for the two human platelet thrombin receptors, PAR1 and PAR4, in thrombus formation

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Introduction. Platelet-dependent arterial thrombosis is the most common cause of death in Australia and drugs that block platelet function are sought for improved anti-thrombotics. Thrombin is a potent platelet activator and works via two receptors, PAR1 and PAR4. PAR antagonists are in clinical development as novel anti-thrombotic agents, however current antagonists target only PAR1 and the function of PAR4 is poorly understood.
Aims. To define the relative contributions of PAR1 and PAR4 to human platelet function in the setting of thrombus formation in order to determine the potential utility of PAR4 antagonists for the prevention of arterial thrombosis.
Methods. We developed a highly specific function blocking anti-PAR4 antibody and synthesised the PAR1 antagonists under clinical investigation. We used these pharmacological tools to examine the role of PAR1 and PAR4 in a range of in vitro assays of human platelet function and an ex vivo whole blood thrombosis model.
Results. In human isolated platelets, thrombin-induced phosphotidylserine exposure was inhibited by the PAR4 blocking antibody (200 μg/ml) alone, and was unaffected by a PAR1 antagonist (E5555, 0.1 μM). No other platelet function examined (aggregation, granule release, or integrin IIbIIIa activation) was inhibited by PAR4 antagonism alone. We next assessed thrombin generation in platelet-rich plasma as a physiological consequence of phosphotidylserine exposure and observed that thrombin-mediated platelet procoagulant activity was also inhibited by antagonism of PAR4, but not PAR1. Finally, an ex vivo human whole blood thrombosis assay showed that both the PAR1 and PAR4 antagonists similarly reduced platelet deposition into growing thrombi, but that fibrin deposition was significantly reduced only by PAR1 inhibition.
Discussion. These results suggest that PAR1 and PAR4 perform divergent functions during platelet activation and indicate that PAR4 antagonists may provide distinct utility to existing PAR1 antagonists for the prevention of arterial thrombosis.

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Pharmacological inhibition or genetic deletion of the AT4 Receptor/IRAP provides protection against Ang II-mediated cardiac hypertrophy and cardiac fibrosis

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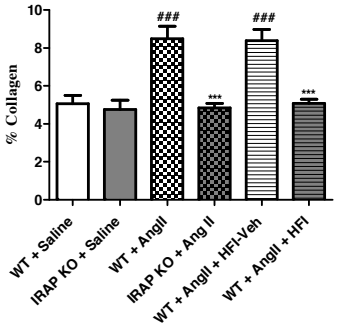
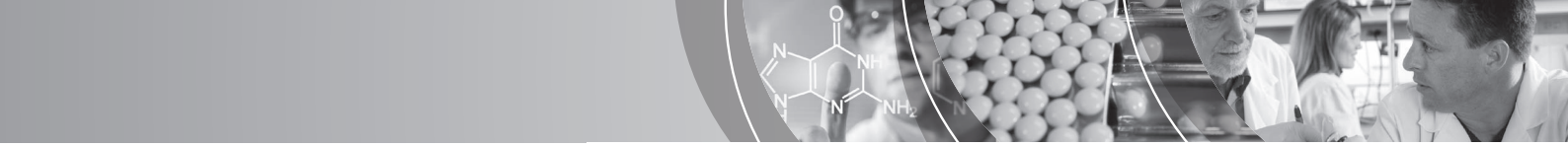


Introduction. Angiotensin (Ang) IV mediates its effects by inhibiting the enzyme insulin-regulated aminopeptidase (IRAP), also known as AT4 receptor (AT4R), with recent studies showing chronic Ang IV treatment is vaso-protective (Vinh et al., 2008a,b).
Aims. We investigated whether IRAP deficiency or pharmacological inhibition of IRAP would prevent development of Ang II-induced cardiac hypertrophy and cardiac fibrosis.
Methods. Male IRAP knockout (IRAP KO) and WT mice aged between 4-6 months were chronically infused with saline or Ang II (4 weeks; 800ng/kg/min). A group of WT mice were also chronically treated with the synthetic IRAP inhibitor, HFI419 (500ng/kg/min) or HFI vehicle for 4 weeks. Ventricular weight to body weight (VW:BW) or tibial length (VW:TL) ratios were assessed for cardiac hypertrophy, while H&E and picrosirius red stained heart sections were used to assess cardiomyocyte hypertrophy and collagen deposition, respectively. Collagen synthesis mechanisms and inflammatory markers were assessed using immunofluorescence and western blot analysis.
Results. Ang II-induced cardiomyocyte hypertrophy was prevented in both IRAP KO mice and HFI419 treated WT mice (n=4; $P<0.05$). Furthermore, Ang II-induced collagen deposition was also attenuated by ~40% in IRAP deficient and inhibition models (n=6; $P<0.001$), with decreased TGF-β expression and reduction in α-SMA expressing myofibroblasts. Both IRAP deficiency and inhibition also exhibited reduced inflammation with a significant decrease in NFκB activation compared to the WT controls (n=6; $P<0.001$).
Discussion. Overall prevention of Ang II-induced collagen deposition most likely results from decreased collagen synthesis via synthetic myofibroblasts, possibly due to decreased expression of TGF-β leading to prevention of fibroblasts differentiating into myofibroblasts. In conclusion, genetic deletion or pharmacological inhibition of IRAP protects against Ang II-mediated cardiac hypertrophy, cardiac fibrosis and inflammation, indicating the potential use of IRAP inhibitors as a therapeutic treatment for cardiovascular disease.
Vinh A et al (2008a) Atherosclerosis 200:37-44
Vinh A et al (2008b) Cardiovasc. Res77:178-187



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The epidermal growth factor receptor ErbB4 mediates neuregulin-induced, but not angiotensin II-induced, cardiomyocyte hypertrophy.

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Introduction. Cardiomyocyte hypertrophy is characterized by increase in cell mass without proliferation. Changes at the molecular level include activation of MAPK signalling, reactivation of the "foetal gene program" and the reorganization of actin. Angiotensin II (Ang II) stimulates cardiac hypertrophy, a process that may involve the transactivation of epidermal growth factor receptors (ErbBs). Three subtypes of ErbBs are present in the postnatal heart (ErbB1, 2, 4), however their precise roles in cardiomyocyte function remain poorly understood.
Aims. To determine if AngII activates cardiomyocyte hypertrophy by transactivation of ErbBs, particularly ErbB4.
Methods. Ventricular cardiomyocytes from neonatal rats were treated with Ang II (100 nM) or the ErbB4 agonist neuregulin-1 (NRG1; 10 nM) to induce cardiomyocyte hypertrophy. ErbB1, 2, 4 were down-regulated using shRNA and siRNA. Reporter assays were used to measure the activity of hypertrophic signalling marker genes myosin light chain 2v (MLC-2v), cyclin D and atrial natriuretic peptide (ANP). MAPK activation was measured via Western blot. Hypertrophic growth was assessed by changes in protein:DNA ratios and sacromeric reorganization.
Results. NRG1 caused robust activation of MLC-2v that was significantly reduced by silencing of ErbB4, but not by ErbB1 or ErbB2, confirming that ErbB4 activation contributes to hypertrophic signalling. However, knockdown of ErbB1, 2 or 4 did not affect Ang II-induced activation of MLC-2v. Similar results were seen for ANP and cyclin D. Similarly, ErbB4 knockdown reduced NRG-induced MAPK activation, whilst Ang II-induced MAPK activation was unaffected. NRG1-induced hypertrophic growth (protein to DNA ratio) and actin reorganization was also reduced by down-regulation of ErbB4.
Discussion. NRG1-induced hypertrophy requires ErbB4 whist Ang II-induced hypertrophic signalling may not involve transactivation of ErbB receptors. We are now extending these findings *in vivo;* we have established a model of cardiac-specific ErbB4 deletion using Cre/Lox recombination, and are undertaking echocardiographic analysis to measure cardiac hypertrophy and function.

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Pharmacokinetic / pharmacodynamic strategies to optimise patient therapy

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Introduction. Despite significant advances in antibiotic therapy, many infections such as osteomyelitis remain challenging. Treatment of such and other infections is becoming increasingly difficult due to the rapid rise in multi-drug resistant bacteria which present one of the most serious threats to global health. Therefore it is critical to optimise the use of available antibiotics based on pharmacokinetic / pharmacodynamic approaches that are ideally suited to design safe and effective treatment strategies.
Aims. To develop innovative dosing strategies via latest, translational experimental and modelling approaches.
Methods. *In vitro* time kill studies were performed to assess the effect of various antibiotics and their combinations on methicillin-resistant *Staphylococcus aureus* (MRSA). New mechanism-based models were developed to describe and predict bacterial killing from combination and monotherapy simultaneously. To assess the concentrations at the site of bone infections, clinical trials were performed in hip replacement patients to quantify the extent and time course of antibiotic bone penetration, utilising state-of-the-art bioanalytical techniques and population modelling methods. Translational pharmacokinetic/pharmacodynamic approaches were applied to design optimal treatment strategies.
Results. Combination regimens with sequential and simultaneous dosing and the first mechanism-based models for antibiotic combinations allowed a quantitative characterisation of different types of antibiotic synergy. To bridge from these *in vitro* insights to optimal patient therapy, we reverse-engineered the pharmacokinetic / pharmacodynamic targets required for successful treatment of osteomyelitis. The employed population pharmacokinetic modelling and Monte Carlo simulations impacted treatment strategies for osteomyelitis by clinicians worldwide and decisions by the US FDA of optimal dosing of children.
Discussion. Latest *in vitro* approaches combined with mechanism-based modelling support an improved understanding of antibiotic synergy and prospective evaluation of combination dosing strategies to combat resistant bacterial 'superbugs'. State-of-the-art bioanalytical, modelling and simulation methodologies enabled the identification of pharmacokinetic / pharmacodynamic targets and significantly impacted regulatory decisions and optimal patient therapy.

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Engineering detergent-stable G protein-coupled receptors enables biophysical profiling of ligand binding and conformational dynamics

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Introduction. The G protein-coupled receptor (GPCR) gene family is the largest in the human genome and one of the most important classes of drug targets. GPCRs sample a range of conformational states, with the equilibrium shifting depending on factors such as the presence of different ligands. This plasticity allows GPCRs to respond to stimuli in different ways depending on the cellular context, but makes pharmacological targeting challenging. Furthermore, structure based drug design efforts targeting GPCRs are encumbered by the high level of difficulty associated with applying biophysical methods such as X-ray crystallography, nuclear magnetic resonance spectroscopy (NMR) and surface plasmon resonance (SPR) to GPCRs. GPCRs need to be extracted from cell membranes using detergents for purification but are typically unstable in detergent micelles. We use a novel directed evolution method, called CHESS, to engineer detergent-stable GPCRs that can be purified and stored in the solubilised form for weeks. This allows us to apply biophysical methods such as NMR to the receptors, probing both the binding of ligands and the conformational dynamics underlying GPCR function.
Aims. To apply NMR based methods to a CHESS engineered neurotensin receptor-1 (NTS_1) variant, $enNTS_1$.
Discussion. Detergent solubilised $enNTS_1$ was purified in the unbound (apo-) state and remained able to bind ligands for at least 2 weeks. The binding of several neurotensin peptide variants was detected using saturation transfer difference NMR (STD-NMR), a method commonly used for screening fragment libraries in structure based drug design campaigns. Furthermore, by isotopically labelling $enNTS_1$ we could record the conformational fingerprints of the receptor in different conditions. This proof-of-principle study demonstrates that stabilised receptors are novel tools for furthering our understanding of the relationship between GPCR structure and function and enables the application of fragment screening and structure based drug design to this important class of membrane proteins.

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Novel roles for magnesium channels in cardiovascular disease and development.

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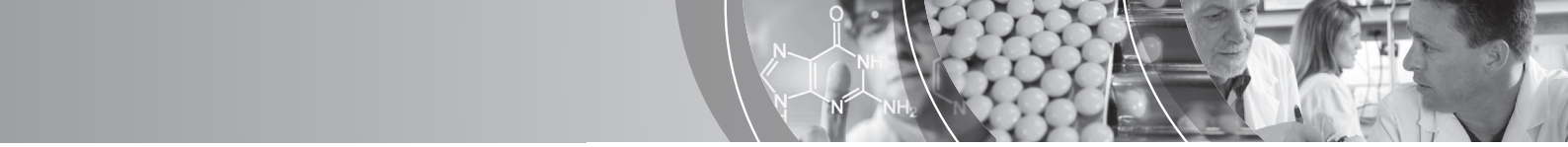
Introduction. Magnesium (Mg^{2+}) is an abundant intracellular cation that is critical for protein synthesis and cellular growth. Clinical and epidemiological evidence indicates a link between Mg^{2+} deficiency and cardiovascular disease, however the underlying mechanisms remain unclear. Two members of the transient receptor potential melastatin channel family (TRPM6 and TRPM7) are now recognised as major regulators of Mg^{2+} transport. Unusually, these channels also contain a kinase domain, making them dual function proteins that can transport cations and activate intracellular signalling. However, to date their function in the heart remains unclear.
Aims. To examine the potential function of these magnesium channels, particularly TRPM7, in the heart.
Methods. qPCR was used to measure channel expression throughout development, and in a mouse model of angiotensin II (AngII)-induced cardiac hypertrophy. Isolated ventricular cardiomyocytes from neonatal rats were used for *in vitro* experiments. TRPM7 was inhibited either pharmacologically (NS8593, 10 μM) or by RNA interference before exposing cells to pro-hypertrophic stimuli (AngII, neuregulin) and measuring indices of growth and remodelling.
Results. Both TRPM6 and TRPM7 are expressed in the heart, with levels ~2-4-fold higher in the fetus than the adult. AngII causes cardiac hypertrophy and increases TRPM6 and TRPM7 expression (4.9 and 2.3 fold respectively vs vehicle). *In vitro*, AngII and neuregulin both activate hypertrophic signalling pathways (re-induction of myosin light chain 2v, atrial natriuretic peptide and cyclinD) and cause sacromeric reorganisation. Inhibition of TPRM7 via RNA interference or NS8593 treatment prevented these pro-hypertrophic alterations.
Discussion. Both Mg^{2+}-permeable channel-kinases (TRPM6 and TRPM7) are expressed in the heart, and AngII can regulate the expression of these channels. The TRPM7 cation channel contributes to hypertrophic signalling, growth and cytoskeletal reorganisation in isolated cardiomyocytes. We are now investigating whether this is due to Mg^{2+} transport by the channel, or due to activity of its innate kinase domain.



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Interventions to improve healthspan and lifespan in mice

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Aging results in a progressive decline in all organ systems having a negative impact on reproductive, cognitive, physical, metabolic function and survival. Understanding the mechanisms underlying these processes has become one of the primary focuses in the field of gerontology. For decades the goal of rodent studies was to identify interventions that lead to increases in both mean and maximal lifespan paying little attention to the impact of that particular intervention on overall health and wellbeing of the organism. The aim of all these studies was to ultimately translate these findings to humans to delay the onset of aging and age-associated diseases. Here we will present data showing the effect of pharmacological and dietary interventions that improve the healthspan and lifespan of mice. Results include responses to oral glucose tolerance tests, locomotor activity in behavioral testing and the effects of the interventions on longevity.

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Drug discovery: lessons from evolution

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A common view within the pharmaceutical industry is that there is a problem with drug discovery and we should do something about it. There is much sympathy for this from academics, regulators and politicians. In this article I propose that lessons learnt from evolution help identify those factors that favour successful drug discovery. This personal view is influenced by a decade spent reviewing drug development programmes submitted for European regulatory approval. During the prolonged gestation of a new medicine few candidate molecules survive. This process of elimination of many variants and the survival of so few has much in common with evolution, an analogy that encourages discussion of the forces that favour, and those that hinder, successful drug discovery. Imagining a world without vaccines, anaesthetics, contraception and anti-infectives reveals how medicines revolutionized humanity. How to manipulate conditions that favour such discoveries is worth consideration.