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Innovation in therapeutics: Fundamental research to clinical impact
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BOOK OF ORAL ABSTRACTS

100 SPARKing translation of fundamental research to clinical impact

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While science and technology are now more innovative and successful than ever, their translation into novel treatments and therapeutics to address key health problems remains a challenge. Recognizing that to close the industry/academia divide, we created the SPARK At Stanford program, in which scientists from both sides work more closely together. SPARK, created twelve years ago, is a partnership between Stanford University and volunteers from the local biotechnology, pharmaceutical, and health care investment industries. SPARK's mission is three-fold: first, to help academic investigators overcome the obstacles intrinsic to moving research discoveries from bench to bedside; second, to educate faculty and trainees about the translational research process so that development of promising new discoveries becomes second nature, and so that trainees are better prepared for potential industry careers; and third, to promote efficient, cost-effective, and innovative approaches to discovery and development. So far, ~60% of the >100 projects have been licensed to companies and/or entered clinical trials. Through weekly meetings, SPARK's activities conducted on campus, provide a rich learning experience that is open to faculty, staff, students, and postdoctoral fellows; this ensures that the know-how remains here and that the out-of-the-box and risk-taking attitude of academia is maintained, while industry's real-life experience is implemented. SPARK has been 'exported' to other academic institutions and we have now formed a Global SPARK community to promote translational research in over three dozen academic institutions on five continents.

Using some examples, I will discuss how SPARK works within the barriers to translating our academic discoveries and what academia can do about them to benefit patient and society.

101 Measuring drug where it matters: the importance of micro-pharmacokinetics on observed receptor pharmacology

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The affinity and kinetics of a drug binding to its target receptor are almost exclusively calculated using equations that assume the interacting molecules are homogeneously distributed in a solvent, with the concentration of drug available to bind target being equal to that in the bulk aqueous phase. While this assumption applies well to soluble enzymes, it is less satisfactory for membrane-associated targets (e.g. GPCRs) where the protein is embedded in a phospholipid bilayer. This is because the inclusion of phospholipid adds an additional amphiphilic compartment into which drugs may partition, depending on their physicochemical properties. In addition, the physical barriers associated with some physiological compartments (e.g. synapses) may restrict drug diffusion away from the receptor-compartment, further promoting drug "rebinding". This talk will introduce the concept of micro-pharmacokinetics and outline our recent efforts to measure local drug concentrations at a sub-cellular level. It will then explore how this influences observed drug action, both *in vitro* and in the clinic, using the extrapyramidal side effects of antipsychotic dopamine D2 receptor antagonists as a case study. Finally it will argue that in certain circumstances, tissue-specificity of drug action might be achieved through careful optimisation of drug rebinding.

102 Functional Divergence of Delta and Mu Opioid Receptor organization in pain circuits

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Cellular interactions between delta and mu opioid receptors (DORs and MORs, respectively), including heteromerization, are thought to regulate opioid analgesia. However, the identity of the nociceptive neurons in which such interactions could occur in vivo remains elusive. To address this question, we examine the distribution and function of DORs and MORs in pain circuits, using mouse genetics, neuroanatomy and circuit tracing, electrophysiology, optogenetics, transcriptomics, and behavioral analysis. We previously showed that DORs and MORs are expressed by predominantly different primary afferent neurons, cutaneous myelinated mechanosensory neurons and peptidergic nociceptors, respectively. We report here that MORs expressed by TRPV1+ nociceptors initiate tolerance and opioid-induced hyperalgesia (OIH) development. RNA sequencing and histological analysis revealed that MORs are expressed by nociceptors, but not by spinal microglia. Deletion of MORs specifically in TRPV1+ nociceptors eliminated morphine tolerance, OIH and pronociceptive synaptic long-term potentiation without altering antinociception. Furthermore, we found that co-administration of methylnaltrexone bromide, a peripherally restricted MOR antagonist, was sufficient to abrogate morphine tolerance and OIH without diminishing antinociception in perioperative and chronic pain models. In the CNS, we found that DOR-MOR co-expression is limited to small populations of excitatory interneurons and projection neurons in the spinal cord dorsal horn and unexpectedly predominates in ventral horn motor circuits. Similarly, DOR-MOR co-expression is rare in parabrachial, amygdalar, and cortical brain regions processing nociceptive information. We further demonstrate that in the discrete DOR-MOR co-expressing nociceptive neurons, the two receptors internalize and function independently. Finally, conditional knockout experiments revealed that DORs selectively regulate mechanical pain by controlling the excitability of somatostatin-positive dorsal horn interneurons. Collectively, these results illuminate the functional organization of DORs and MORs in pain circuits and reappraise the importance of DOR-MOR cellular interactions for developing novel opioid analgesics.

Corder at al., Nature Medicine, 2017. Wang et al., Neuron, 2018

103 CGRPα within the TRPV1-cre population contributes to visceral nociception

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The role of CGRPα in visceral and somatic nociception is incompletely understood. We have investigated the effects of deletion of the CGRPα gene from the TRPV1-cre population in visceral and somatic nociception. Cre-lox recombination was associated with significant reductions in CGRPα-mCherry fluorescence, but not total in CGRP immunoreactivity within dorsal root ganglia (DRG), suggesting that selective deletion of CGRPα was associated with upregulation of CGRPβ. The amount of time mice spent stationary, following injection of acetic acid (0.6%, i.p), was significantly less in α*CGRP*$^{lx/lx}$;Trpv1-Cre mice. However, there was no difference in the writhing reflex (number of abdominal stretches), nor number of abdominal scratches in both genotypes. It was found that α*CGRP*$^{lx/lx}$;Trpv1-Cre mice developed a more pronounced hyperalgesia in comparison to their baseline values when compared to controls. However, Hargreaves test revealed no difference between the two genotypes at any time point after the injection. Mechanosensitivity of spinal afferent nerve endings innervating the mouse colon was unaffected in α*CGRP*$^{lx/lx}$;Trpv1-Cre mice; as was the relative proportion of stretch-sensitive and insensitive afferents. Odour avoidance test, odour preference test and buried food test also revealed no differences in α*CGRP*$^{lx/lx}$;Trpv1-Cre mice. The findings suggest that αCGRP-mediated transmission within the Trpv1-Cre population is not essential for the development and transmission of heat or inflammatory heat hyperalgesia. However, the data suggest that CGRPα in TRPV1-cre population plays a significant role in duration of visceral nociception. The observation that the writhing reflex was not similarly affected, suggests that different pathways control these two different visceral nociceptive behaviours.

104 Using novel toxin peptides to understand bladder mechanosensation

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The bladder is innervated by extrinsic afferents that project into the dorsal horn of the spinal cord, providing sensory input to the micturition centres within the central nervous system. Under normal conditions, the continuous activation of these neurons during bladder distension goes mostly unnoticed. However, for patients with chronic urological disorders such as overactive bladder syndrome (OAB) and interstitial cystitis/painful bladder syndrome (IC/PBS), exaggerated bladder sensation and altered bladder function are common debilitating symptoms. There is now significant clinical and pre-clinical evidence that both OAB and IC/PBS are related to structural, synaptic, or intrinsic changes in the complex signaling pathways that mediate bladder sensation, however, determining the specific mechanisms responsible for such changes remains challenging. In these studies, we used single-cell reverse-transcription polymerase chain reaction of retrogradely traced bladder innervating dorsal root ganglia (DRG) neurons to determine the expression profile of voltage gated sodium (Na_v) channels, and patch-clamp recordings to characterise the contribution of tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) Na_v channels to total sodium current and neuronal excitability. We also used *ex-vivo* bladder afferent recordings to characterise populations of mechanosensitive afferents in response to graded distension in the presence of novel toxin peptides that exhibit selectivity to activate or inhibit specific Na_v channel subtypes. These data demonstrate an essential role for TTX-S Na_v channels in the regulation of bladder-innervating DRG neuroexcitability, bladder afferent responses to distension, and nociceptive signalling to the spinal cord.

105 Defining Opioid Receptor Function in the Enteric Nervous System

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Introduction. Opiates are effective analgesics for controlling moderate to severe pain. However, their clinical utility can be greatly limited by several on-target and centrally-mediated side effects, including the development of dependence and analgesic tolerance. Endogenous opioids, such as enkephalin, are also major inhibitory regulators of intestinal motility and secretion. Opioid analgesics can therefore have significant negative effects on gastrointestinal function, including the development of intractable opiate-induced constipation. Existing approaches to target the mu opioid receptor (MOR) to promote analgesia while limiting constipation have largely been ineffective and many of the advances in this area have been incremental. This reinforces the need to better understand the unique mechanisms underlying opioid-induced constipation and to test new pharmacological approaches to therapy in the most clinically appropriate systems. The delta opioid receptor (DOR) has been proposed as an alternative therapeutic target to MOR for the treatment of chronic pain, migraine, anxiety and depression. However, whether acute and chronic treatment with DOR agonists leads to equivalent adverse effects of on gastrointestinal function has not been examined in detail. This presentation describes recent studies by our group into the signalling, trafficking and function of opioid receptors in the enteric nervous system. Specifically, the development of tolerance by enteric neurons to DOR agonists, the potential for MOR-DOR interactions in the enteric nervous system, and the possible use of positive allosteric modulators of DOR for the treatment of gastrointestinal motility disorders will be discussed.

106 Electroceuticals as innovative strategies to target inflammatory bowel disease

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The therapeutic use of electric stimulation to activate peripheral autonomic nerves is in use or has been trialled in humans for a range of gastrointestinal and metabolic disorders including gastroparesis, incontinence, constipation and obesity (Payne et al 2018). The discovery that stimulation of autonomic pathways, in particular the vagus nerve, can inhibit inflammation has opened up a new approaches to the treatment inflammatory bowel diseases including Crohn's disease and ulcerative colitis. Crohn's disease is generally seen in young people and currently has no cure. Despite pharmaceutical interventions including anti-inflammatory drugs and biologicals, surgery to remove the inflamed bowel becomes necessary in 9/10 patients and signs of recurrence develop in 8/10 cases within 1 year after surgery. A small scale clinical trial of cervical vagal stimulation has reported some efficacy in Crohn's disease, but with substantial side effects (Bonaz et al). We are currently working to better understand the mechanisms by which vagal stimulation inhibits gut inflammation and develop a sub-diaphragmatic vagal implant for therapeutic use in humans. We provide evidence that an inflammogen applied to the lumen of the small intestine can act via peripheral reflexes to increase efferent sympathetic nerve activity to the gut. Data from previous studies suggests that this activation could inhibit and/or exacerbate gut inflammation depending on the types of sympathetic efferent nerves activated. Other studies have demonstrated that vagal stimulation inhibits systemic inflammation via activation of afferent pathways and modulation of spinal/sympathetic efferent pathways to various abdominal organs. We have confirmed that stimulation of the abdominal vagus inhibits gut inflammation in a rat model of ileitis, and we are investigating the mechanisms and the optimum stimulus parameters. We have tested an implant to stimulate the abdominal vagus, monitor electrode impedance and record compound nerve action potentials. We have demonstrated its long term efficacy in stimulating the nerve, its safety and its lack of off-target effects when placed below the cardiac vagal branches in a large animal (sheep). We are currently planning a safety trial in post-surgical Crohn's patients.

Payne, Furness and Stebbing (2018) Nature Rev Gastro Hep (in press) https://doi.org/10.1038/s41575-018-0078-6
Bonaz et al (2016) Neurogastroenterol. Motil. 28, 948–95

107 Endosome and mitochondrial reactive oxygen species as novel targets in respiratory infectious disease

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Influenza A virus infections are a substantial global burden, resulting in significant morbidity and mortality. The WHO estimates ~650,000 deaths associated with influenza A virus annually, which costs the Australian Health Care System ~$115 mill per year and US system ~$11 billion per year (Putri *et al*., 2018; ISG). The current prophylactic treatment strategies include vaccines and antivirals but both of these have limitations. Vaccines provide little protection against new or emerging strains of viruses that enter the population. Antivirals can be effective in alleviating clinical symptoms of influenza virus infection, but usually have a narrow window of administration; they can cause adverse effects; and are subject to strain resistance. There is therefore an urgent need for alternative therapeutic approaches for influenza virus infection, regardless of the strain or pathogenicity. We and others have recently shown that oxidative stress underpins respiratory infectious disease and have highlighted several enzymatic and non-enzymatic sources of ROS as novel therapeutic targets. ROS are either deliberately generated by the immune system, by specific NADPH oxidases or produced during cellular metabolism by the mitochondria. Given that ROS are highly reactive molecules, their site of production governs their cellular targets and biological function. For example, phagocytic cells engulf bacteria and initiate phagocytosis and then within the confines of the phagosome, deliver bursts of ROS to these pathogens. Moreover, virus internalization by endocytosis results in ROS generation in endosomes via NOX2-containing NADPH oxidase (To *et al*., 2017). These emerging paradigms of subcellular compartmentalization of ROS production have unravelled novel organelle-specific ROS inhibitors including endosome targeted NOX2 inhibitors, and mitochondrial-targeted antioxidants. Pre-clinical animal model studies from our laboratory have shown suppression of influenza A virus pathology by use of endosome- and mitochondrial-targeted ROS inhibitors. Therefore, understanding subcellular ROS production during influenza virus infection will likely reveal new targets for treating these debilitating respiratory infectious diseases.

Putri *et al*., (2018). Vaccine, 36, 3960-3966.
Influenza specialist Group (ISG)- http://www.isg.org.au/index.php/about-influenza/impact-of-influenza/
To *et al*., (2017). Nature Communications, 8, 69. DOI: 10.1038/s41467-017-00057-x

108 Angiotensin II Type 2 receptors as targets in inflammatory and fibrotic disease

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Cardiovascular disease (CVD) is associated with pathological alterations including left ventricular (LV) dysfunction and increased organ fibrosis such as in heart and kidneys that can lead to end organ damage/failure. The treatment of pathological fibrosis is now recognised as a large unmet clinical need as current therapies are only moderately successful in ameliorating CVD progression. One of the main drivers of fibrosis is inflammation, which illustrates the multi-faceted nature of extracellular matrix deposition, which is also influenced by conventional risk factors such as hypertension and ageing. Much of our laboratory's work has focused on less established aspects of the renin angiotensin system, including angiotensin type 2 receptors (AT_2R). When stimulated, AT_2R are now thought to exert cardiovascular-protective effects in CVD and thus counter-balance excessive AT_1 receptor stimulation by angiotensin II (Wang et al 2017). It has taken considerable time to recognise AT_2R as a potential therapeutic target. This issue has arisen because of the difficulty in studying AT_2R acutely in functional assays and because research was hampered by a lack of pharmacological tools with which to probe AT_2R function. In this presentation, the cardiovascular effects of a number of novel AT_2R agonists will be discussed in the context of their potential anti-inflammatory and anti-fibrotic effects, thus confirming AT_2R as a novel target in CVD.

Wang Y et al (2017) Front Pharmacol 8:564. doi: 10.3389/fphar.2017.00

109 Nanoparticles as delivery vehicles in inflammatory disease

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Introduction. Vaccines are a cost-effective intervention to limit the spread of disease, with the ultimate aim of eradication. New vaccines are needed for complex diseases, which require novel delivery systems and adjuvants to induce protective and long-lasting immunity. Non-inflammatory nanoparticles in the viral size range can interact with the immune system and enhance immune responses. Incorporating these nanoparticles into vaccine formulations may be one possible method to target complex diseases.
Aims. To examine the protective and immunogenic responses of nanoparticles and their interaction with immune cells, such as antigen presenting cells.
Methods. Nanoparticles of different material compositions (i.e. polystyrene and iron oxide) were assessed in animal models for their ability to induce immune responses and protective effects against different diseases, including malaria and cancer. Lung models were also utilised to assess the anti-inflammatory properties of nanoparticles.
Results. Particle size is critical to elicit the desired immune response. Inorganic polystyrene nanoparticles in the viral size range are non-inflammatory, target dendritic cells but do not induce suppressive cell subsets, and can protect against cancer, malaria and acute lung inflammation. Novel biodegradable vaccine delivery systems are also comparable to conventional adjuvants (i.e. CpG) at inducing antibodies and T cells.
Discussion. Vaccines incorporating nanoparticles interact with cells of the immune system to induce beneficial and protective immune responses. Ideally these vaccines will include biodegradable nanoparticle formulations for future translation.

Fifis *et al* (2004) Journal of Immunology 173:3148-3154

110 Aging and caloric restriction research: a biological perspective with translational potential

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Metabolic dysfunction is coincident with the onset and progression of numerous age-related diseases and disorders. Several diseases of aging, including diabetes, cancer, and neurodegeneration, have an established metabolic component. Our studies have focused on links between metabolic status and disease vulnerability. Caloric restriction (CR) delays aging and the onset of age-related disease in diverse species, including rodents and nonhuman primates (NHP). Our work demonstrates that CR animals are metabolically distinct from their control counterparts. Molecular profiling identifies CR responsive elements that are highly enriched for metabolic pathways and in particular mitochondrial processes. The complexity of the CR response is enabled through regulation at multiple levels from gene expression and transcript abundance, to RNA processing, to post-translational mechanisms including protein modification. We find that the cellular consequence of small changes in metabolism is quite dramatic and extends to numerous cellular functions from metabolism to growth to intracellular crosstalk. The relationship between growth and metabolism is bi-directional and integral in the response to CR in mice and NHPs. The striking parallels between the human and NHP biometric and systemic response to CR suggest that insights gleaned from these studies may be clinically relevant for human aging, and may serve as the basis for development of interventions to counter age-related increases in disease vulnerability

111 Nutrition, ageing and drug discovery

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Introduction. Ageing is delayed by caloric restriction, an outcome that is mediated by canonical nutrient sensing pathways. Modulation of these pathways by metformin, rapamycin and resveratrol increases lifespan in some animal models.
Methods. We have studied other nutritional interventions to determine whether aging can be influenced by ad libitum-fed diets in mice.
Results. Using nutritional geometry to evaluate the effects of 25 diets varying in protein, carbohydrates, fat and energy content, we found that diets low in protein and high in carbohydrate increased lifespan and improved latelife health, an effect associated with altered mTOR activation and altered levels insulin, IGF-1 and FGF21 levels. Further, this effect correlated with circulating levels of branched chain amino acids (BCAA). Therefore we studied the effects of altering dietary BCAAs. High BCAAs were associated with increased food intake, obesity, increased body fat, impaired insulin/glucose metabolism and shorter lifespan. This was mediated by altered ratio of BCAA and tryptophan, leading to reduced serotonin and altered expression of hypothalamic appetite genes. We are also studying whether the beneficial effects of metformin, rapamycin and resveratrol on metabolic outcomes are determined by the underlying dietary macronutrient and energy composition. Initial results indicate that this is the case for several cardiometabolic outcomes.
Discussion. Lifelong studies of the effects of diet on aging in animals may contribute to dietary guidance for healthy aging in humans while identifying potential targets for the development of drugs that act on aging and age-related health outcomes.

112 Pharmacologic manipulation of DNA repair and metabolism to extend lifespan and reproduction

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One of the earliest declines with advancing biological age is female reproductive capacity. This has primarily been attributed to a depletion of the ovarian reserve, yet a decline in oocyte quality occurs well before ovarian depletion. The reasons for this are unclear. Here, we present data from our lab showing that a decline in an interaction between the NAD dependent deacylase SIRT2 and the kinetochore attachment protein BubR1 is responsible for this decline in oocyte quality. Animals which transgenically over-express SIRT2, or which have been treated with the NAD precursor nicotinamide mononucleotide (NMN) are resistant to this decline. NMN treatment also prevents chemotherapy induced infertility, and prevents other adverse events of chemotherapy including memory loss, inactivity and cardiovascular toxicity. These data suggest that the NAD precursor NMN could be used as a therapeutic to treat infertility and diseases of ageing, and to pursue this, we also present data from isotope labelled metabolic flux experiments to determine how exogenous NMN impacts endogenous NAD metabolism, and the metabolic route that this exogenous material follows.

Given the role of DNA damage from chemotherapy in mediating biological ageing, we also investigated the biochemical requirements of ageing cells, which are dependent upon activation of the pentose phosphate pathway for the generation of new nucleotides, and for the regeneration of reduced glutathione to neutralise reactive oxygen species. We recently completed a high-throughput drug screen to identify small molecule activators of the pentose phosphate pathway enzyme glucose-6-phosphate dehydrogenase (G6PD). We have identified four small molecule activators of G6PD which robustly increase lifespan in the nematode *C. elegans*, these may be further candidates for treating diseases of ageing.

Finally, given the role of chemotherapy in accelerating DNA damage and biological ageing, we sought to determine the contribution of type II DNA transposable elements to ageing, which are normally suppressed, but can be de-repressed during DNA damage or ageing. We performed an RNAi screen against these type II DNA transposons in *C. elegans*, and identified a single DNA transposon whose silencing robustly extends lifespan. Interestingly, the transposase protein encoded by this element has a signal peptide indicative of extracellular secretion, which has important implications for the non-cell autonomous nature of biological ageing.

113 Targeting the ageing liver microcirculation to prevent age related disease

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Age-related changes in liver function have a significant impact on systemic aging and susceptibility to age-related diseases. Recently, a role for liver sinusoidal endothelial cells (LSECs) in the relationship between aging and disease has also been proposed. Age-related loss of fenestrations within LSECs impairs the transfer of substrates (such as lipoproteins and insulin) between sinusoidal blood and hepatocytes, resulting in post-prandial hyperlipidemia and insulin resistance, significant risk factors for the development of cardiovascular and metabolic diseases. Through understanding the biological controls of LSECs and their fenestrations a significant therapeutic target for the treatment and prevention of age-related disease has been uncovered. Research has now commenced on the development of targeted therapeutics specifically designed to facilitate the direct delivery of drugs that regulate fenestrations in LSECs, providing an innovative approach to ameliorating age-related diseases and increasing healthspan.

114 Improving the understanding of an old drug to treat the youngest patients: population pharmacokinetics of pentoxifylline and its metabolites in critically ill, very pre-term infants

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Introduction. Infection-induced inflammation is associated with adverse long-term outcomes in preterm infants. Pentoxifylline (PTX) is a candidate for adjunct immunomodulatory therapy in preterm infants with late-onset sepsis (LOS) and necrotising enterocolitis (NEC), but pharmacokinetic data in this population are extremely limited.
Aims. To characterise the pharmacokinetic properties of intravenous PTX and three of its metabolites (M1, M4, M5) in very preterm infants with suspected LOS or NEC.
Methods. An open label pilot clinical study of intravenous PTX as an adjunct therapy in preterm infants (gestation <32 weeks) with suspected LOS or NEC was undertaken. PTX was infused for 12h for two days (60 mg kg^{-1} per 12h), and in infants with confirmed diagnosis of LOS or NEC, for 6h for another four days (30 mg kg^{-1} per 6h). Plasma concentrations of PTX and its principal metabolites were measured using a validated LC-MS assay. NONMEM was used to analyse the data using population pharmacokinetic modelling.
Results. The preterm infants (n = 26) had a median (range) gestation of 24.8 weeks (23.3-30.4) and birthweight of 689g (370-1285). After changes in size and maturation were successfully modelled using allometric scaling, clearance increased with postmenstrual age by approximately 30% per week for PTX and M1 (lisofylline). Simulations of current dosing demonstrated a six-fold difference in AUC between 24 and 35 weeks postmenstrual age.
Discussion. The developed model can be used to explore dosing strategies based on size and maturation for pre-term infants. The developed model can be used to assist exploring PK/PD relationships in planned studies with larger cohorts

115 Association between drug burden index and geriatric syndromes in a large, single-centre general medicine cohort

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Introduction. The drug burden index (DBI) is a measure of sedative and anticholinergic exposure for grading potential impact on physical and cognitive function (Hilmer S et al, 2007). The relationship between DBI and geriatric syndromes in hospitalised patients has only been examined in small cohorts.
Aims. Examine the relationship between DBI and geriatric syndromes in patients admitted to a general medicine unit.
Methods. A retrospective analysis of general medicine inpatients at a large metropolitan hospital between 1st July 2012 and 1st July 2018. Patients aged < 18 and those admitted for < 24 hours were excluded. Geriatric syndrome diagnoses were determined by ICD-10-AM coding and DBI was calculated from non-PRN medications administered in the first 24 hours of admission. The relationship between high DBI (≥1) and delirium was examined using multivariable logistic regression. Model covariates were age, polypharmacy and previous diagnosis of dementia (defined by ICD-10-AM coding). Polypharmacy and DBI cut-points were chosen to be consistent with previous work (Best O et al, 2013).
Results. 22,658 (16,033 patients) admissions met the criteria. The median age at time of admission was 81 years (IQR 71 – 87) and 11.9% of admissions were coded for delirium. Compared with DBI of 0, there was a statistically-significant association between high DBI and delirium (OR 1.55, 95% CI 1.28 - 1.89) and somnolence (OR 1.84, 95% CI 1.08 – 3.13), but not falls (OR 1.13, 95% CI 0.92 – 1.39). Polypharmacy wasn't significantly correlated with these outcomes.
Discussion. We found an association between high DBI and two geriatric syndromes in patients admitted under a general medicine unit. This study is limited by low-sensitivity ICD-10-AM coding to define geriatric syndrome diagnoses and DBI calculations based on inpatient administration rather than home medicines. Prospective deprescribing of anticholinergic and sedative medications is required to establish whether reducing DBI will have an impact on this risk.
Best O et al (2013) Intern Med J 43(8):912–918
Hilmer S et al (2007) Arch Intern Med 167(8):781–787

116 Adalimumab exposure and pregnancy outcomes in women with inflammatory bowel disease: a systematic review and meta-analysis

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Introduction. Tumour necrosis factor (TNF)α inhibitors are increasingly prescribed for the treatment of inflammatory bowel disease (IBD) in women of reproductive age. Active IBD during pregnancy leads to poorer pregnancy outcomes, and immunosuppression is often necessary to ensure quiescence. Three recent systematic reviews and meta-analyses showed that TNFα inhibitors, as a class, do not increase the risk of adverse pregnancy outcomes (APOs) compared with disease-matched controls. However, most studies focused on infliximab (IFX), with minimal data on adalimumab (ADA).
Aims. We conducted a systematic review and meta-analysis to assess the effects of ADA exposure, vs IFX or no treatment for IBD, on maternal, fetal and infant outcomes when administered 90-days preconception and/or antepartum.
Methods. Online databases were screened between January 1999 and June 2018 for studies describing the use of ADA preconception or antenatally in women with IBD, and maternal, fetal and infant outcomes.
Results. We identified 26 studies for systematic review; the studies were heterogeneous and outcome measures varied.
Eleven studies were suitable for meta-analysis. Ten studies with 423 pregnancies compared ADA with IFX, whereas 3 studies with 211 pregnancies compared ADA with disease-matched controls (2 studies compared ADA with both comparators). Compared with IFX there were no significant differences in live birth rate (OR 1.31, [95% CI 0.52 – 3.31]), premature delivery (OR 0.49, [95% CI 0.17 – 1.42]), spontaneous abortion (OR 0.73, [95% CI 0.25 – 2.13]), elective termination (OR 1.97, [95% CI 0.27 – 14.28]), low birth weight (OR 0.66, [95% CI 0.22 – 1.98]), congenital abnormalities (OR 0.47, [[95% CI 0.09 – 2.38]), severe infant infection within 12 months requiring hospitalisation (OR 0.76, [95% CI 0.22 – 2.70]), antepartum IBD flare (OR 1.12, [95% CI 0.53 – 2.35]) or IBD flare within 3 months postpartum (OR 1.04, [95% CI 0.47 – 2.30]). ADA compared with disease-matched controls showed no significant differences in live birth rate (OR 0.92, [95% CI 0.45 – 1.90]), premature delivery (OR 2.19, [95% CI 0.96 – 5.01]), spontaneous abortion (OR 1.10, [95% CI 0.49 – 2.46]), elective termination (OR 0.50, [95% CI 0.04 – 5.60]), or congenital abnormalities (OR 2.11, [95% CI 0.40 – 11.15]).
Discussion. ADA does not appear to increase the risk of APOs when compared with IFX or disease-matched controls.

117 Paracetamol for pain and fever: a systematic review of systematic reviews

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Introduction. Paracetamol is one of the most widely used and prescribed analgesic and antipyretic medicines worldwide. Despite this there has not been a comprehensive synthesis of the efficacy and safety of paracetamol across the pain and fever conditions for which it is used.
Aims. To evaluate and summarise the available evidence from existing, recent systematic reviews of paracetamol for any pain condition or fever.
Methods. Two authors extracted efficacy and adverse events outcomes from eligible systematic reviews. The quality of systematic reviews was assessed using the AMSTAR checklist. We used the GRADE criteria to make an assessment about the overall quality of evidence for a given outcome.
Results. Thirty four systematic reviews covering over 30 medical conditions were eligible. Surprisingly, there are only a few conditions where there is high quality evidence that paracetamol is effective, namely hysterectomy pain, perineal pain in the early post-partum period, reducing fever in the critically ill and reducing febrile reactions in children being vaccinated. There is high quality evidence that paracetamol is ineffective for acute low back pain and neck pain. There are ten common conditions for which paracetamol may to be effective, but due to the limited number of studies and sample of participants, this is currently based on low quality evidence. There are a large number of conditions for which the current available evidence is insufficient to determine the true effectiveness of paracetamol.
Discussion. This review provides clinicians and patients with the most current and comprehensive overview of the effectiveness and safety of paracetamol and the findings will guide clinical decision making around the appropriate use of this medicine globally. There is a need for further studies to resolve the uncertainty around the efficacy of paracetamol for some common pain conditions.

118 Adverse drug reactions to opioids in electronic health records: implications for prescribing alerts

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Introduction: Allergy to opioids is the second most common drug allergy label in electronic health records (EHR). Adverse drug reactions (ADRs) to opioids cause significant morbidity and contribute to increased healthcare costs. Computerised prescribing alerts may reduce ADRs but are often ignored due to poor specificity and alert fatigue.
Aims: To examine the documentation of opioid ADRs in a large-scale hospital-based EHR and consider the implications for prescribing alerts.
Methods: A cross-sectional retrospective review of EHR documentation of opioid ADRs at four public hospitals in South Australia, from August 2013 to September 2017, was conducted. These hospitals use the Enterprise Patient Administration System (EPAS) electronic health record. Data was extracted from all ADR entries including the reported allergen, ADR category (allergy or intolerance), reaction details and occupation of the healthcare professional entering the information
Results: A total of 86,727 unique ADR reports were examined. Of these 13,781 involved opioids (15.9% of all ADRs), the majority entered as allergy (n=8913, 64.7%) rather than intolerance (n=4868, 35.3%). The most commonly identified opioids were codeine (n=5570, 40.4%), morphine (n=4130, 30.0%), oxycodone (n=1772, 12.9%) and tramadol (n=1397, 10.1%). Over half (n=7483, 54.3%) of opioid ADR reports were entered by nurses, followed by doctors (n=4259, 30.9%), and pharmacists (n=1798, 13.0%). The most commonly documented reactions were nausea/vomiting (n=3912, 28.4%), rash (n=647, 4.7%), itch (n=642, 4.7%) and hallucinations (n=527, 3.8%). Only 2.6% (n=362) of opioid ADR labels were for anaphylaxis.
Conclusions: This large hospital-based study demonstrates the high rate of opioid ADR labels in EHRs. The majority of these labels were for gastrointestinal, dermatological and neurological symptoms suggestive of pharmacological intolerance. Labels suggestive of true allergy were uncommon. Prescribing alerts need to be tailored to the reaction type as most warrant pre-medication, dose reduction, or opioid rotation rather than avoidance.

119 Bayesian networking reveals links between toxicities, genetics and treatment parameters following 5-fluorouracil (5-FU)-based treatment

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Introduction. Severe gastrointestinal (GI) toxicity and other frequently reported toxicities following 5-FU-based treatment are highly prevalent and negatively affect therapy. We have previously identified *TLR2* and *TNF* genetic markers that predict GI toxicity risk (Coller et al, 2015). However, we have not yet examined links with other toxicities or comorbidities.
Aims. To investigate relationships between the genetics, toxicities and comorbidities after 5-FU-based treatment.
Methods. 106 participants who previously received 5-FU-based treatment for mixed cancers were recruited and immune response genetics determined by custom multiplex analysis (Coller et al, 2015). GI toxicity, neuropathy, pain, skin toxicity and cardiotoxicity data (toxicity: symptoms Grade ≥ 3 National Cancer Institute's CTCAE v4, requiring treatment cessation or reduction), demographics, comorbidities (BSA, smoking status, alcohol use, type 2 diabetes, cardiovascular disease, arthritis, asthma, GORD, thyroid activity) and treatment parameters were mined from clinical records. Bayesian network analysis based on lowest Akaike Information Criteria score determined direct relationships.
Results. The final Bayesian network revealed direct links between: GI toxicity with cancer type and skin toxicity; skin toxicity with chemotherapy regimen, *CASP1* genotype; pain with chemotherapy regimen; neuropathy with sex, chemotherapy regimen and cardiotoxicity; and cardiotoxicity with diabetes and treatment hospital.
Discussion. This preliminary Bayesian network revealed the complexity of relationships between toxicities, genetics, cancer and regimen that frequently occur following 5-FU-based treatment.

Coller JK et al (2015) Support Care Cancer 23:1233-1236

120 The effect of chronic polypharmacy, the Drug Burden Index (DBI) and deprescribing on physical function in aged mice

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Introduction. Polypharmacy (use of ≥ 5 drugs) and increasing DBI (measure of total exposure to anticholinergic and sedative drugs) is associated with impaired physical function in observational studies of older adults.
Aims. We aim to determine the longitudinal effect of polypharmacy, DBI and deprescribing (withdrawing drugs) on functional outcomes in aged mice.
Methods. From 12 to 21 months of age male C57BL/6 mice were fed control or treatment containing therapeutic doses of five drugs with Zero DBI (simvastatin, metoprolol, omeprazole, paracetamol, irbesartan), Low DBI (simvastatin, metoprolol, omeprazole, paracetamol, citalopram), High DBI (simvastatin, metoprolol, oxybutynin, oxycodone, citalopram), or single drug (simvastatin, metoprolol, oxybutynin, oxycodone or citalopram) (n=40/ group). At 21 months, animals either continued treatment or were deprescribed (n=20/ group). Functional tests were conducted at 12, 15, 18, 21 and 24 months.
Results. From 15-24 months, compared to control diet, locomotor activity (open field), grip strength (wire hang) and nest making declined following Low DBI, High DBI and citalopram treatment and frailty index score increased following Low DBI, High DBI, oxybutynin, metoprolol and citalopram treatment (p<0.05). At 24 months, Zero DBI, High DBI and oxybutynin impaired short-term memory (Barnes maze; p<0.05). Deprescribing improved locomotor activity for Zero DBI, Low DBI, High DBI and citalopram treatment (p<0.05). Deprescribing also improved nesting activity for Low and High DBI (p<0.05), grip strength for Low DBI animals (p<0.05) and had no effect on memory.
Discussion. Our results show for the first time in a preclinical model that chronic polypharmacy with DBI or individual sedative or anticholinergic drugs impair function, which may be reversed with deprescribing. Future studies will investigate the mechanism.

121 Application of cytochrome P450 (CYP) phenotyping procedure in clinical setting; a feasibility study

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Introduction. CYP phenotyping using a cocktail approach is a valuable tool to evaluate the impact of a drug or intervention on the human drug metabolising capacity and to assess the potential drug-drug interactions. Cardiac surgery with cardiopulmonary bypass (CPB) has the potential to alter CYP enzyme activities.
Aims. To investigate the feasibility of using an *in vivo* CYP phenotyping cocktail to evaluate the extent of the impact of CPB on drug metabolism.
Methods. Caffeine (50 mg), midazolam (1 mg), dextromethorphan (30 mg), losartan (5 mg), and omeprazole (20 mg) as selective substrates of CYP1A2, 3A, 2D6, 2C9 and 2C19, respectively, were administered by the oral route to five patients undergoing cardiac surgery with CPB. Blood samples were collected at times 0, 1, 2, 4 and 6h after receiving the CYP substrates. The ratios of areas under the plasma concentration versus time profile ($AUC_{0\text{-}6h}$) of CYP-mediated metabolites to their corresponding CYP substrates for individual patients were used as the measure of CYP activity. The *in vivo* CYP phenotyping was conducted twice in each patient; on the day of surgery and one day before or 3 days after surgery.
Results. The mean activities of CYP2D6, 3A, 2C9 and 2C19 were reduced by 71, 42, 33 and 50%, respectively while the CYP1A2 activity increased by 45% on the day of surgery compared with that determined one day before or 3 days after surgery. The plasma concentrations of IL-6 and IL-10 increased significantly by 5- and 3.5-fold, respectively, and IL-8 and TNF-α plasma concentration were increased 1.9 and 1.2-fold, respectively during CPB.
Discussion. I*n vivo* CYP isoenzyme activities were altered during cardiac surgery with CPB, and these changes may be linked to CPB-induced release of pro-inflammatory and/or anti-inflammatory cytokines. The CYP phenotyping cocktail was well-tolerated with no adverse effect.

122 Investigating potential functional crosstalk of co-located metabotropic glutamate receptor 5 and adenosine A1 receptors in primary neurons and glia

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Introduction. Alzheimer's disease (AD) is the most prevalent cause of dementia in the elderly. Reactive astrocytes and activated microglia are associated with two AD pathological hallmarks: amyloid β plaques and neurofibrillary tangles. Emerging evidence suggests metabotropic glutamate receptor 5 (mGlu5) inhibition (Hamilton et al, 2016) and adenosine A_1 receptor (A_1R) activation (Angulo et al, 2003) reduces Aβ deposition and rescues memory deficits in animal models. Thus, mGlu5 and A_1R are promising therapeutic targets. However, mGlu5 and A_1R pharmacology is poorly characterised. Our preliminary data suggests mGlu5 and A_1R functionally interact in neurons.
Aims. We tested the hypothesis that mGlu5 and A_1R functionally interact in healthy neurons and glia, which is altered in AD pathological context.
Methods. Immunopanning and shake-off methods were employed to culture resting and activated forms of primary astrocytes and microglia. Gene and protein expression of mGlu5 and A_1R were confirmed by using qRT-PCR and western blotting. The purity of astrocytes and microglia cultures was assessed by immunocytochemistry with an Operetta High-Content Imaging System. Intracellular calcium mobilization (iCa^{2+}) was used to interrogate pharmacology and functional interactions in primary neurons and glia.
Results. mGlu5 and A_1R were detected in both astrocytes and microglia cultures for up to 6 days, with minimal levels of other adenosine receptor subtypes. VU04024465 (mGlu5 selective allosteric agonist) and MeCCPA, (A_1R selective agonist) mobilize iCa^{2+} in glia. Co-addition of MeCCPA significantly increased the maximal response to VU04024465 in neurons.
Discussion. Functional mGlu5 and A_1R are present in astrocytes and microglia. Coincident activation of A_1R enhances $mGlu_5$ activity in neurons. Future studies will investigate the effect of coincident activation and modulation of A_1R on mGlu5-mediated signalling in neurons and glia and determine molecular mechanisms governing A_1R/mGlu5 crosstalk.

Angulo E et al (2003) Brain Pathol 13:440-451; Hamilton A et al (2016) Cell Rep 15:1859-186

123 Does gabapentin or pregabalin directly modulate the Mu Opioid Receptor?

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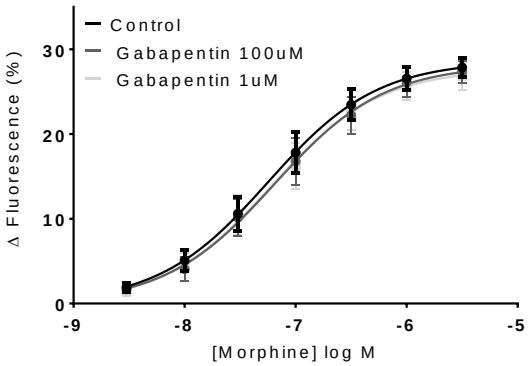
Introduction. Pregabalin and Gabapentin improve neuropathic pain symptoms (1) but their use for opioid augmentation is recognised, and fatalities associated with co-ingestion of both drugs and opioids are increasing (2). Gabapentin and pregabalin may reduce opioid tolerance, but the molecular mechanism of this is unclear.
Aims. To determine if gabapentin and pregabalin modulate responses to morphine wild type human μ-opioid receptor (MOR) in AtT-20 and HEK293 cell lines.
Methods. Stably transfected AtT20 and HEK293-GIRK4 cells were used to measure opioid-induced hyperpolarizations with FLIPR membrane potential dye.



Results. No change in fluorescence was detected with addition of pregabalin or gabapentin in doses of 100uM to 1nM. There was no difference in Morphine CRC when preincubated with pregabalin or gabapentin at 100uM or 1uM compared to control Morphine CRC (figure). Preincubation with either drug also did not affect MOR signalling desensitisation.
Discussion. Gabapentin and pregabalin are GABA analogues whose efficacy in neuropathic pain is mediated through action on the α2δ subunit presynaptic calcium channel. Activity of pregabalin or gabapentin on the MOR was not identified. Our data do not support the hypothesis that gabapentin or pregabalin augment opioid effect through allosteric modulation at the MOR.

1. Finnerup NP et al Pharmacotherapy for neuropathic pain in adults. Lancet Neurol. 2015;14(2):162-73
2. Lyndon A et al Risk to heroin users of polydrug use of pregabalin or gabapentin. Addiction 2017;112(9) 1580-1589

124 Lithium reverses mechanical allodynia through a mu opioid receptor-dependent mechanism

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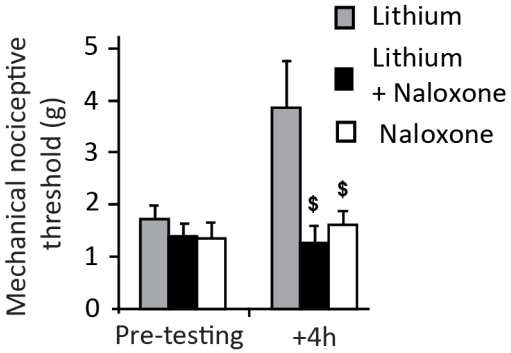

Introduction. Lithium is widely used to treat bipolar disorders and displays mood stabilizing properties. In addition, lithium relieves painful cluster headaches and has a strong analgesic effect in neuropathic pain rat models.
Aims. We have investigated the analgesic effect of lithium on the cuff model of neuropathic pain.
Methods. We used behavioural and pharmacological approaches to study the analgesic effect of a single injection of lithium in wild-type and mu opioid receptor (MOR) null cuffed neuropathic mice. Mass spectrometry and ELISA allowed to measure the levels of endogenous MOR agonist beta-endorphin, as well as monoamines in brain and plasma samples 4 h after lithium administration.
Results. A single injection of lithium chloride (100mg/kg, ip) alleviated mechanical allodynia for 24 h and this analgesia is significantly reversed by naloxone in wild-type mice (figure). The analgesic effect of lithium was absent in MOR null neuropathic mice. Biochemical analyses highlight a significant increase of beta-endorphin levels by 30% in the brain of lithium-treated mice compared to controls. No variation of beta-endorphin was detected in the blood.
Discussion. Together, our results provide evidence that lithium induces long-lasting analgesia in neuropathic mice presumably through elevated brain levels of beta-endorphin and the activation of MORs.

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125 AE Succinimide, an analogue of Methylylcaconitine MLA stabilizes the closed state of the α4β2 nAChR.

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Introduction. Nicotinic Acetylcholine Receptors (nAChRs) are pentameric ligand-gated ion channels. The α4β2 nAChR is highly expressed in the brain and exists in two functional isoforms: the $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$ that differ by an ACh-binding site at the α4-α4 interface of $(\alpha4)_3(\beta2)_2$ receptors. Methylylcaconitine (MLA) is a natural toxic potent antagonist that competes with ACh at α4β2 nAChRs. Identifying ligands that are selective for this specific nAChR may have significant therapeutic potential and contribute to the understanding of the physiological roles of these subtypes *in vivo*.
Aim. In this study, evaluated AE succinimide, a simplified MLA analogue at α4β2 nACh receptors for inhibitory effects at rat α4β2 nAChRs.
Method. AE succinimide was assessed at $(\alpha4)_3(\beta2)_2$ and $(\alpha4)_2(\beta2)_3$ stoichiometries expressed in *Xenopus* oocytes using the two-electrode voltage clamp technique. Site-directed mutagenesis, substituted cysteine accessibility methods and cross-linking experiments were used to investigate the channel lumen as a potential binding site.
Results and Discussion. AE succinamide was a non-competitive inhibitor of α4β2 nAChRs that bound within the channel lumen. Using α413' cysteine mutation, we found that upon binding, AE succinamide induces a conformational change that caused two adjacent mutated α4 subunits to cross react in an act to stabilize the closed state which was reversed by the reducing agent, dithiothreitol (DTT). This is the first example of a compound that induces a conformational change in the α4β2 nAChR to stabilize the closed state.

126 In vitro cytotoxic activity of natural products and derivatives in different nanoformulations against brain cancer cells

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Introduction. Glioblastoma is the most aggressive type of brain cancer and the current standard chemotherapy temozolomide is inadequate due to tumour resistance and recurrence. Natural products including ursolic acid (UA), a pentacyclic triterpenoid, have previously shown cytotoxicity against glioblastoma cells. However, their poor physiochemical properties, pharmacokinetics and pharmacodynamics have restricted their clinical use.
Aims. In this study, different nanoparticle (NP) formulations of natural products (UA, derivatives and curcumin) were prepared to improve their solubility and cytotoxicity.
Methods. The cytotoxicity of pure UA, UA-1 and nanoparticles alone or in combination with curcumin against U87MG glioblastoma cells was evaluated using cell viability assays. The particle size and loading capacity of the fabricated nanoparticles were assessed by using dynamic light scattering and HPLC, respectively.
Results. The highly soluble nanoparticles were shown to enhance the cytotoxicity of UA and UA-1 ($p < 0.05$). Excitingly, the formulations significantly lowered the IC50 of glioblastoma chemotherapeutic temozolomide required in drug combination experiments ($p < 0.05$)
Discussion. We successfully improved the solubility and cytotoxicity of the natural products using these nanoformulations. We also improved temozolomide sensitivity in glioblastoma cells when used in combination formulations. We suggest that glioblastoma's increased sensitivity to temozolomide when used in combination may result in reduced tumour resistance and recurrence, reduced side effects for patients via dose reduction, and thus improve patient outcomes.

127 Betulinic acid derivatives protect Müller cells from glutamate-induced oxidative stress

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Introduction. Müller cells are the predominant glial cells in the retina. One of the key roles of Müller cells is to uptake the neurotransmitter glutamate and convert it into glutamine. Dysfunction of Müller cells due to oxidative stress causes an excess accumulation of glutamate, which can lead to excitoxicity progressing to many retinal diseases such as glaucoma and diabetic retinopathy.
Aims. This study aims to investigate the anti-oxidative effects of betulinic acid (BA), betulin (BE) and their derivatives on human Müller cells.
Methods. Human Müller MIO-m1 cells were pre-treated with or without BA, BE or their derivatives (named H3-H20) and followed with the incubation of glutamate. Cell viability was assessed using MTT and calcein AM assay. The reactive oxygen species (ROS) level of MIO-m1 cells was measured with flow cytometry. The cellular apoptosis and necrosis profile was analyzed with annexin/PI staining method. The modulation of signaling pathways was investigated with immunoblotting.
Results. The derivatives H3 and H5 have minimal cytotoxicity and possess optimal anti-oxidative effect. These compounds significantly suppressed ROS production and attenuated cellular apoptosis and necrosis induced by glutamate. The anti-oxidative effect of H3 and H5 impacted on the glutamate-induced activation of Akt, ERK1/2, p38 and JNK pathways.
Discussion. H3 and H5 compounds have protective effect against glutamate-induced oxidative stress on MIO-m1 cells. These two compounds are leading candidate agents to be used in the prevention of human retinal diseases.

128 The sources, distribution, human toxicity and pharmacologic effects of cannabis contaminants

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Introduction. Medicinal cannabis is a topical domain of clinical pharmacology research. Agricultural grade Cannabis is inherently predisposed to harbouring a variety of biotic and abiotic contaminants, however in many jurisdictions this is the product used for therapeutic purposes. An understanding of these contaminants and subsequent altered cannabinoid profile and effects on pharmacokinetic disposition is required in order to ensure patient safety.
Aims. We thus aimed to undertake a systematic review of the academic literature to critically appraise the evidence base investigating cannabis contaminants, their human health and pharmacological effects, and therewithal identify any pertinent gaps in current knowledge.
Methods. We examined all indexed biological and biomedical databases and the Cochrane library were systematically searched from inception to December 2017. Filtered results were assessed for eligibility by two independent reviewers. Selected articles were aggregated into a qualitative narrative synthesis addressing four domains concerning cannabis contaminants: sources and distribution, human toxicity, the effect of different routes of administration on contaminant bioavailability and potential interactions with phytocannabinoid pharmacokinetic and dynamic profiles.
Discussion. This Review demonstrated that microbes, heavy metals and pesticides were commonly reported contaminants of cannabis. Infection, carcinogenicity, reproductive and development effects comprise their known human toxicity and these may be contributed to by toxins in plant, and effects of heating and cooling plant for human use. However, deficiency of aggregate data frustrates adverse event quantification. The Review described how the various administration routes and dosing formulations of cannabis impact the subsequent transformation and bioavailability of contaminants and carcinogens. Although of key interest to clinicians, this Review was unable to elicit likely effects on important pharmacokinetic and pharmacodynamics interactions between phytocannabinoids and contaminants due to lack of data.

129 The effects of chronic polypharmacy and deprescribing on the livers of aged mice

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Introduction. Ageing causes progressive impairments in liver function. Polypharmacy (the use of ≥5 medications) occurs in 66% of Australians aged ≥75 years. Polypharmacy and increasing Drug Burden Index (DBI: measures total exposure to sedatives and anticholinergic medication) are associated with functional impairments in older people. Deprescribing, the withdrawal of one or more medications, has been shown to improve some clinical outcomes. The effect of polypharmacy and deprescribing on the ageing liver is not well known.
Aims. To investigate the effect of chronic polypharmacy or monotherapy with increasing DBI on liver histology, and determine whether deprescribing medications affects any observed changes.
Methods. Male mice aged 12 months were randomly assigned to control or treatment diet containing therapeutic doses of commonly prescribed medications with Zero DBI (simvastatin, metoprolol, omeprazole, paracetamol, irbesartan), Low DBI (simvastatin, metoprolol, omeprazole, paracetamol, citalopram), High DBI (simvastatin, metoprolol, oxybutynin, oxycodone, citalopram), or monotherapy (simvastatin, metoprolol, oxybutynin, oxycodone or citalopram) (n=40/group). At 21 months, treated mice continued with the treatment or underwent deprescribing (n=20/group). At age 26 months, livers were collected for histological analysis (n=8-13/group) staining with Haematoxylin and eosin for morphology, Sirius Red for fibrosis, and quantification of Kupffer cells using F4/80 immunohistochemistry. Treatment groups were compared using a one-way ANOVA on SPSS.
Results. Preliminary data shows, compared to control and other treatment groups, livers of mice treated with citalopram had higher steatosis and ballooning degeneration (n=4, $P<0.05$). These effects were not seen with deprescribing citalopram (n=4, $P>0.05$). Neither total inflammation (sum of portal and lobular inflammation scores) nor fibrosis differed significantly from control in any treatment group (n= 4, $P>0.05$).
Discussion. Our preliminary results show that chronic polypharmacy and monotherapy with selected medications does not contribute towards total inflammation and fibrosis but citalopram may cause steatosis and ballooning degeneration, which may be reversible on deprescribing. Further analysis will confirm these results.

130 The effect of long-term polypharmacy on cardiovascular functions and cardiac fibrosis in aged mice

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Introduction. Polypharmacy (concurrent use of ≥ 5 medications) and exposure to drugs with increasing Drug Burden Index (DBI: the cumulative exposure to anticholinergic and sedative drugs) are associated with impaired function in older adults. Preclinical studies can provide a mechanistic understanding of these exposures on organ function.
Aims. We aim to evaluate the effect of chronic polypharmacy and monotherapy with increasing DBI and deprescribing (cessation of medications) on cardiovascular function and histology in aged mice.
Methods. 12-month-old male C57BL/6 mice received control chow or medicated feed containing polypharmacy regimens of Zero DBI (simvastatin, metoprolol, omeprazole, paracetamol and irbesartan), Low DBI (simvastatin, metoprolol, omeprazole, paracetamol and citalopram), High DBI (simvastatin, metoprolol, oxybutynin, oxycodone and citalopram) or monotherapy, with each medication independently from the High DBI regimen, all at therapeutic doses. At age 21 months, animals were re-randomised to continue treatment or were deprescribed. BP and rotarod endurance were assessed every three months. Hearts were collected at age 26 months for collagen quantification.
Results. At 21 months, compared to control, systolic and diastolic BP decreased in Zero DBI, Low DBI, metoprolol and simvastatin treated mice ($P<0.05$) but not in High DBI (also has metoprolol and simvastatin) group ($P>0.1$). At 21 and 24 months, compared to control and High DBI, rotarod latency-to-fall, adjusted for weight and cohort, increased in metoprolol group ($P<0.05$). Neither BP nor rotarod endurance differed significantly with each treatment compared to deprescribing of that treatment. Preliminary results (n=5) indicate that compared to control, High DBI diet increased myocardial collagen ($P<0.05$) while Zero DBI, Low DBI, metoprolol and simvastatin showed no significant effect.
Discussion. Our results suggest that chronic treatment with this High DBI polypharmacy regimen may impair therapeutic effects of antihypertensives and increase myocardial collagen. Future studies will continue to investigate morphological changes of the heart including wall thickness and cardiomyocyte damage

131 Therapeutic role of rAAV6-BMP7 to limit diabetic cardiomyopathy in type 2 diabetic mice

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Introduction: Diabetic cardiomyopathy is a complication characterized by structural and functional changes in the heart including cardiomyocyte hypertrophy, cardiac fibrosis and diastolic dysfunction. Bone morphogenetic protein 7 (BMP7) is an anti-fibrotic protein shown to counterbalance transforming growth factor β-induced cardiac fibrosis in settings of renal fibrosis and in the setting of type 1 diabetes-induced cardiomyopathy.
Aims: To determine whether cardiac-selective rAAv6-BMP7 gene therapy attenuates cardiac fibrosis and improves cardiac function in a murine model of type 2 diabetic mellitus (T2DM) induced diabetic cardiomyopathy.
Methods: 6-week-old male FVB/N mice were administered streptozotocin or citrate vehicle (55mg/kg/d) via ip injections for 3 consecutive days. Diabetic mice were placed on a high-fat diet, while non-diabetic controls received a standard laboratory diet. After 18 weeks of untreated diabetes, echocardiography was performed with anaesthetic (ketamine/xylazine/atropine; 60/6/0.6 mg/kg; ip) to confirm the presence of diastolic dysfunction, prior to administration of rAAV6-BMP7 or a null vector (2x10^11 vector genomes) gene therapy via a single tail vein injection.
Results: Blood glucose levels increased with the onset of T2DM ($p<0.05$) and the mice developed insulin resistance observed through glucose tolerance testing. T2DM resulted in an increase in total interstitial collagen ($p<0.05$) and collagen type I ($p<0.01$) and III ($p<0.001$). This was associated with an increase in the pro-fibrotic markers such as plasminogen activator inhibitor-1 and procollagen I at an mRNA level. rAAV6-BMP7 treatment attenuated levels of interstitial collagen type I and type III as well as procollagen I mRNA levels ($p<0.01$). rAAV6-BMP7 treatment also reduced the presence of cardiomyocyte hypertrophy ($p<0.05$) and apoptotic cardiomyocytes ($p<0.01$) in T2DM induced diabetic cardiomyopathy. Endpoint echocardiography showed a reduction in E/A ($p<0.05$) and e'/a' ($p<0.01$) ratio as well as an increase in deceleration time ($p<0.05$). Treatment with rAAV6-BMP7 improved deceleration time ($p<0.01$) but no other markers of diastolic function were altered with treatment.
Conclusion: Treatment with rAAV6-BMP7 attenuates characteristics of diabetic cardiomyopathy including cardiac fibrosis, cardiomyocyte hypertrophy, apoptosis and some functional markers in a model of T2DM.

132 Investigating the therapeutic targeting of myofibroblast-inflammasome activity to treat cardiac fibrosis

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Introduction. Serelaxin (recombinant human relaxin; RLX) has been shown to exert its anti-fibrotic effects *in vitro* and *in vivo* by inhibiting the pro-fibrotic influence of a number of cytokines including TGF-β1 and/or interleukin (IL)-1β. Recent studies have shown that RLX can also attenuate NLRP3 inflammasome activation (which is known to promote IL-1β and IL-18 activity) in mice subjected to myocardial infarction[1]. Whether RLX inhibits the pro-fibrotic effects of IL-1β by suppressing NLRP3 inflammasome activation in myofibroblasts *in vitro* and *in vivo* remains unknown.
Aim. To determine the extent to which the anti-fibrotic effects of RLX are mediated via inhibition of NLRP3 inflammasome activity in human cardiac fibroblasts (HCFs) *in vitro* and a mouse model of cardiomyopathy *in vivo*.
Methods. Primary human cardiac fibroblast (HCFs; ScienCell, USA) were stimulated with TGF-β1 (T; 5ng/ml) alone to undergo differentiation into myofibroblasts or with T+LPS (100ng/ml)+ATP (5mM) (T+L+A) for 8hr or 72hr to stimulate NLRP3 inflammasome activation. T- and T+L+A-stimulated cells were also treated with RLX (100ng/ml) over both time-points (n=5-10 separate experiments). 7-8 week-old male mice (n=5-6/group) were subjected to isoprenaline (ISO; 25mg/kg)-induced cardiomyopathy *in vivo*, then s.c-treated with either RLX (0.5mg/kg/day), the NLRP3 inhibitor (MCC950; 10mg/kg/day) or both via osmotic mini-pumps from days 7-14 post-injury. In each case, human myofibroblasts *in vitro* and mouse left ventricular (LV) tissues were assessed for changes in NLRP3, ASC, pro-caspase-1, pro-IL-1β, pro-IL-18, TLR4, α-SMA and collagen-I by Western blotting and densitometry of the relevant bands.
Results. T+L+A-stimulated HCFs had significantly increased expression of NLRP3, ASC, pro-caspase-1, pro-IL-1β and pro-IL-18 after 8hrs and pro-caspase-1, TLR4, α-SMA and collagen-I after 72hrs; while the LV of ISO-injured mice had increased expression of all these measures after 14 days (all by 25-105%; all $p<0.05$ vs T or saline, respectively). However, RLX significantly inhibited or decreased measures of inflammasome activity and fibrosis *in vitro* and *in vivo* (all $p<0.05$ vs T+L+A or ISO, respectively). MCC950 also inhibited inflammasome activity without affecting fibrosis; whereas the combined effects of RLX+MCC950 negated the inflammasome- and fibrosis-inhibitory effects of RLX *in vivo*.
Discussion. RLX appears to suppress NLRP3 inflammasome activity in myofibroblasts as part of its anti-fibrotic effects.

[1]Valle Raleigh J et al., Cardiovasc Res, 2017. 113:609-619

133 The single chain-derivative, B7-33, retains the cardioprotective effects of relaxin

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Introduction. The pleiotropic hormone, relaxin (RLX), exerts various organ-protective effects *in vivo* independently of etiology. However, its complex two-chain, disulfide-rich insulin-like structure is a limitation to its facile preparation and affordability, while its strong ability to activate cAMP signalling (which can lead to various detrimental effects) may preclude its long-term use as a treatment for human disease. Hence, a single chain-derivative of RLX, B7-33, was developed and shown to retain the anti-fibrotic effects of RLX *in vitro* and *in vivo*[1]. Whether B7-33 can retain the other cardioprotective effects of RLX is required to determine the extent to which it can replace RLX as a safer therapeutic.
Aim. To compare the cardioprotective effects of B7-33 to RLX and an ACE inhibitor in a murine model of fibrotic cardiomyopathy *in vivo*.
Methods. 7-8 week old male 129sv/ev mice (n=6-8/group) were subjected to isoprenaline (ISO; 25mg/kg)-induced cardiomyopathy, then s.c-treated with either RLX (0.5mg/kg/day), B7-33 (0.25mg/kg/d; equivalent dose corrected for MW) or perindopril (1mg/kg/d) via mini-pumps from days 7-14 post-injury. Control mice received saline instead of ISO. Changes in animal body weight (BW), blood pressure (BP), cardiomyocyte hypertrophy, and various measures of vascular dysfunction and rarefaction, left ventricular (LV) inflammation and fibrosis were assessed at day 14 post-injury.
Results. ISO-injured mice had significantly increased LV inflammation and fibrosis, cardiomyocyte hypertrophy and vascular rarefaction (by 0.5-8-fold) in the absence of any changes in BW, BP or vascular dysfunction (all $p<0.05$ vs saline-controls). Both B7-33 and RLX equivalently reduced LV fibrosis by ~40% and normalised the ISO-induced increase in LV inflammation and cardiomyocyte hypertrophy, while restoring blood vessel density (all $p<0.05$ vs ISO alone). On the other hand, perindopril lowered BP, LV inflammation and vascular rarefaction, but not fibrosis.
Discussion. B7-33 appears to retain the cardioprotective effects of RLX, and provides more rapid anti-fibrotic effects compared to perindopril. Extending these findings in other experimental models will validate B7-33 as a future therapy.

[1]Hossain MA et al., Chem Sci, 2016. 7:3805-3819.

134 G protein-coupled estrogen receptors: novel therapeutic targets in aldosterone/salt-induced hypertension?

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Introduction. The G protein-coupled estrogen receptor 1 (GPER) may modulate some of the effects of aldosterone. G-1, a GPER agonist, can acutely lower blood pressure and promote T cell-mediated anti-inflammatory responses.
Aims. To test the effects of G-1 and G-15 (GPER antagonist) on aldosterone/salt-induced hypertension in male and female mice, and to examine the cellular mechanisms involved.
Methods. C57Bl/6, RAG1-deficient and GPER-deficient mice were treated with either vehicle, aldosterone/salt (0.72 mg/kg/d SC plus 0.9% NaCl for drinking) ± G-1 (0.03 mg/kg/d SC) and/or G-15 (0.3 mg/kg/d SC) for 14 d. Some females were ovariectomised 7 d prior to treatment. T cells were adoptively transferred intravenously 21 d prior to treatment. Results. In male C57Bl/6, aldosterone/salt caused a sustained increase in BP of ~25 mmHg within 7 d and G-1 attenuated this increase by ~50 % (n=11-13, $P<0.05$). G-15 alone did not alter hypertension caused by aldosterone/salt but it prevented the anti-hypertensive effect of G-1. G-1 had no effect on aldosterone/salt induced hypertension in male GPER-deficient mice (n=8). The pressor response to aldosterone/salt was delayed in intact female C57Bl/6 where an increase in BP was only observed at 14 d; however, co-administration of aldosterone/salt with G-15 resulted in a marked increase of ~20 mmHg by d 7 (n=6-8, $P<0.05$). Administration of aldosterone/salt produced a robust increase in BP by d 7 in ovariectomised female C57Bl/6 (n=5-9, $P<0.05$) and female GPER-deficient mice (n=8, $P<0.05$), similar to that observed in males. T cells in kidneys of male and female C57Bl/6 mice were found to express GPER. In male RAG1-deficient mice, aldosterone/salt had virtually no effect on BP (n=8, $P<0.05$). However, adoptive transfer of T cells from male C57Bl/6 into male RAG1-deficient mice fully restored the pressor response to aldosterone/salt, which could be attenuated by co-treatment with G-1 (n=8-14, $P<0.05$). Preliminary data suggests that aldosterone/salt has no effect on BP by d 7 in female RAG1-deficient mice that have received T cells from female C57Bl/6 donors (n=4), whereas BP is increased in females receiving T cells from GPER-deficient donors (n=4).
Discussion. Our findings indicate that activation of GPER by G-1 and/or endogenous estrogen profoundly attenuates aldosterone/salt-induced hypertension in mice. T cells are key contributors to aldosterone/salt-induced hypertension and there appears to be sex differences in the pressor response to aldosterone/salt.

135 Relaxin mediates its anti-fibrotic actions in human cardiac myofibroblasts via AT2 receptor-dependent phosphatases

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Introduction. The ovarian and cardiovascular hormone, relaxin, was recently found to mediate its anti-fibrotic effects in primary renal myofibroblasts *in vitro* and a mouse model of obstructive nephropathy *in vivo* via Relaxin Family Peptide Receptor 1 (RXFP1)/angiotensin II type 2 receptor (AT_2R) heterodimers, such that TGF-β1-induced myofibroblast differentiation and aberrant collagen deposition were inhibited[1]. Whether relaxin is able to signal through AT_2R-dependent mechanisms, downstream of RXFP1/AT_2R interactions, remains unknown.
Aims. To determine whether recombinant human relaxin (RLX) mediates its anti-fibrotic effects via AT_2R-dependent phosphatase activity in primary human cardiac myofibroblast (HCFs) *in vitro*.
Methods. HCFs (fetal ventricular and atrial fibroblasts; ScienCell, USA) were stimulated with TGF-β1 (5ng/ml) to undergo differentiation into myofibroblasts, and treated with RLX (16.8nM/100ng/ml) or the AT_2R-agonist, Compound 21 (C21; 1μM), in the absence or presence of the AT_2R antagonist (PD123319, 0.1μM) or an RXFP1 antagonist (1μM) for 72 hours. HCFs were also stimulated with TGF-β1 (5ng/ml) and treated with RLX (16.8nM) or C21 (1μM), in the absence or presence of the PP2A inhibitor (okadaic acid, 10nM) or MKP-1 inhibitor (NSC95397, 1μM) for 72 hours. AT_2R-phosphatase expression and known end-points of RLX activity: phosphorylated (p-)ERK1/2, p-nNOS, α-SMA (myofibroblast differentiation) and collagen I (the basis of fibrosis) were then assessed by Western blotting and densitometry of the appropriate bands (from 3-4 separate experiments conducted in duplicate in each case).
Results. TGF-β1-stimulated HCFs were found to express the PP2A (serine/threonine) and MKP-1 (MAP kinase), but not SHP1/SHP2 (tyrosine), phosphatases. RLX or C21 significantly increased p-ERK1/2 and p-nNOS (by 55-175%), but decreased α-SMA and collagen I expression (by 25-50%) in TGF-β1-stimulated HCFs; which were abrogated by co-administration of either PD123319, RXFP1 antagonist, okadaic acid or NSC95397 (all $p<0.05$ vs RLX or C21 alone).
Discussion. RLX appears to mediate its anti-fibrotic effects in HCFs via AT_2R-dependent PP2A and MKP-1 phosphatase activity, suggesting for the first time that it can signal through AT_2R-dependent mechanisms via RXFP1/AT_2R interactions.
[1]Chow B et al (2014) Kidney Int, 86:75-85.

136 Multiple adenosine receptor subtypes stimulate wound healing in human EA.hy926 endothelial cells.

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Introduction. Wound healing is a vital outcome associated with tissue damage and can be stimulated by adenosine released from cells during events such as tissue injury, inflammation or ischaemia. Several studies have demonstrated a role for adenosine via adenosine receptor subtypes in stimulating endothelial cell proliferation, angiogenesis and wound healing (see Feoktistov et al, 2009).
Aims. To determine the potency and efficacy of selective adenosine A_1, A_{2A} and A_{2B} receptor agonists on the rate of wound healing and cell proliferation in human EA.hy926 endothelial cells.
Methods. The permanent human endothelial hybrid cell line (EA.hy926) was grown in high glucose Dulbecco's Modified Eagle's Medium (DMEM). Adenosine receptor subtype mRNA and protein levels were assessed using real time PCR and Western blot techniques. EA.hy926 endothelial cell proliferation was measured using the scratch wound healing and CCK-8 assays.
Results. Real time PCR data showed that only adenosine A_1, A_{2A} and A_{2B} receptor mRNA was expressed in this cell line. All three adenosine A_1, A_{2A} and A_{2B} selective receptors agonists, CPA, CGS21680 and NECA, significantly increased the rate of wound healing in human EAhy926 endothelial cells (n=6 per group, $P<0.05$). The selective adenosine A_1, A_{2A} and A_{2B} receptor antagonists, DPCPX, ZM241385 and MRS1754 (10 nM) reversed the effects of their respective agonists. EAhy926 endothelial cell proliferation was also significantly increased with the selective adenosine A_1 and A_{2B} receptor agonists, CPA and NECA (n=6 per group, $P<0.05$). Western blot analysis demonstrated that adenosine A_{2A} and A_1 receptor protein levels were highly expressed compared to the adenosine A_{2B} receptors in the EAhy926 endothelial cell lines.
Discussion. All three adenosine A_1, A_{2A} and A_{2B} receptor subtypes contribute to cell proliferation and wound healing in human EAhy926 endothelial cells. Treatments selectively targeting the adenosine receptor subtypes may enhance wound healing and should be investigated as a therapy for conditions such as diabetes mellitus that slow wound healing.

Feoktistov I et al (2009) Handb Exp Pharmacol 193;383-397

137 Effects of cytokines, toll-like receptors and signal transduction receptor gene polymorphisms on acute rejection in kidney transplant patients

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Introduction. Acute rejection remains the biggest challenge early after kidney transplantation. Besides the adaptive immune system, innate immunity can trigger acute rejection [1]. Pro-inflammatory cytokine levels of IL-1β, IL-6 and TNF-α are associated with acute rejection [2] but the impact of SNPs in *IL1B*, *IL6* and *TNFa* is inconsistent [3]. Similarly, inconsistencies exist regarding the role of SNPs of the T-cell activating cytokine *IL2* and anti-inflammatory cytokines *IL10* and *TGFB1* in predicting acute rejection [3]. Toll-like receptors (TLRs) also contribute to acute rejection with MyD88 being essential for TLR function [4]. The effect of these genetic polymorphisms on acute rejection has not been adequately assessed.
Aims. To investigate the impact of *IL1B*, *IL2*, *IL6*, *IL10*, *TGFB1*, *TLR2*, *TLR4, TNFa* and *MYD88* SNPs on acute rejection in kidney transplant recipients in the first two weeks post-transplant.
Methods. All kidney transplant recipients (n= 165) received tacrolimus, mycophenolate and prednisolone. Acute rejection was based on Banff classification. Three SNPs in *IL1B*, two in *IL10* and *TLR4*, respectively, and one in *IL2*, *IL6*, *TGFB1*, *TLR2, TNFa* and *MYD88*, respectively, were genotyped. Genotype differences in acute rejection incidence within the first two weeks post-transplantation were compared by Chi-square or Fisher's exact tests.
Results. Thirty-eight patients (23%) developed acute rejection in the first two weeks post-transplantation. None of the genetic polymorphisms significantly impacted on acute rejection (*TNFa* rs1800469, lowest point-wise P = 0.051).
Discussion. Although the impact of cytokine, TLR and *MYD88* SNPs on acute rejection was not statistically significant, given the relatively limited sample size in this study, further assessment on these SNPs, especially the impact of *TNFa* rs1800469 on acute rejection warrants further investigation.

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138 Reducing inappropriate polypharmacy and deprescribing: An online education module for hospital clinicians

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Introduction. Hospitalisation is an under-utilised opportunity to reduce inappropriate polypharmacy in older adults. Clinician barriers and enablers to deprescribing include awareness of the problem and self-efficacy, which is a prescriber's belief and confidence in their ability to address inappropriate polypharmacy. The effectiveness of online education in deprescribing is unclear. This is an RACP Evolve Trainee demonstration project, which promotes ASCEPT Evolve guideline 5: Reduce use of multiple concurrent therapies (hyperpolypharmacy).
Aims. 1) Develop an online NSW Health, Education and Training Institute (HETI) module on inappropriate polypharmacy and deprescribing to educate multi-disciplinary clinical staff involved in older inpatient care. 2) Describe hospital clinician awareness of inappropriate polypharmacy and self-efficacy for deprescribing post-completion of the module.
Methods. An 11-minute animated case-based module has been developed with subject matter experts as part of a multi-factorial intervention. A piloted online questionnaire investigating awareness and self-efficacy was developed using literature and expert opinion. A convenience sample of hospital clinicians was recruited from six metropolitan NSW hospitals to complete the post-module questionnaire.
Results. At interim analysis, questionnaire completion rate is 35% of module viewers. Seventy six hospital clinicians completed the questionnaire: 72% female, 87% medication reviewers (pharmacists or hospital doctors), and ~50% have <5 years experience. Awareness of inappropriate polypharmacy was high (88%), but confidence managing is lower (72%, χ^2_1=12.84, p≤0.00). Most participants had low awareness of the available tools for deprescribing. The lowest domain for self-efficacy in medication reviewers was 'making a deprescribing plan'. Interestingly, 66.7% of non-medication reviewers (nurses and allied health) would like to participate in future deprescribing discussions with patients.
Discussion. An online module to address barriers to inappropriate polypharmacy has been developed, and further exploration of multi-discipline clinician understanding of deprescribing may direct future in-hospital interventions.

139 Combination therapy with an SGLT2 inhibitor as initial treatment for type 2 diabetes: a systematic review and meta-analysis

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Introduction. Debate exists whether patients with type 2 diabetes (T2DM) should have anti-hyperglycaemic agents initially prescribed sequentially or in combination. Sodium-glucose cotransporter 2 inhibitors (SGLT2i) may be appropriate agents for first-line combination therapy, particularly due to their extra-glycaemic benefits.
Aims. To compare the efficacy and safety of initial combination SGLT2i/metformin therapy with either metformin monotherapy, or SGLT2i monotherapy. To compare high dose and low dose SGLT2i combinations with metformin.
Methods. PubMed, EMBASE and Cochrane Library were searched for randomised controlled trials (RCTs) of SGLT2 inhibitors. RCTs were selected if they (1) enrolled treatment-naïve T2DM participants (2) compared combination therapy with an SGLT2i to monotherapy (each agent in the combination) (3) treatment duration was ≥ 12 weeks (4) change from baseline in haemoglobin A1c (HbA1c), weight, and adverse events were reported.
Results. Four RCTs were included in the meta-analysis that focused on the effects of the SGLT2i/metformin combination (n=3749). Combination SGLT2i/metformin resulted in a greater reduction in HbA1c (-0.55% [95% CI -0.67, -0.43]) and weight (-2.00 kg [95% CI -2.34, -1.66]) compared with metformin monotherapy after 24-26 weeks of treatment and a greater reduction in HbA1c (-0.59% [95% CI -0.72, -0.46]) and weight (-0.57 kg [95% CI -0.89, -0.25]) compared with SGLT2i monotherapy over the same treatment period. Compared with combination low dose SGLT2i and metformin, high dose SGLT2i and metformin resulted in no HbA1c difference (0.02% [95% CI -0.08, 0.13]) but greater weight reduction (-0.47 kg [95% CI -0.88, -0.06]).
Discussion. Initial combination therapy with SGLT2i/metformin has HbA1c and weight benefits, compared with either agent alone. High dose SGLT2i/metformin combination therapy appears to have modest weight but no glycaemic benefits compared with the low dose SGLT2i/metformin combination therapy.

140 Predictive Performance of a Commercial Bayesian Forecasting Program in Estimating Vancomycin Drug Exposure

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Introduction. Current TDM guidelines for vancomycin (IV) in adults identify drug exposure (as indicated by area-under-the-curve, AUC) as the best pharmacokinetic (PK) indicator of therapeutic outcome. We aimed to assess the predictive performance of a Bayesian forecasting program in estimating vancomycin AUC with limited PK sampling.
Methods. The application of a 1-compartment Bayesian forecasting program (DoseMeRx; DoseMe Pty Ltd, Brisbane, QLD) for vancomycin was examined retrospectively in adult patients (n = 22) with stable renal function. Patients were intensively sampled following a single 1g dose (if weight < 50 kg, 15 mg/kg) infused over at least 0.67h. For each patient, 'AUC_{TRUE}' was calculated from fitting all vancomycin concentrations to a 2-compartment model using weighted, nonlinear least-squares regression. AUC_{TRUE} was then compared to the following Bayesian-estimated AUC values: '$AUC_{DATA\text{-}RICH}$' (using all vancomycin concentrations), '$AUC_{TRUNCATED}$' (using all concentrations >1h post-infusion), and '$AUC_{DATA\text{-}POOR}$' (using a single concentration sampled at either 1.5, 2, 4, 6, 10, 16, or 24h post-infusion).
Results. When comparing DoseMe-estimated $AUC_{DATA\text{-}RICH}$ to AUC_{TRUE}, the Bayesian program was biased towards underestimating drug exposure, with a median % prediction error (MDPE%) of -27.9 (IQR 19.5). Precision was also poor, with a median absolute % prediction error (MDAPE%) of 29.7 (IQR 17.1). When comparing DoseMe-estimated $AUC_{TRUNCATED}$ to AUC_{TRUE}, bias was lower (MDPE% -12.0 [IQR 14.8]), as was imprecision (MDAPE% 16.0 [IQR 10.4]). Furthermore, when comparing $AUC_{DATA\text{-}POOR}$ to AUC_{TRUE}, at all time points the Bayesian program tended to underestimate AUC. Predictive bias was smallest when inputting a single vancomycin concentration sampled at the earliest time post-infusion (MDPE% ranged from -2.8% [IQR 29.5] at 1.5h to -26.2% [IQR 11.5] at 24h).
Discussion. Overall, the Bayesian program tended to under-predict AUC. Compared to $AUC_{DATA\text{-}RICH}$, $AUC_{TRUNCATED}$ was a more accurate estimation of AUC_{TRUE}. This is likely due to the 1-compartment Bayesian model requiring serum vancomycin concentrations beyond the α-distribution phase (>1h post-infusion). When predicting $AUC_{DATA\text{-}POOR}$ with a single vancomycin concentration, predictive performance was less biased with vancomycin concentrations at the beginning of the β-distribution phase (~1.5 h post-infusion).

141 Total and unbound mycophenolic acid pharmacokinetics before and after kidney transplantation

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Background: An increased incidence of acute rejection is seen with underexposure to mycophenolic acid (MPA) in the initial week post kidney transplantation, particularly in high immunological risk patients. Up to 25% of individuals in contemporary drug regimens are below the proposed therapeutic range at this time, and these individuals cannot be identified *a priori.*
Objectives: To quantify the change in MPA pharmacokinetics, and unexplained between-occasion variability, from pre- to post-kidney transplantation.
Methods: The ADOPT Trial (Dose Optimization Prior to Transplant) collected MPA and metabolite concentration data prior to kidney transplantation after steady-state dosing, and on multiple post-transplant occasions, from 45 transplant recipients. Analysis was performed using NONMEM 7.4.1.
Results: Total and unbound MPA concentration data were fit to a two-compartment pharmacokinetic model with first order absorption and lag-time, and first order elimination with allometric scaling. There was a -13% relative prediction error (RPE) in model-predicted post-transplant clearance using the pre-transplant individual clearance estimate, which is an improvement compared with using the population clearance (40% RPE).
Conclusion: Pre-transplant measurements of MPA can be used to improve the prediction of the dose required post-transplant to achieve target exposure.

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142 Ebselen prevents cigarette smoke-induced endothelial-dependent dysfunction in mouse thoracic aorta

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Introduction. Chronic obstructive pulmonary disease (COPD) is a disease characterised by persistent airflow limitation, lung inflammation and increased oxidative stress. COPD is predominantly caused by cigarette smoking (CS) and is currently the 4th leading cause of death globally, with ~50% of patients dying from a cardiovascular event. Although increased oxidative stress and systemic inflammation alter blood vessel structure by promoting vascular remodeling, stiffness and atherosclerosis, how this leads to worsened cardiovascular outcomes in COPD patients is unknown.
Aim. To investigate the effect of CS exposure on blood vessel endothelial and smooth muscle function.
Methods. Male BALB/c mice were exposed to either room air (sham) or CS generated from 9 cigarettes per day, 5 days a week for 8 weeks. Mice were treated with the anti-oxidant ebselen (5mg/kg, oral gavage once daily) or vehicle (5% CM cellulose) 1 h before CS exposure. The mice were culled, the thoracic aorta removed, cut into ~2 mm rings, mounted in a myograph and set to a resting tone of 5mN of force. The aortic rings were pre-constricted to their maximum tension using the thromboxane A2 receptor agonist U46619 (100nM). The vessels were then washed with Krebs and pre-constricted to 50% maximum, using U46619. Concentration-response curves to acetylcholine (Ach) or sodium nitroprusside (SNP) were then performed to investigate endothelial and smooth muscle dilator responses, respectively.
Results. Ach (10^{-8} to 10^{-5} Mol/L) caused an ~80% relaxation of U46619-contracted aorta obtained from sham-exposed, vehicle-treated mice (n=6). However, aorta taken from CS-exposed, vehicle-treated mice had significantly impaired relaxant responses to Ach (n=6, ~40 % R_{max}, P<0.0001), which was prevented by ebselen treatment in CS-exposed mice (~80% relaxation). Relaxant responses for aorta taken from sham-exposed, ebselen-treated mice were similar to sham-exposed, vehicle-treated mice (n=6, ~80 % R_{max}). SNP (10^{-8} to 10^{-5} Mol/L) caused an ~80% maximum relaxation of aorta taken from both sham and CS-exposed mice, irrespective of ebselen treatment.
Discussion. Endothelial function of mouse aorta is impaired following CS exposure. Moreover, targeting oxidative stress with ebselen reduces CS-induced endothelial dysfunction suggesting that this approach is a novel means for treating cardiovascular comorbidities in COPD.

143 Drugs used in pregnancy and neonatal medicine: how likely are they to access the developing brain?

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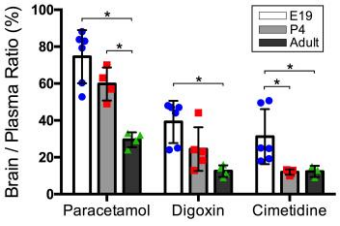

Introduction. Over 60% of pregnant patients take medications that cannot be classified as safe by the FDA (Andrade et al, 2004). The need for treatments is high, however information about the transfer of common drugs into the developing brain, where they could cause long-term harm, is limited. Understanding which drug classes are least likely to access the developing brain would assist in safe and effective patient dosing.
Aims. (i) To measure the transfer of 3 commonly used medications (paracetamol, digoxin and cimetidine) across the placental and blood-brain barriers of rats during development. (ii) To measure whether repeat drug exposure can lead to the placental and blood-brain barriers acquiring resistance to drug transfer.
Methods. Sprague Dawley rats (E19, P4, adult) were injected i.p. with ^{3}H labelled paracetamol (15 mg/kg), cimetidine (11mg/kg) or digoxin (30μ/kg) either acutely (sampling 30 minutes post-injection) or chronically (4 days bi-daily injection, then as acute on the 5th day). The amount of ^{3}H radiolabel was measured in the blood, cerebrospinal fluid and brain using liquid scintillation counting. For RT-qPCR studies unlabelled drugs were used in the same protocol.
Results. All 3 drugs transferred from blood to the fetal brain in higher amounts than the newborn, which once again had higher transfer than in the adult (Fig 1). In pregnancy 10-35% of the drug in maternal blood reached the fetal brain (paracetamol 34%±2; digoxin 16%±2, cimetidine 14%±3; n=8). Chronic treatment resulted in significantly less transfer of paracetamol/digoxin across the adult blood brain barrier (P<0.05) but not in newborns or fetuses. Decreased brain entry correlated with increased expression of specific blood-brain interface efflux transporters.
Discussion. Efflux transporters are functionally active at blood-brain interfaces at all ages tested, as each drug entered the brain at levels below that predicted by their lipid solubility. At no age did these transporters completely prevent drug entry and in immature brains up-regulation of these transporters following chronic exposure did not occur. These results are of clinical importance, providing an estimation of neurological transfer of different drug classes.
Andrade et al (2004) A Am J Obstet Gynecol 191:398-407.

144 Novel AT_2 receptor agonists reverse high salt diet-induced cardiac and renal fibrosis

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Introduction. Targeting the angiotensin type 2 receptor (AT_2R) has demonstrated anti-fibrotic effects in various organs. We have successfully developed a single β-substitution to a number of Ang III analogs that markedly increased AT_2R:AT_1R selectivity from 20,000-fold up to 200,000-fold.
Aims. To compare the potential anti-fibrotic effects of novel AT_2R ligands (β-Pro^7-Ang III, β-Pro^7-Trp^8-Ang III, D-Arg^2-β-Pro^7-Trp^8-Ang III and N-Ac-β-Pro^7-Trp^8-Ang III) in a high salt (HS) diet-induced heart and kidney disease model.
Methods. Male FVB/N mice were fed a HS (5% NaCl) diet for 8 weeks. From weeks 5-8, sub-groups of mice (n=8-9/group) were treated with either β-Pro^7-Ang III (0.1 mg/kg/day) ± PD123319 (1 mg/kg/day), β-Pro^7-Trp^8-Ang III, D-Arg^2-β-Pro^7-Trp^8-Ang III or N-Ac-β-Pro^7-Trp^8-Ang III (all 0.1 mg/kg/day) via subcutaneous mini-pumps. Markers of cardiac and renal inflammation, fibrosis, collagen turnover and reactive oxygen species production were measured.
Results. Compared to normal salt (NS) diet, HS increased cardiac and renal interstitial fibrosis (picrosirius red-staining) by ~1.5 fold and renal glomerulus fibrosis by ~2 fold, which was confirmed by hydroxyproline analysis (all n=7-9, $P<0.05$). These changes were associated with increased myofibroblast differentiation (α-smooth muscle actin) and inhibited collagen degradation (MMP-13). β-Pro^7-Ang III reversed HS-induced cardiac fibrosis, which was abolished by AT_2R antagonist PD123319. β-Pro^7-Trp^8-Ang III, D- Arg^2-β-Pro^7-Trp^8-Ang III and N-Ac-β-Pro^7-Trp^8-Ang III all reversed HS-induced inflammation (MCP-1; phosphorylated-IκB; F4/80 levels) and fibrosis in heart and kidney (all n=7-9, $P<0.05$). The anti-fibrotic effects caused by these AT_2R agonists in the heart were associated with downregulated myofibroblasts differentiation while cardiac TIMP-1 expression was significantly inhibited by N-Ac-β-Pro^7-Trp^8-Ang III (n=8, $P<0.05$). Moreover, HS-induced cardiac and renal oxidative stress was reversed by β-Pro^7-Trp^8-Ang III, D- Arg^2-β-Pro^7-Trp^8-Ang III and N-Ac- β-Pro^7-Trp^8-Ang III (all n=7-9, $P<0.05$).
Discussion. Novel selective AT_2R selective agonists are cardio- and reno-protective, highlighting a potential therapeutic role of AT_2R in cardiovascular disease.

145 Relationship between academic outcomes in a pharmacology unit of a biomedical science degree, and attending lectures or accessing lecture recordings: is it detrimental to access lecture recordings?

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Introduction. We have previously shown that nursing students who attend lectures have better academic outcomes in pharmacology than those that do not, even though these students had access to lecture recordings.
Aims. To determine the relationships between academic outcomes in a pharmacology unit of a biomedical science degree, and attending lectures or accessing lecture recordings.
Methods. In 2017, in a 3rd year pharmacology unit for biomedical science students, 39 of 42 students consented to undertake the study. Attendance was documented in nine of 10 face-to-face lectures, and the number of lecture recordings used to ≥ 60% completion was collated. Regression analysis of final unit mark against lecture attendance or access of lecture recordings was undertaken and Pearson's correlation coefficient (r) determined.
Results. Sixty percent of the students attended the first lecture, and then lecture attendance declined to an average of 41%/lecture. The final marks were significantly higher for students who attended ≥ 50% of lectures (86% ± 3, n = 21) than those who attended < 50% of lectures (71% ± 4, n =18). There was a weak positive association (r = 0.3148) between lecture attendance and the final unit mark in pharmacology. Thirteen of the 39 students did not access any lecture recordings, and an additional 10 students did not access any lecture to ≥ 60% completion. For the 26 students who accessed the lecture recordings, there was a weak negative association (r = -0.4875) between number of lectures accessed to ≥ 60% completion of the recording and final mark. When the 10 students who accessed lecture recordings, but not to ≥ 60% completion, were removed from the regression analysis, there was still a weak negative association (r = -0.3282) between number of lectures accessed to ≥ 60% completion of the recording and final mark.
Discussion. As the pharmacology students attending lectures had better academic outcomes than those that did not, it still seems important to provide face-to-face lectures. Lecture recordings were accessed more by the students who subsequently had poorer outcomes. It seems unlikely that this access was detrimental, and may have improved the outcomes for these individuals.
Doggrell SA & Schaffer S (2017) https://www.asceptasm.com/wp-content/uploads/2017/12/APSA-ASCEPT-poster-abstracts-51217.pdf

146 Is a genotype-phenotype relationship predictive of 5-fluorouracil-induced severe GI toxicity incidence? A pilot study.

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Introduction. Severe gastrointestinal (GI) toxicity following 5-fluorouracil (5-FU)-based treatment is highly prevalent and negatively affects therapy. Our immune genetic study revealed *TNF* and *TLR2* variants predicted severe GI toxicity risk[1]. However, matching diagnostic phenotypic markers are yet to be identified.
Aims. To determine if the secretion of proinflammatory cytokines, IL-1β and TNF-α, can predict the incidence of severe GI toxicity in patients after 5-FU-based treatment.
Methods. 24 participants (9 with severe GI toxicity) who previously completed our immune genetic study were recruited, with their toxicity data, demographics, treatment parameters and *IL1B*, *TNF*, *TLR4* and *TLR2* genotypes known. *Ex vivo* quantification of LPS- and PAM3CSK4-induced stimulation of IL-1β and TNF-α secretion from PBMCs was determined by ELISA. Changes in secretion from baseline were determined and compared between participants with and without severe GI toxicity using Mann-Whitney tests. In addition, the association between *IL1B*, *TNF*, *TLR4* and *TLR2* genotypes and IL-1β and TNF-α secretion was also examined using Mann-Whitney or Kruskal-Wallis tests.
Results. There was a 2- and 5.7-fold decrease (P = 0.02 and P = 0.001, respectively) in LPS- (100 ng/mL) and PAM3CSK4- induced (50 pg/mL) TNF-α secretion in participants with severe GI toxicity compared to participants with no/mild GI toxicity. In addition, LPS-induced secretion of IL-1β was decreased in participants that were heterozygous and homozygous variant for the *IL1B* rs1143634 SNP compared to wild-types: 50 μg/mL - 1.4- and 11.2-fold, respectively (P = 0.001); and 100 μg/mL - 1.55- and 8.4-fold, respectively, (P = 0.002).
Discussion. This study is ongoing. Further participants are being recruited to expand the study cohort to 46.

[1]Coller JK et al (2015) Support Care Cancer 23:1233-1236

147 Yohimbine, a new phenotyping measure for CYP2D6 activity

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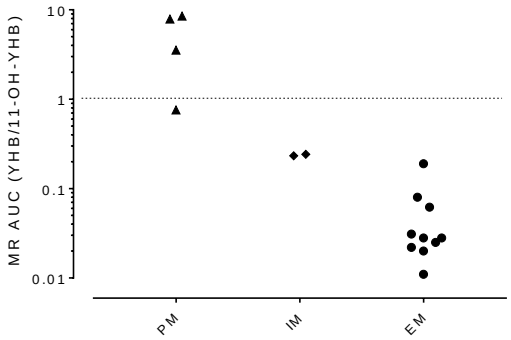
Introduction. CYP2D6 is one of the important drug metabolising enzymes exhibiting a genetic polymorphism. Genotyping is not as predictive for the enzyme activity; hence a specific and precise marker is required.
Aims. To develop CYP2D6 phenotyping using yohimbine as a marker substance with high specificity.
Methods. A clinical study protocol was approved by the competent authority and the ethics committee. Sixteen healthy participants took 5 mg yohimbine as a single oral dose. A 24 hour pharmacokinetic profile was obtained. Participants were genotyped for CYP2D6 alleles by means of long PCR and SNaPShot-Kit. Plasma concentrations of yohimbine and 11-OH-yohimbine were quantified using an according to EMA guidelines validated high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay with a LLOQ of 0.5 ng/ml.



Results. Pharmacokinetics of yohimbine showed a high variability with respect to CYP2D6 genotype. AUC $_{0-\infty}$ was 16.9 h*ng/ml in EMs (n=10), 89.4 in IMs (n=2) and 2140 in PMs (n=4). Correspondingly clearance decreased from 9496 ml/min (EMs), to 932 (IMs), and to 39 (PMs). The metabolic ratio of the AUCs of yohimbine and 11-OH-yohimbine can be used to identify the CYP2D6 phenotypes (see Figure).
Discussion. Because of the significant differences between the CYP2D6 genotypes in yohimbine pharmacokinetics covering at least two orders of magnitude, yohimbine could be a suitable substance for CYP2D6 phenotyping.

148 Failure to account for phenoconversion may lead to misleading pharmacogenomic (PGx) reports

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Introduction. Common polymorphisms in the genes encoding CYP2D6, CYP2C19 and CYP2C9 enzymes play an important role in predicting adverse effects and efficacy of substrate medications. PGx testing is increasingly being used in clinical practice to guide prescribing, however, recommendations are often made using genotype information alone. Drug-induced changes to the enzyme's phenotype through inhibition or induction, a process called phenoconversion, may contribute to significant variability in drug exposure. The impact of phenoconversion has not be well described in clinical practice.
Aims. To quantitate the extent of phenoconversion in an Australian patient population and evaluate its potential impact on drug-drug interactions.
Methods. Adapting the method by Borges et al, 2010, we estimated the phenoconversion frequencies using the records of 2905 Australian patients who underwent pharmacogenomic testing of CYP2D6, CYP2C19 and CYP2C9 genes to guide the prescribing of psychotropic medications. Patients predicted by genotype to have normal or reduced enzyme function were phenoconverted to PMs in the presence of a moderate or strong enzyme inhibitor.
Results. A 5-fold increase in the frequency of CYP2D6 and CYP2C19 PMs due to phenoconversion was observed compared to genotype-predicted PMs (5.4% to 24.7% and 2.7% to 17%, respectively). For strong inhibitors only, a 3-fold and a 4-fold increase in PMs was observed for CYP2D6 and CYP2C19 enzymes respectively, (5.4% to 17.6% and 2.7% to 11%). Due to the low number of CYP2C9 inhibiting drugs taken by this patient population, no significant change to the genotype-predicted phenotype frequencies for CYP2C9 were identified.
Discussion. These figures describe a high frequency of drug-induced CYP2D6 and CYP2C19 phenoconversion rates to PM status among patients receiving psychotropic medications. It is important for treating clinicians to be aware of phenoconversion risk in this patient group to ensure drug-drug interactions are appropriately addressed.

Borges S et al (2010) J Clin Pharmacol 50:450-458.

149 Genomic Guided Pharmacotherapy; A Pilot Study in Danish Patients Suffering from Chronic Non-Cancer Pain

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Introduction. Efficacy and safety of drugs vary significantly between individuals. Approximately 40 % of this variation can be ascribed to genetic variability. The CYP450 enzyme system is responsible for the metabolism of most drug substances. In Denmark (DK) the first genetic test "Personalised Medicine Profile" (PMP) was recently made available by GeneTelligence. PMP is a genotyping test, which identify genetic variation, translates genotype into phenotype and generates a personalised and evidence-based report (PMP-report) on the influence of genetic variation on drug pharmacokinetics and – dynamics. The PMP-report includes data on variations in 14 different genes and evidence based recommendations for individual therapy of 158 commonly prescribed drugs.
Aims. To evaluate practical issues using the PMP test and the interpretation of the resulting report in a small selected population of patients suffering from chronic non-cancer pain and further to assess the number and types of drug-gene interactions identified by the PMP-test.
Methods. Patients were recruited from three Pain Centres in DK. Patients received a buccal swab DNA-kit. After genotyping, phasing, imputation and annotation a personalised report (decision support tool) was generated, using the PMP software platform.
Results. Data from 14 patients, was included in the analyses. In general, the patients found it easy to perform the buccal swab. One swab contained insufficient DNA material to perform the genotyping and was repeated. The patients received from 1 to 14 different medications. In 8 out of the 14 patients the PMP-test identified $\geq$ 1 actionable drug-gene interaction. A selected case will be presented to illustrate the types of of drug-gene interactions identified.

Papasterigiou J (2017) japha 57:624-629

150 Effect of chronic polypharmacy and the Drug Burden Index (DBI) on muscle function and structure in aged mice

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Introduction. Ageing, polypharmacy (use of ≥5 medications) and increasing DBI (measures total anticholinergic and sedative medication exposure) are associated with falls and impaired physical function. Preclinical ageing models can assess underlying mechanistic changes
Aims. We investigated whether chronic polypharmacy or monotherapy, with increasing DBI and/or cessation (deprescribing), affected physical function and/or muscle histology in mice.
Methods. 12-month-old male C57BL/6 mice received either control diet or study drug(s) at therapeutic doses. Polypharmacy diets consisted of Zero DBI (metoprolol, simvastatin, omeprazole, paracetamol, irbesartan), Low DBI (metoprolol, simvastatin, omeprazole, paracetamol, citalopram) and High DBI (metoprolol, simvastatin, citalopram, oxycodone, oxybutynin). Individual drugs (High DBI regimen) were tested as monotherapy. At 21-months, animals were randomised to continue treatment or gradual withdrawal. Rotarod performance was assessed at 12-24-months, and balance beam (6mm) at 24-months. Gastrocnemius muscle samples were collected at 26-months.
Results. Rotarod performance at 21-24-months indicated significantly increased endurance for metoprolol treated mice (n=15-36) compared to control (n=24-29) and to High-DBI (n=18-38), and for Zero-DBI mice (n=15-34) compared to Low-DBI (n=19-40); ($p<0.05$). Balance assessment showed deprescribed High-DBI (n=18) and metoprolol (n=16) mice performed significantly better than their continuously prescribed comparators (n=10-15; $p<0.05$). Preliminary histology results suggest a trend towards less muscle fibres per field in control (n=3), compared to High-DBI (n=3; $p=0.119$) and citalopram animals (n=2; $p=0.049$), while collagen quantification suggests no difference between control (n=5), High DBI (n=6) and High DBI deprescribed (n=4).
Discussion. Rotarod detected differences between drug treatments and deprescribing affected balance. Our preclinical results suggest polypharmacy and certain monotherapy drug regimens impact measures of muscle function and may affect structure. Future research will continue to characterise histological changes in muscle.

151 The role of UGT enzymes in cytotoxic drug resistance in breast cancer cells and cancer stem cells

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Introduction. UDP-glucuronosyltransferases (UGTs) conjugate sugars to lipophilic chemicals as part of a larger network of drug metabolizing enzymes (DMEs) involved in elimination of drugs and toxins. Epirubicin (EPI) is a cytotoxic often used in combination with other drugs to treat breast and other cancers, generally in later stages. UGT2B7 is thought to be the only UGT that metabolizes EPI; our studies show UGT2B7 is induced by EPI in liver cells, which may enhance systemic clearance. However, the role of UGT2B7 in intratumoural clearance, and hence resistance, is unknown.
Aims. To assess whether EPI increases UGT2B7 expression in breast cancer cells and breast cancer stem cells (BCSC), and to determine whether UGT2B7 plays a role in drug resistance in breast cancer cells and/or BCSC.
Methods. MDA-MB-231 breast cancer cells were treated with EPI and UGT2B7 mRNA was quantified by qPCR. UGT2B7-overexpressing cell lines were generated and characterized for response/resistance to EPI. An 'induced BCSC' (iBCSC) model was established by reprogramming MDA-MB-231 cells with pluripotency factors (Oct4/Sox2/Klf4); these were characterized for stem-cell like behaviour, response/resistance to EPI, and gene expression.
Results. Treatment of MDA-MB-231 cells with EPI for 72 hrs induced expression of UGT2B7 by ~4 fold. Increases in the expression of other UGTs and drug efflux ABC transporters were also observed. Ectopic overexpression of UGT2B7 in MDA-MB-231 cells led to increased EPI resistance; the increase in half maximal inhibitory concentration (IC50) averaged ~1.5 fold (n=2). The iBCSC model showed a gene expression profile consistent with epithelial mesenchymal transition (EMT) and constitutive drug resistance. Although UGTs (including UGT2B7) were not constitutively elevated in iBCSC, treatment with EPI resulted in a much higher UGT2B7 induction (~47 fold) relative to the parental cell line.
Discussion. EPI transcriptionally induces UGT2B7 (and efflux transporters) in breast cancer cells contributing to short-term resistance of these cells to EPI toxicity. BCSC may have both constitutive elevation of genes that contribute to drug resistance (such as efflux transporters) but may also be epigenetically primed to rapidly induce additional mediators of resistance, such as UGT2B7. Understanding the roles of UGTs and transporters in the drug resistant phenotype of BCSC may provide new avenues to enhance the efficacy of cytotoxics in this pathogenic cell population.

152 How are we using immunosuppressant medicines in Australasian elderly renal transplant recipients?

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Introduction. Kidney transplantation is first-line treatment for most patients with end-stage renal failure. Optimising immunosuppressant regimens is crucial; current guidelines make no specific recommendations for elderly patients.
Aims. To explore current immunosuppressant medicine prescribing practices in elderly and younger adult renal transplant recipients across Australia and New Zealand.
Methods. A descriptive study of data obtained from the ANZDATA (Australia and New Zealand dialysis and transplant) registry, including all patients transplanted from 2000-2015 was conducted. Patients were categorised as younger adults (<65 years) or elderly (≥65 years). The choice and doses of immunosuppressant medicines prescribed initially and at one-year post transplant was compared using descriptive statistics (Mann-Whitney test or chi-statistic).
Results. A total of 6,930 patients were included in the analysis; 39% of younger adults and 38% of elderly patients were female, with an average age of 47 and 67 years respectively. The most commonly prescribed immunosuppressant drugs were prednisolone, mycophenolate, cyclosporine A and tacrolimus; with 86% of younger adults and 84% of elderly patients taking three immunosuppressant medicines (initially and at one-year). Initial doses of tacrolimus were significantly lower in the elderly, and this trend continued at one-year ($p<0.05$; Figure 1). The elderly also had greater median reductions from initial to one-year post transplant in their doses of mycophenolate, cyclosporin A and azathioprine ($p<0.05$).

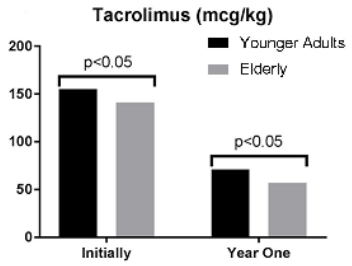

Figure 1: Median tacrolimus doses prescribed initially and at one-year post transplant in elderly and younger adult recipients

Discussion. In our sample, immunosuppressant doses were reduced more in elderly patients. In order to determine reasons for these differences, further investigation of drug exposure and side effects in the elderly is warranted.

153 Microsampling as an alternative collection method to venous blood to quantify capecitabine and its metabolites by LC-MS/MS

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Introduction: Dose individualisation of many anticancer therapies has been shown to significantly improve cancer outcomes by enabling optimum drug exposure or reducing major toxicity. Pharmacokinetic-guided dose individualisation of capecitabine and 5-fluorouracil may be associated with an increase in overall survival and/or lower toxicity. However, this is difficult to achieve for remote patients where specialised facilities are unavailable. Volumetric absorptive microsampling collection as an alternative to venepuncture may facilitate this process in remote locations or in the home.
Aims: To evaluate the use of the Mitra microsampling device for its applicability in determination of capecitabine and its metabolites by LC-MS/MS.
Methods: Exact volume of whole blood (10 µL), obtained from volunteers, spiked with various analyte concentrations, was absorbed on Mitra microsampling devices and dried at ambient temperature for at least 3h. Sample tips containing the absorptive pad were placed into the microcentrifuge tubes and acetonitrile containing stable isotope-labelled internal standards was added. Samples were sonicated, evaporated under vacuum and then re-suspended in 0.1% formic acid before injected into a Shimadzu 8060 LC-MS/MS. Chromatographic separation was on a Luna Omega Polar C_{18} (100 x 2.1 mm, 1.6 µm) column using gradient elution of 0.1% formic acid and acetonitrile.
Results: The intra and inter-day imprecision ranged from 3.0-8.1 and 6.3-13.3% respectively, for capecitabine, 5'-deoxy-5-fluorocytidine, 5'-deoxy-5-fluorouridine and 5-flurouracil. Accuracy ranged from 95 -116%. LLOQ with imprecision of < 18.8 % and accuracy between 89-114 % was 50 µg/L for 5-flurouracil and 10 µg/L for all other analytes. Assays were linear from 50-50,000 µg/L for 5-flurouracil and 10-10,000 µg/L for all other analytes.
Discussion: Microsampling with LC-MS/MS provides a method as reliable as conventional blood collection for capecitabine and metabolites. This may lead to less invasive and better timed sample collection for therapeutic drug monitoring supporting optimised cancer practice.

154 Mechanism of cell-specific signalling by a peptide mimetic of the relaxin receptor RXFP1

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Introduction. The peptide hormone H2 relaxin activates the GPCR relaxin family peptide 1 (RXFP1) receptor. Recombinant H2 relaxin has demonstrated considerable promise as a treatment for acute heart failure (AHF). While it did not meet primary endpoints in a Phase IIIb study, patients showed improvements in markers of cardiac, renal and hepatic damage consistent with the prevention of organ damage. Due to these actions, drugs targetting RXFP1 are undergoing continued clinical development by numerous pharmaceutical companies. However, RXFP1 has a complex mechanism of ligand-mediated activation that complicates the development of small molecules and peptide mimetics that exactly mimic the mode of relaxin-mediated activation. In line with this, we recently developed a H2 relaxin mimetic peptide, B7-33, and showed it has cell-specific actions (Hossain et al., 2016. Chem. Sci. 7:3805-3819).
Aim. To investigate the cell-specific mechanism of action of B7-33 in parallel with the small molecule biased agonist ML290 in human cells relevant to the clinical actions of H2 relaxin.
Methods. We compared B7-33, ML290 and relaxin-mediated cAMP and cGMP signalling in HEK cells overexpressing RXFP1 (HEK-RXFP1) and cells natively expressing RXFP1; THP1, human cardiac fibroblasts and rat renal myofibroblasts, and the human dermal fibroblast cell line BJ3 with or without RXFP1 miRNA-mediated knockdown (KD). Angiotensin (AT) receptor-mediated actions were studied in fibroblasts using AT antagonists and agonists.
Results. B7-33 is a weak agonist of cAMP in HEK-RXFP1 and THP1 cells whereas it potently activates cGMP in human and rat myofibroblasts. cGMP activation by B7-33 and H2 relaxin in BJ3 cells was dependent on RXFP1 as miRNA-mediated KD abolished activity. Additionally, cGMP activation in fibroblasts could be blocked by RXFP1, AT1 and AT2 antagonists. Moreover, cGMP activation by the AT2 agonist, Compound 21, was also blocked by RXFP1, AT1 and AT2 antagonists highlighting that both RXFP1 and AT2 agonists are acting via a mechanism involving all three receptors.
Discussion. This mechanism extends our previous results showing that the AT2 receptor is essential for relaxin's anti-fibrotic actions (Chow et al., 2014, Kid Int, 86, 75–85) and provides a potential explanation for the cell-specific actions of B7-33 in myofibroblasts whereby B7-33 has a higher affinity for the RXFP1/AT1/AT2 complex than for RXFP1 alone.

155 Probing the binding and function of polyamines at the calcium-sensing receptor

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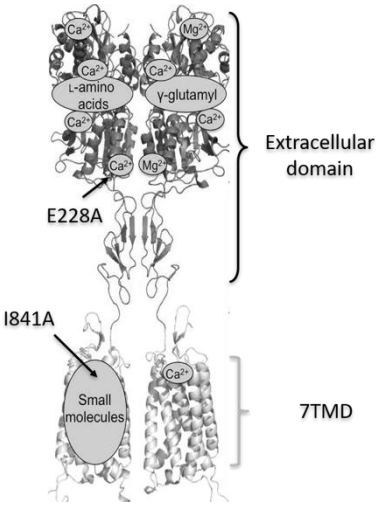

Introduction. The calcium sensing receptor's (CaSR) ability to detect changes in extracellular calcium (Ca^{2+}_o) to maintain Ca^{2+}_o homeostasis is well characterised. However, the CaSR also supports various other physiological functions, including airway inflammation. Thus, the CaSR is a putative drug target in asthma, since its allosteric agonists, polyamines, induce bronchoconstriction. Importantly, CaSR negative allosteric modulators can reduce asthma related pathology. However, the structural basis underlying polyamine-mediated CaSR activation is unknown.
Aim. To determine amino acid residues important for the binding and transmission of efficacy of the polyamines: agmatine, putrescine, spermidine, and spermine.
Methods. Calcium mobilisation assays in CaSR-HEK293 cells and an operational model of agonism were used to quantify agonist affinity and efficacy at the wild type and mutated CaSR. FACS analysis was used to determine the effect of amino acid substitutions on CaSR expression.
Results. Mutagenesis suggests that polyamines may bind to both the extracellular and seven transmembrane domains (7TMD) of the CaSR to induce Ca^{2+} mobilisation. Although polyamines share similar chemical structures, they are differentially affected by different amino acid substitutions. For example, E228A, located in the extracellular binding domain, enhances the affinity of agmatine and putrescine but has no effect on spermidine or spermine. I841A, located in the 7TMD binding site for small molecules, reduced the efficacy of spermidine and spermine but not agmatine or putrescine.
Discussion. Mutagenesis suggests that polyamines may differentially bind and activate the CaSR. Understanding how polyamines interact with the CaSR will inform future drug development that aims to specifically block polyamine binding and activation networks in the CaSR.

156 A molecular ruler for ligand efficacy: Using NMR to probe ligand induced changes in α1A-adrenoceptor conformational equilibria and characterize fragment hits

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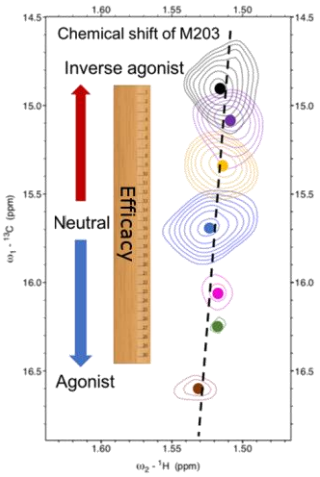

α_1-adrenoceptors (α_1-ARs) comprise three G protein-coupled receptor (GPCRs) subtypes that stimulate smooth muscle contraction in response to binding adrenaline and noradrenaline. α_{1A}-AR and α_{1B}-AR are clinically targeted for treating hypertension and benign prostatic hyperplasia but are putative drug targets for neurodegenerative diseases. New subtype-selective tool compounds are required to probe the role of these receptors in the brain and to validate them as drug targets for neurodegenerative diseases. GPCRs are allosteric machines that sample multiple conformations existing in equilibrium. Agonist binding shifts the equilibrium to active states to promote G protein signaling. Recent crystal structures give us snapshots of inactive and active states, but not the dynamics that underlie GPCR activation. Here, we isotopically labeled six methionines in α_{1A}-AR to probe how different ligands modulate the conformational equilibrium of this GPCR using NMR. Met292 sits in the orthosteric ligand binding pocket and its chemical shift was unique upon binding different ligands. Met203 on-the-other-hand, is located on the intracellular side of the receptor where G proteins interact. We found the resonance of Met203 shifts upfield in the presence of inverse agonists, downfield upon agonist binding and that the chemical shift changes correlated well with ligand efficacy. The linear dependence of the chemical shifts is consistent with a conformational selection mechanism, while the resonance broadening in the presence of agonist suggests increased microsecond motion. We subsequently used this molecular efficacy ruler to validate the pharmacology of two novel hits from a trial fragment screen and the peptide toxin, τ-Tia. In conclusion, this study validates the current conformational equilibrium-based hypothesis of GPCR function and establishes NMR for screening and characterizing novel GPCR ligands.

157 Cryptic pocket formation underlies subtype selectivity at the M1 muscarinic Acetylcholine receptor (M_1 mAChR)

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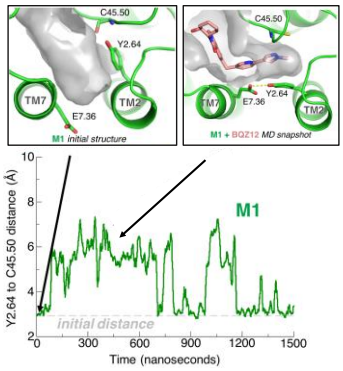

Introduction. Allosteric modulators of G protein-coupled receptors (GPCRs) are hotly pursued as novel drugs, predominantly because they offer a means to achieve selectivity between closely related GPCR subtypes. However, the mechanisms by which many allosteric modulators achieve selectivity, and particularly at the muscarinic acetylcholine receptor (mAChR) family, remain elusive. In light of recent structures that reveal virtually identical allosteric binding sites in M_1-M_4 mAChRs, we hypothesised that, a highly dynamic rearrangement of the M_1 mAChR must underlie the unprecedented selective nature of M_1 mAChR selective positive allosteric modulators (PAMs).
Aims. The aim of our study was to show that M_1-selective PAMs achieve their exquisite subtype selectivity by occupying a dynamic pocket, called *cryptic pocket*, that is not observed in existing solved inactive M_1-M_4 mAChR structures.
Methods. We used Molecular Dynamic (MD) simulations at M_1-M_4 mAChRs to simulate receptor activation, and radioligand binding studies at M_1 and M_4 mAChRs, wild type and rationally designed mutants, to validate our hypothesis.
Results. The spontaneous formation of the *cryptic pocket* occurs far more frequently in MD simulations of the M_1 mAChR compared to M_2-M_4 mAChRs. Binding of the M_1-selective PAM, BQZ-12, in this pocket reconciles mutagenesis data that previously appeared contradictory. Additionally, further mutagenesis experiments validated our computational prediction that prevention of the opening of the cryptic pocket decrease the affinity of this M_1-selective PAM, whilst un-affecting the M_4-selective PAM, LY2033298, at the respective M_4 mAChR mutants.
Discussion. Our findings suggest opportunities for the design of subtype specific allosteric drugs that exploit differences in the conformational ensembles of receptors whose static structures are nearly identical.

158 Assessing adenosine A1 receptor-mediated G protein dissociation using a NanoBiT assay

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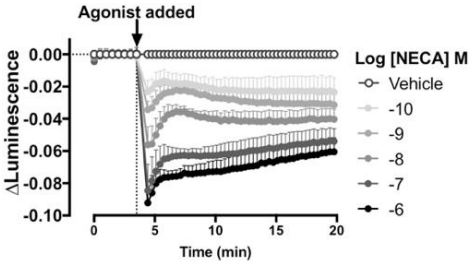

Introduction. Activation of the adenosine A_1 G protein-coupled receptor (A_1AR) represents a potent cardioprotective strategy for the management of cardiac ischaemia reperfusion injury (IRI). However, A_1AR agonists have failed to translate to the clinic for IRI due to dose-limiting bradycardia. We have identified A_1AR biased agonists, VCP746 and capadenoson, which display potent cardioprotective activity with minimal bradycardia.[1,2]

Aims. To identify whether ligand-dependent differences in Gα protein activation may underpin the different signalling profiles of prototypical and biased adenosine A_1 receptor agonists.

Methods. HEK293A cells with all G proteins deleted by CRISPR/Cas9 genome editing were stably transfected with the human A_1AR (3x HA A_1AR-ΔGall) and transiently transfected with a Gα subunit ($G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, or $G\alpha_z$) conjugated to a large luciferase fragment, $G\beta_1$, and $G\gamma_2$ conjugated to a small luciferase fragment. The change in luminescence, generated upon subunit dissociation, was determined upon A_1AR agonist addition.

Results. A_1AR agonists (NECA, MeCCPA, VCP746, capadenoson) stimulated a concentration-dependent reduction in luminescence for each $G\alpha_{i/o}$ subunit assessed, with a similar rank-order of potency. The prototypical agonists NECA and MeCCPA exhibited a biphasic kinetic profile with a rapid G protein dissociation followed by subunit re-association. In contrast, the kinetic profiles for the biased agonists, VCP746 and capadenoson, were characterised by slower and sustained subunit dissociation.

Discussion. The rank-order potency was retained for each of the agonists, suggesting biased agonism was not being driven through preferential coupling to the α subunits assessed. However, ligand-dependent differences in luminescence profiles provide preliminary evidence that biased agonism may involve different G protein kinetics.

1. Baltos J et al (2016) Biochem Pharmacol 99:101-112
2. Valant C et al (2014) Proc Natl Acad Sci USA 111(12):4614-1619

159 Structural insights into the allosteric binding sites of the M5 muscarinic acetylcholine receptor

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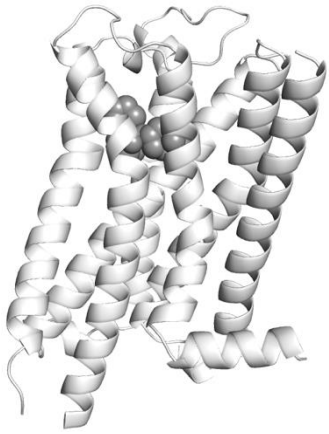

Introduction. The five muscarinic acetylcholine receptors (mAChRs M1-M5) are an important family of G protein-coupled receptors that are widely expressed throughout the central and peripheral nervous systems. The M1, M4, and M5 mAChR subtypes have been implicated in the pathophysiology of major neurological and psychiatric diseases, including Alzheimer's disease, schizophrenia, and drug addiction. As such, mAChRs have remained essential targets for drug discovery, despite the difficulty associated with designing molecules to selectively target the highly conserved orthosteric binding site. While crystal structures of the M1- M4 mAChRs have been determined, there are only a few ligand-bound structures that exist, including no structures of the M5 receptor. In addition, recently discovered highly selective M5 allosteric modulators have provided the opportunity for a deeper understanding of the receptor's physiological and therapeutic relevance; however, questions remain about how such ligands interact with the receptor.

Aims. Determine the structure of the M5 mAChR and determine the binding site for M5 allosteric ligands.

Methods. Crystal structures of the M5 receptor were determined using lipidic cubic phase X-ray crystallography. Molecular insights into allosteric modulator binding were determined using biophysical and pharmacological assays.

Results. Here we report the first structure of the M5 mAChR allowing a full comparison of all five subfamily members and provide mechanistic insight into how allosteric modulators bind the M5 receptor.

Discussion. Overall, our results indicate that the highly selective M5 allosteric modulators do not bind to the prototypical mAChR allosteric site.

160 Mechanistic pathways of metformin induced reversal of age-related pseudocapillarization in mice liver endothelium

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Introduction: Age-related pseudocapillarization leads to impaired transfer of lipoproteins, pharmacological agents and insulin from the blood to the liver. This transfer occurs via fenestrations contained within the liver sinusoidal endothelial cells (LSECs) that line the sinusoids of the highly vascularized liver. Metformin is an extensively used treatment for type II diabetes that has been shown to delay age-related pseudocapillarization and this may be a mechanism for improved insulin and lipoprotein clearance in old age[1]. Here we have investigated the pathways involved in the metformin induced re-fenestration of LSECs isolated from old (18m, n=3) and young mice (4m, n=3).
Methods: Isolated LSECs were incubated for 30 mins with metformin (100, 50, 25, 10, 5 µM). Cells were fixed and prepared for visualization under scanning electron microscopy and direct stochastic optical reconstruction microscopy to examine fenestration porosity, diameter and frequency, and actin and transgelin densitometry. SK-HEP1 cell lines were used for p-AMPK, AMPK, p-eNOS, eNOS and actin measurements by western blot; lactate production, cGMP and ATP production were measured with assay kits and mitochondrial activity with immunofluorescence.
Results: *In vitro* single dose metformin treatment promoted increased fenestration porosity and frequency in young and old mice and the SK-HEP1 cell line ($p<0.05$). Western blot analysis demonstrated upregulation of pAMPK/AMPK and peNOS/eNOS with peaks in expression correlating with changes in fenestrations. ATP, cGMP and mitochondrial activity were also upregulated following metformin treatment in SK-Hep1. L-lactate and MTT assays did not demonstrate changes in metformin treated cells compared to controls. Increasing metformin dosages were associated with changes in transgelin and the actin cyto-architecture ($p<0.05$) consistent with what has previously been reported.
Conclusion: This study has demonstrates that reversal of age-related pseudocapillarization may be pharmaceutically driven in an AMPK and eNOS dependent manner.
[1]Alfaras I et al (2017) *NPJ 3*(1):16

161 Ca^{2+} imaging in organoids and *ex vivo* tissue: New insights into spontaneous and agonist-induced Ca^{2+} responses

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Introduction. Ca^{2+} signaling has essential roles in connective, muscular, nervous and epithelial tissue types. The nature of the intracellular Ca^{2+} signal in different cell types is shaped by the Ca^{2+} signaling toolkit (i.e. the expression and localization of Ca^{2+} channels, pumps and binding proteins) as well as the cellular microenvironment (i.e. extracellular signals and cell- and stromal-interactions). Numerous studies have investigated properties of intracellular Ca^{2+} signaling in isolated primary cells (e.g., immune cells) and tissue pieces (e.g., brain slices). In epithelial tissues, however, intracellular Ca^{2+} signaling is often studied in immortalized cells that are serially-passaged on glass or plastic.
Mammary epithelial cells are highly dependent on their physical and chemical cellular microenvironment. These cells are also Ca^{2+} signal dependent at various stages of development. Studies to examine intracellular Ca^{2+} responses in primary mammary epithelial cells with 3-dimensional context are therefore imperative.
Aims. To compare agonist-evoked and spontaneous intracellular Ca^{2+} signals in luminal and basal mammary epithelial cells isolated from mice with lineage-specific expression of the fast, genetically-encoded Ca^{2+} indicator GCaMP6f.
Methods. We generated mammary organoids (mini-organs)—with distinct luminal and basal cellular compartments, hormone receptor expression and lineage-restricted GCaMP6f expression—enabling us to compare agonist-induced Ca^{2+} responses in luminal and basal cells in both the 2D and 3D context. We also developed and tested a protocol to visualize agonist-induced intracellular Ca^{2+} responses in thick *ex vivo* mammary tissue pieces.
Results. We visualized intracellular Ca^{2+} responses in mini-organs and intact tissue in response to specific agonists. Using this approach, we demonstrate that oxytocin induces a robust increase in intracellular Ca^{2+} in basal mammary epithelial cells of the lactating mammary gland, followed by a phase of spontaneous Ca^{2+} oscillations immediately prior to contraction of individual mammary alveolar units, a finding that could only be revealed by studying these cells in their native context.
Discussion. Visualization of intracellular Ca^{2+} responses in cells in 3-dimensions, under conditions where cellular morphology, polarity, differentiation and cell-cell interactions, faithfully mimic *in vivo* biology is paramount if we are to fully understand the roles of intracellular Ca^{2+} signaling in both development and disease, and in response to pharmacological modulation.

200 Evaluating student perceptions of novel feedback methods

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Introduction. Practical classes form a key component of undergraduate pharmacology curricula. Thus, students' practical reports are a fundamental form of assessing their skills in data presentation, interpretation and written communication. We have previously evaluated undergraduate pharmacology students' perceptions of video feedback, in an effort to increase their engagement with the feedback process. Although students responded positively to this feedback method, this cohort consisted of a small number of students, approaching the very end of their degree.
Aims. The present study investigated video feedback in a second student cohort, to assess whether this has any influence on how it is perceived.
Methods. Participants were undergraduate Bachelor of Science / Biomedical Science students undertaking a third year core pharmacology unit in semester 1, 2018 (n=153). This unit included three assessed practical reports contributing to each student's overall unit mark. Feedback for the second report was provided in written or video format. At the conclusion of the unit's practical teaching program, students were invited to complete an anonymous hard copy survey asking them about their perceptions of the feedback they received.
Results. 153 from 174 students enrolled in the unit completed the survey (88% response rate). Approximately 90% of respondents indicated that they review feedback on any task either most or all of the time, with the majority indicating that the feedback format of their practical report made no difference to whether they reviewed it. Irrespective of feedback format, nearly 70% of respondents indicated that the feedback and mark they received were consistent. Approximately 40% of respondents indicated that the future usefulness of their practical report feedback would largely depend on the task being assessed.
Discussion. Feedback format appears to make little difference to the likelihood of its review, particularly if the majority of students already review the feedback they receive. The student cohort and nature of the assessment task might also influence how students respond to particular modes of feedback.

201 Evidence-based clinical practice guideline for deprescribing cholinesterase inhibitors and memantine in people with dementia

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Introduction. Ensuring optimal medication use in people with dementia involves both prescribing medications that will help achieve care goals and deprescribing (supervised withdrawal) of medications for which risk outweighs benefit. A lack of drug-specific deprescribing guidelines has been reported by health care professionals as a significant barrier to optimising medication use.
Aims. The purpose of this project was to develop a guideline to assist healthcare professionals to determine when it might be suitable to trial withdrawal of cholinesterase inhibitors (ChEIs) and memantine.
Methods. The Guideline Development Team (GDT) consisted of content experts, end-users, methodology experts and consumers. We followed the process of developing class-specific deprescribing guidelines, based on a comprehensive checklist for successful guideline development, the AGREE-II criteria and GRADE (Grading of Recommendations Assessment, Development and Evaluation).
Results. Four recommendations and three practice points were developed to guide deprescribing of ChEIs and memantine. The recommendations assist clinicians to identify individuals who may be suitable for a trial of deprescribing (such as those who do not have an appropriate indication, those who have never experienced a benefit, those who appear to be no longer benefitting, and those who have severe/end stage dementia). The practice points provide tapering and monitoring recommendations and other situations in which trial deprescribing could be considered. The guideline recommendations have been approved by the NHMRC and are published with supporting material at http://sydney.edu.au/medicine/cdpc/resources/deprescribing-guidelines.php.
Discussion. While there were limitations to the available evidence, the GDT was able to provide recommendations to guide deprescribing of ChEIs and memantine with the aim of improving quality of life in people with dementia. The recommendations should be considered in the context of the individual and deprescribing should be conducted as a process with consumer engagement throughout.

202 Spatial and temporal dimensions of mu-opioid receptor (MOR) signalling

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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 (MOR) subtype. Understanding the spatial and temporal dynamics of MOR signalling by different agonists could be important for the design of novel drug candidates with effective analgesia but with minimal adverse side effects.
Aims. To delineate the subtleties of MOR 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 and confocal imaging. Receptor organisation and dynamics at the plasma membrane were assessed using Ground State Depletion super-resolution microscopy in Total Internal Reflection Fluorescence mode (GSD/TIRF), Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Recovery After Photobleaching (FRAP).
Results. DAMGO and morphine induced distinct spatiotemporal signalling profiles. DAMGO activation of MOR caused a transient activation of cytosolic and nuclear ERK, that was dependent on a GRK2-mediated lateral redistribution of the receptor within the plasma membrane prior to, and independent of, endocytosis. In contrast, morphine activation of MOR caused a sustained increase in PKC activity at the plasma membrane that restricted MOR movement, and resulted in a sustained increase in cytosolic ERK.
Discussion. Activation of MOR by DAMGO and morphine causes distinct spatiotemporal signalling profiles dependent on the lateral organisation of the receptor at the plasma membrane, and independent of receptor internalisation. We are currently investigating how the development of tolerance affects these compartmentalised signals.

203 Mechanistic basis of signalling bias at μ-opioid receptors

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Biased signalling is property of GPCRs whereby different agonists at a single receptor type can differentially activate different downstream signalling pathways. Biased signalling at the μ-opioid receptor (MOPr), the main drug target for opioid analgesics, is well established and supposedly biased opioids with improved side effect profiles have entered late stage clinical trials. Clinically used opioid agonists produce analgesia and other actions by selectively activating MOPr and G-protein signalling. Additional pathways such as ß-arrestin dependent signalling & regulation have also been postulated contribute to their adverse effects. However, the mechanism of bias remain unclear. While structurally distinct agonists receptor states appear to contribute to bias for some GPCRs, theoretical considerations suggest agonist residence might be more important. We determined the rate kinetics of a range of opioid peptide agonists from natural sources, semisynthetic analogues and alkaloids in the G-protein-arrestin-signalling pathways to test this notion and find both that bias is correlated with efficacy and systematically increasing residence time by increasing aliphatic extensions on oxycodone increases bias towards ß-arrestin. We have developed a novel tetrapeptide, bilorphin, based on a novel opioid active peptide from an Australian estuarine isolate of *Penicillium sp.* MST-MF667 with an unusual LDLD chirality. Molecular dynamics simulations of bilorphin and endomorphin-2 interaction with MOPr suggested distinct interactions and receptor conformations that could underlie their large differences in bias. While bilorphin is systemically inactive, a glycosylated analog, bilactorphin, is orally active with similar in vivo potency to morphine. This provides a new avenue to explore the importance of signalling bias at MOPr for opioid safety.

204 Neuroimmune genetics of acute postoperative pain and opioid analgesia

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Opioids remain the first-line pharmacological treatment for the relief of moderate to severe acute postoperative pain, but pain severity and opioid dose requirements vary substantially between patients. There are major genetic components to acute pain and analgesia, and polymorphisms in neuronal pain signalling and opioid receptor- and pharmacokinetics-related genes play some role in postoperative pain and opioid response, respectively. However, much of the genetic component of acute pain and analgesia remains unexplained.
Activation of the innate immune system and its subsequent modulation of neuronal pain signalling has been implicated generally in pain and opioid analgesia, and genetic variability in this pathway is now being investigated due to its plausible mechanisms. Studies so far show that polymorphisms in neuroimmune signalling pathway genes (e.g. *TLR2/4*, *IL1B*, *TGFB1*, *IL6R*, *BDNF*) can contribute to variability in post-operative pain and morphine requirements, in some cases to a greater extent than classical pain/opioid somatosensory related signalling genes such as *OPRM1* and *COMT*. Ethnic groups also differ significantly in the frequency of most neuroimmune signalling pathway polymorphisms investigated, meaning genotype effects are often ethnicity-dependent, but can also explain some interethnic variability in postoperative pain and opioid requirements (Somogyi et al. 2016).
We have so far only covered a tiny fraction of genetic variability in the pathway, making neuroimmune signalling pathway genetics an exciting and largely unexplored avenue to understanding variability in postoperative pain and analgesia.
Somogyi A et al (2016) Pain 157:2458-66

205 Non-opioid receptor-mediated actions of opioids: preclinical and clinical studies

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In this study, we quantified the ability of opioids and their metabolites, present in biological samples, to activate the MOR and TLR4. Alphascreen cyclic AMP (cAMP) assay and MOR overexpressing HEK293 cells were employed to quantify the MOR activation. HEK-Blue hTLR4 were utilized to measure TLR-4 activation. Both assays were standardised using morphine, its MOR-active metabolite morphine-6 glucuronide (M6G) and its MOR-inactive, but TLR4-active metabolite morphine-3 glucuronide (M3G) in the presence/absence of plasma. Specificity was verified using opioid and TLR-4 antagonists or inhibitors. These methods were then employed to measure the receptor activation potential in the circulation of morphine-treated mice (1 or 10 mg/kg every 12 h for 3 days) or lower limb joint replacement surgery patients administered with opioids.
Morphine administration to mice led to TLR4 activation at time points where M3G is measured in plasma, but also to TLR4-independent NF-κB activation via elevation of circulating cytokines such as TNFα at time points where M3G is not detected. More importantly, morphine administration led to inhibition of LPS-induced TLR4 activation. Patient post-operative plasma samples displayed the ability to activate MOR and to inhibit LPS-induced TLR4 activation. Linear mixed model analysis revealed that MOR activation had a significant effect on inhibition of LPS-induced TLR4 activation. Furthermore, TLR4 had a significant effect to explain pain scores.
Our results show for the first time that (i) opioids administered to mice or surgery patients result in modulation of ligand-induced TLR4 activation and (ii) postoperative pain is associated with increased circulating TLR4 activation potential.

206 Beneficial effects of prazosin treatment during radiotherapy for prostate cancer

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Introduction. We have shown prazosin induces apoptosis in human prostatic cancer cell lines and selectively radiosensitises hypoxic prostate cancer cells.
Aim. To investigate the potential for prazosin to serve as an adjuvant treatment to radiotherapy for prostate cancer.
Methods. We retrospectively evaluated data from patients with prostate cancer who were receiving radiotherapy, but were also being treated with an alpha1-blocker to control urinary symptoms.
Results. Patients receiving radiotherapy were found to have a 3.9 times lower relative risk of biochemical relapse if they were being treated with prazosin. They also had significantly lower rates of biochemical relapse at both the two and five-year points (2.7%, 8.8%) when compared to the controls who received no alpha-blocker (22.6%, 34.5%). Discussion. These data suggest that prazosin reduces the risk of prostate cancer recurrence following radiotherapy.

207 Therapeutic potential of medical cannabis in gastrointestinal disorders

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The endogenous cannabinoid system (ECS) is a key modulator of gastrointestinal (GI) physiology, influencing motility, secretion, epithelial integrity and immune function. All elements of the ECS are expressed within the GI tract, including the major endocannabinoids anandamide and 2-AG, cannabinoid receptors and metabolic enzymes such as FAAH. Altered expression of the ECS occurs in some GI disorders such as colitis, and altered ECS genotypes are linked with clinical disorders such as Crohn's disease. There is anecdotal evidence that medical cannabis is used with varying efficacy in the treatment of IBD, IBS and other gut disorders, however clinical trials are needed before conclusive evidence of effectiveness can be determined. This is compounded by the diversity of available cannabis strains and chemotypes, encompassing hundreds of constituent cannabinoids and other phytochemicals with potential bioactivity. The loosening regulatory barriers for access to medical cannabis in Australia will facilitate greater access to standardized medical cannabis preparations to equip further basic and clinical research in this field, where such studies are needed to inform both the medical cannabis industry and clinical practice.

208 Non-hormonal male contraception using a combination of adrenoceptor and purinoceptor antagonists

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Introduction. According to the World Health Organization, there are 85 million unwanted pregnancies worldwide each year. Aside from the issue of global overpopulation, this is also a problem in affluent societies such as the U.S.A. and Australia. The NIH reported that in 2002, 49% or 2.65 million pregnancies (including abortions) in the U.S.A. were reported as unintended. Similarly, the Australian Bureau of Statistics recorded a teenage birthrate of ~15 per 1000 women in Australia in 2012, higher than most other developed countries. While present contraceptive methods are effective, there is clearly a need to develop additional methods of contraception for males, a market which is clearly lacking. Therapeutic targets for male contraception are associated with numerous problems due to their focus on disrupting spermatogenesis or hormonal mechanisms to produce dysfunctional sperm. This project describes the dual genetic deletion of α_{1A}-adrenoceptors and P2X1-purinoceptors in male mice thereby blocking sympathetically mediated sperm transport through the vas deferens during the emission phase of ejaculation.
Results. This modification produced 100% infertility without effects on sexual behaviour or function. Sperm taken from the cauda epididymides of double knockout mice were microscopically normal and motile. Furthermore, double knockout sperm were capable of producing normal offspring following intracytoplasmic sperm injection into wild type ova and implantation of the fertilized eggs into foster mothers. Blood pressure and baroreflex function was reduced in double knockout mice but no more than single knockout of α_{1A}-adrenoceptors alone.
Discussion. These results suggest that this autonomic method of male contraception appears free from major physiological and behavioural side effects. In addition, they provide conclusive proof of concept that pharmacological antagonism of the P2X1-purinoceptor and α_{1A}-adrenoceptor provides a safe and effective therapeutic target for a non-hormonal, readily reversible male contraceptive. A synthetic medicinal chemistry approach is currently under way to discover a suitable P2X1-purinoceptor antagonist to use in combination with tamsulosin for this purpose.

209 Towards a personalised approach for the treatment of BPH

Dr Betty Exintaris, Monash Institute of Pharmaceutical Sciences, Monash University

Introduction. A greater insight into human spontaneous prostatic contractility is fundamental for understanding the mechanism of action of current pharmacotherapies that target prostatic smooth muscle tone, thereby improving the lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH). However, a clear limitation with recent studies on contractility within the human prostate gland includes the lack of acquisition of patients' data. In this study, we demonstrate that there are individual differences in the spontaneous contractile profile recorded in the transition zone in fresh prostatic specimens from 34 men, where individual responses to current pharmacotherapies also differ.
Aims. To correlate the variability in prostatic responses to clinically used drugs with patient characteristics.
Methods. Transition zone tissue (10mm X 15mm) from the prostate gland was obtained from consenting patients undergoing radical prostatectomy. Contractile recordings were made from prostatic preparations (5mm X 10mm) using standard tension recording techniques as we have previously described. A paired Student's t-test was used to test for statistical significance ($P < 0.05$).
Results. In all 34 specimens, robust spontaneous contractions were recorded at a frequency of 1.92 +/- 0.14 contractions per minute and an average duration of 13.2 +/- 1.2 seconds per contraction. The basal tension was 4.58 +/- 0.23 mN and the amplitude of each contraction was 0.23 +/- 0.02 N/g. Overall, the PDE5 inhibitor, sildenafil [10-5M] significantly ($p< 0.05$, $n= 12$) decreased the basal tension and frequency of spontaneous contractions within the transition zone specimens. However, there was notable interpatient variability, with a subset of patients unresponsive to Sildenafil. Regression analysis determined that age was significantly ($R2= 0.448$, $p< 0.05$) negatively correlated with responsiveness to Sildenafil, with younger men having a greater reduction in the frequency of spontaneous contractility. In contrast, older men responded better to the current gold standard pharmacological agent, tamsulosin ($R2= 0.362$, $p< 0.05$)
.Discussion. Consideration of individual patient's clinical data may explain the varying parameters measured in the contractility profiles of different patients in this study. In addition, this study demonstrated that pharmacotherapies that have a direct modulatory effect on human prostatic smooth muscle tone in some patients, may not modulate the contractility of others. Such information may lead to the development of a more personalised treatment approach to better manage the symptoms associated with BPH, in addition to improving the quality of life for patients.

210 Insulin regulated aminopeptidase – what is it and why should we target it in cardiovascular disease?

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Cardiovascular disease (CVD) is exemplified by pathological alterations including left ventricular (LV) dysfunction, increased organ fibrosis, increased arterial stiffness and impaired endothelial function, with changes in one system affecting others, eventually leading to chronic heart failure and/or end organ damage. The multi-faceted nature of CVD makes it very challenging to treat and the effectiveness of many strategies depends on the underlying aetiology, severity and extent of disease with pathological fibrosis a major driver in many situations, and current standard of care causing only modest regression of fibrosis. We have now identified a novel target in the treatment of CVD, the enzyme insulin regulated aminopeptidase (IRAP). IRAP has a broad tissue distribution (brain, aorta, heart, kidney, liver, lung and uterus) and has diverse roles in the body, including trafficking of the glucose transporter 4 (GLUT4) in response to insulin and cleavage of various peptides[1]. Although the role of IRAP in CVD is not fully understood, there is clear evidence of IRAP upregulation in various pathophysiological states[2]. However, it is unclear whether increased IRAP expression/activity is a cause or consequence of CVD. To gain insight into the potential of targeting IRAP in CVD, we have assessed the anti-fibrotic effects evoked by removal of IRAP activity, by both pharmacological inhibition with novel specific IRAP inhibitors and genetic deletion using global IRAP deficient (IRAP KO) mice. Indeed, we now show for the first time that genetic deletion or pharmacological inhibition of IRAP is anti-fibrotic and is associated with recovery of left ventricular (LV) function, and enhanced vascular function. These studies provide compelling proof-of-principle that a reduction in IRAP activity markedly inhibits cardiac, renal and vascular tissue fibrosis, decreases inflammation and is vaso-protective and thus identifies IRAP as a novel target in CVD.

1. Chai SY et al. (2004) Cell Mol Life Sci 61, 2728-2737
2. Vinh A et al. (2008) Cardiovasc Research 77, 178-187

211 Disease-specific context of biased agonism and its application for cardiovascular disease

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Context-specific biased agonism refers to the ability of a drug to enrich a distinct spectrum of receptor conformations to those of the reference drug in a disease context-specific manner. Context- specific adenosine receptor (AR) biased agonism presents an opportunity to design drugs that selectively enhance cardioprotective and anti-remodelling signalling within post-MI cardiac cells, with the relative exclusion of on-target adverse effects. Ours is the first research program to directly investigate the influence of disease context on biased agonism. Recently we identified two A1AR/A2BAR biased agonists, VCP746 and capadenoson, that confer potent A1AR-mediated cardioprotection with *minimal* effects on heart rate or blood pressure; enabling, for the first time, separation of desired from adverse effects. Furthermore, we have demonstrated that AR biased agonism decreases secondary pathological hypertrophy and fibrosis, through A1AR and adenosine A2B receptor (A2BAR) activation, respectively. Importantly, new data demonstrate context-specific A1AR/A2BAR biased agonism promotes superior cardioprotection during the different stages of post-MI pathobiology, relative to prototypical AR agonists. Taken together, these studies reveal the need to understand the effect of the signalling context (age, disease, cell differentiation) when developing novel cardiovascular therapeutics, and even highlight a potential new drug discovery space that such a signalling context entails.

212 Exploiting biased agonism for managing cardiac ischaemia

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Introduction: Myocardial infarction remain the leading cause of mortality and morbidity, thus developing innovative approaches against this condition is highly desirable**.** Formyl peptide receptor (FPR) are GPCRs integral to regulation and resolution of inflammation, but it can elicit opposing downstream cellular response. This study tested the hypothesis that FPR biased ligand is superior than balanced ligand providing cardio-protection against MI.
Method: Signalling fingerprints of the small-molecular agonist Compound 17b and Compounds 43 at FPR1 and FPR2 were systemically assessed in CHO cells stably-expressing human FPR1 and FPR2. This was contrasted to their impact on cardiac injury response, both in cardiomyocytes, cardiofibroblasts and across 4 different time points post ischaemic insult in adult male mice.
Results: Compound 17b exhibited a distinct signaling fingerprint to Compound 43 at both hFPR subtypes. Compound 17b post-receptor signaling was biased away from potentially detrimental intracellular calcium mobilisation by 30 fold, while maintaining the beneficial reperfusion risk salvage pathway. The biased agonist agonist Cmpd17b (50 mg/kg/day, i.p.) elicited significant cardioprotection when administered at reperfusion, reducing cardiac necrosis (44⍰4% to 29⍰5%), cardiac neutrophil content at 48h from 3.5⍰0.4 to 1.9⍰0.2 AU, early cardiac remodeling and more importantly preserving cardiac function (25⍰4 to 35⍰5%) when compared to vehicle treated mice subjected to ischaemic insult (n=6-14, p<0.05, one way ANOVA with Tukey's post-hoc).
Conclusion: We demonstrated that FPR1/2-biased agonism with the prototype molecule Compound17b is superior than the balanced ligand Compound 43 in vitro and in vivo. These finding supports the development of small molecule FPR agonist to treat myocardial infarction.

213 Orphan G protein-coupled receptor GPR37L1: a cardiovascular regulator with phenotypic variability

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Introduction. G protein-coupled receptors (GPCRs) are major pharmaceutical targets for cardiovascular (CV) disease treatment, with approximately a third of all FDA-approved drugs acting on GPCRs. Thus, the discovery that deletion of the brain-expressed, orphan GPCR (no identified ligand), GPR37L1, caused a ~62 mmHg increase in murine blood pressure (Min et al., 2010) led to the hypothesis that GPR37L1 is a potential target for the treatment of hypertension.
Aims. To investigate the mechanism by which GPR37L1 affects blood pressure (BP) and cardioprotection.
Methods. Multiple GPR37L1 null (KO) lines were obtained (Min et al., 2010) or created (Coleman et al., 2018), and their BP determined at 12 weeks using multiple methods: anaesthetised micromanometry; conscious tail cuff or radiotelemetry with spectral analysis. Responses to a 7-day AngII infusion or aging were also assessed.
Results. We found no BP elevation in the original KO line (Min et al., 2010), while our KO mice showed a BP elevation confined to females from multiple cohorts (+11 mmHg micromanometry, +9 mmHg telemetry; Coleman et al., 2018). Responses to AngII infusion were also sexually dimorphic; male KO mice advanced to heart failure and female KO mice showed protection from cardiac fibrosis. One year-old KO mice of both sexes developed left ventricular hypertrophy despite unaltered BP. Radiotelemetry BP measurement of an independent cohort of our KO mice revealed a paradoxical absence of the previously observed female KO BP elevation, yet a female-specific blunted CV response to aversive stressors was detected.
Discussion. We have consistently observed CV phenotypes in GPR37L1 null mice, yet the identification of these phenotypes is variable. We attribute this variability to the marginal increases in BP (maximum of 11 mmHg difference; sex- and age-dependent) and to differences in BP recording protocols. These findings suggest that GPR37L1 is not a robust or druggable target for the treatment of essential hypertension.
Min et al (2010) Biochem Biophys Res Commun, vol. 393(1), pp55-60.
Coleman et al (2018) Biol. Sex. Diff., vol. 9(1), pp14.

214 Can cystic fibrosis patients finally catch a breath with cystic fibrosis transmembrane conductance regulator drugs?

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Introduction: Cystic fibrosis is a life limiting disease caused by defective or deficient cystic fibrosis trans-membrane conductance regulator (CFTR) activity. The recent FDA approval of lumacaftor/tezacaftor combined with ivacaftor targets patients with the F508del-CFTR.
Aim: The question remains, is this breakthrough combination therapy the 'magic-bullet' cure the vast majority of patients with CF?
Methods & Results: This study covers the contemporary clinical and scientific knowledge-base for CFTR modulator drugs including drug-drug plasma protein binding and cytochrome interactions and unexpected 'side-effects' such as antimicrobial activity or CNS potential.
Conclusion: The study highlights the prospectus of these future treatment options.
Table: CFTR gene mutation classes

CFTR Class	Effect on protein	Example
I	Complete loss of protein function	1078delT; 1717-1G→A; 3659delC; 621 + 1G→T
II	Defective regulation processing	G85E, F508del, I507del, R560T, N1303K
III	Defective protein regulation	G178R, S549N, S549R, G551D, G551S, G970R, G1244E, S1251N, S1255P, G1349D
IV	Defective protein conductance	R117H, R334W, R347P
V	Reduced protein synthesis	2789 + 5G→A, 3849 + 10KbC→T, A455E
VI	Impacted protein surface retention	120del23, N287Y

215 Understanding and informing medicines use among older Australians receiving residential aged care services

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More than 270,000 older Australians receive residential aged care services annually. Residents of aged care facilities are often frail, with multiple health conditions, and many have complex care needs. More than half of all residents are living with dementia. Polypharmacy is prevalent in residential aged care facilities (RACFs) and is increasing over time. Residents have more complex medication regimens than community-dwelling people of the same age. Although residents of aged care facilities consume the highest amounts of medicines, this population is among the least researched. Few interventions have focused on optimising medicines use in Australian RACFs, with varying success. This presentation will summarise recent pharmacoepidemiological research undertaken to better understand patterns of medicines use and health outcomes among residents of South Australian RACFs. Quantitative and qualitative findings from the 'SImplification of Medications Prescribed to Long tErm care Residents' (SIMPLER) study, a cluster randomised controlled trial involving 242 residents from eight South Australian RACFs, will also be discussed.

216 Structure-function studies of drug metabolising enzymes: application of computational approaches for the characterisation of structural plasticity and ligand recognition

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Cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT), which comprise enzyme 'superfamilies', are together responsible for the elimination of approximately 80% of drugs cleared by hepatic metabolism. Although three-dimensional structures from X-ray crystallography have been invaluable for understanding the structural basis of the ligand selectivity of CYP enzymes, elucidation of the dynamic aspects of protein structure and the implications of plasticity for ligand binding and catalysis are somewhat limited with this approach. In contrast to CYP, understanding structure-function relationships of UGT enzymes are less advanced due to the unavailability of an X-ray crystal structure of a full-length human protein. Computational approaches, particularly Molecular Dynamics (MD), provide an alternative approach for modelling the structural flexibility of CYP and UGT enzymes, and its implications for ligand binding. Three examples of the application of computational approaches, namely MD and comparative modelling, to the elucidation of CYP and UGT structure-function will be provided in this presentation. The first example involves the use of MD simulations to demonstrate plasticity in the active site of CYP2C9, and characterise the mechanisms by which dapsone acts as a heterotropic activator of NSAID metabolism. The validation of time-dependent effects of abiraterone on CYP17A1 activity by MD simulations will also be demonstrated, including elucidation of a two-step induced fit mechanism. The third example involves the application of comparative modeling to investigate cofactor binding in UGT enzymes. Overall, the studies demonstrate the potential of MD simulation and other computational methods for the rationalisation and prediction of CYP and UGT structure-function.

217 Pharmacoepidemiologial studies in dementia: findings from Swedish national registers

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Introduction. Medication use in people with dementia is challenging. There is limited guidance for prescribing in people with dementia and this population are particularly vulnerable to adverse drug events. Inappropriate medication use can lead to an increased risk of morbidity and mortality. Big data provides opportunities to study the effects of medications and the ways to optimise their use in people with dementia.
Aims. To investigate medication use in the treatment and care of people with dementia and their association with various clinical outcomes.
Methods. Nationwide data linkage studies using data from the Swedish Dementia Registry (SveDem), the Swedish National Patient Register, the Swedish Total Population Register, and the Swedish Dental Health Register were conducted.
Results. A total of 57658 people were registered in SveDem between 2007 – 2015. Acetylcholinesterase inhibitor (AChEI) users had a lower risk of stroke (hazard ratio [HR]: 0.85, 95% confidence interval [CI]: 0.75–0.95) and all-cause death (HR: 0.76, 95% CI: 0.72–0.80) compared to non-use. AChEI use was also associated with a lower risk of antipsychotic (HR: 0.89, 95% CI: 0.82–0.98) and anxiolytic (HR: 0.91, 95% CI: 0.85–0.97) initiation in those with Alzheimer's disease and Lewy Body dementia. Anticholinergic cognitive burden (ACB) increased the risk of developing stroke and all-cause death (ACB score of 1 [HR: 1.09, 95% CI: 1.04–1.14] and ACB score of ≥2 [HR: 1.20, 95% CI: 1.14–1.26]. Greater use of xerostomic medications was associated with an increased rate of tooth extractions.
Discussion. Medication use may be associated with outcomes including stroke, death and poor oral health in people with dementia. Careful consideration should be made when prescribing certain medication classes in this population.

218 Continuing pain – the importance of collaboration

Prof Andrew McLachlan, Sydney Pharmacy School, The University of Sydney

Musculoskeletal pain carries a major health burden and available treatments provide variable results and the risks of harms.

This talk will discuss at recent, current and future trials focussing on the management of low back pain, sciatica and osteoarthritis. Pain is a multidimensional health condition, so finding effective solutions requires multidisciplinary collaboration.

300 Novel psychoactive substances – what's happening out there and how do we know?

John P Thompson, School of Medicine, Cardiff University, UK

The number of new recreational drugs appearing on the market over the last few years has been unprecedented. This has posed challenges for regulators, analytical laboratories and clinicians alike.

Many of the 'novel psychoactive substances' (NPS) drugs are analogues and modifications from previously known chemicals including piperazines, phenyethylamines, tryptamines and opioids. However, these may be sold recreationally as 'branded' products, and the identity of the contents may not be known to the user. This poses a challenge in following trends, a problem that may be complicated by identically branded products having different chemical contents at different times or in different locations.

In many instances, and certainly when a new psychoactive substance hits the streets, its chemical identity and clinical effects will not be known, nor will there be much 'practical experience' within users of a dose response curve, or even time of onset. This can result in accidental overdoses, and advice being sought at a time when the clinical experience and evidence base for appropriate treatment strategies are limited. For practical purposes it may be necessary to have a syndromic classification, to facilitate treatment, until more is known about the particular toxin.

To obtain a reasonable understanding of which NPS are out there requires the use of multiple different techniques. These may include timely analysis of drug seizures by law enforcement and border control agencies, voluntary submission of samples by users, hospitals and local government to harm-reduction sites (e.g. WEDINOS), analysis of biological samples from patients and even analysis of pooled urine samples from nightclubs!

301 The structural basis of class B G protein-coupled receptor activation and signalling

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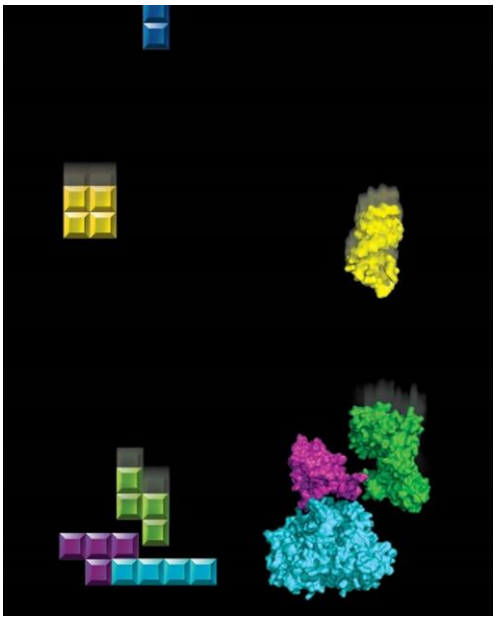
Introduction. Class B G protein-coupled receptors (GPCRs) bind critically important physiological peptides of 30-40 amino acids, and are important targets for major diseases including diabetes, obesity and osteoporosis. This family of receptors predominantly couple to heterotrimeric Gs proteins to promote cAMP signalling, however they can pleiotropically couple to multiple other effectors. This makes them subject to biased agonism, a phenomenon that described the ability of different ligands acting at the same receptor to promote different signalling profiles. This paradigm has gained traction in recent years as a novel avenue to target GPCRs for drug discovery. Class B receptors can also couple to receptor activity modifying proteins (RAMPs), which has the potential to modulate receptor function through altering ligand selectivity and/or effector coupling.
Methods. Single molecule cryo-electron microscopy (cryo-EM) was applied to determine structures of class B GPCRs in complex with their canonical transducers, heterotrimeric Gs proteins. Alanine scanning mutagenesis, pharmacological profiling and analytical modelling were employed to identify key residues within these receptors that are important for receptor ligand interactions, receptor and effector activation and signalling.
Results. Structures of minimally modified receptors bound to peptide agonists, including the human calcitonin receptor, the glucagon-like peptide-1 receptor (GLP-1R), and the calcitonin-gene-related peptide receptor (CGRPR), which consists of the calcitonin-related receptor and the single pass transmembrane protein RAMP1, were solved at 3.8Å. 3.3Å and 3.3Å respectively. Combining structures of these peptide:receptor:Gs complexes with mutagenesis and pharmacology data revealed key receptor domains important for ligand-specific interactions, signal transduction and biased agonism.
Discussion. Collectively, these studies reveal differences in modes of ligand binding and initiation of activation across different subfamilies of class B GPCRs, in addition to common macromolecular changes associated with activation and Gs coupling. Moreover, analysis of the structural data combined with pharmacology, receptor mutagenesis and analytical modelling reveals critical insights into GPCR modulation by RAMPs and the structural basis of GLP-1R biased agonism.

302 Designing modulators of protein-protein interactions

Jessica K Holien[1]. ACRF Rational Drug Discovery Centre, St Vincent's Institute[1], Fitzroy, VIC, Australia



Protein-protein interactions (PPIs) play important regulatory roles in cells, for example in the cell division cycle or in cell signalling and often become disregulated in cancers. Thus it is not surprising that modulators of PPIs – ideally small "drug-like" molecules - are urgently being sought and developed by the Pharmaceutical Industry to treat unmet medical need. However, the characteristics of the PPI interface make this task non-trivial. Structure-based computational molecular modelling is at the heart of PPI modulator design. However, the established design principles need to be significantly modified and revised for the difficult and unique nature of PPI interfaces.
Using cancer examples, I present the current methods used in the ACRF Rational Drug Discovery Centre to modulate PPIs including; homology modelling, protein-protein docking, virtual screening, and biophysical analysis. These methods have been used in conjunction with biological results to structurally explore novel PPIs and subsequently design potent and selective small molecule modulators for these interactions.

Yuriev E, Holien J, Ramsland PA (2015) Improvements, trends, and new ideas in molecular docking: 2012-2013 in review. J Mol Recognit. 28(10):581-604
Nero TL, Morton CJ, Holien JK, Wielens J, Parker MW. (2014) Oncogenic protein interfaces: small molecules, big challenges. Nat Rev Cancer. 14(4):248-62

303 Discovery of first-in-class adenosine A_1 receptor negative allosteric modulators by virtual ligand screening

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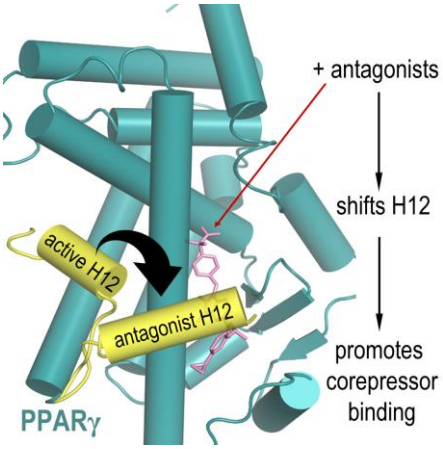

The adenosine A_1 receptor (A_1AR) is a potential therapeutic target for acute heart failure, a major global health burden also associated with renal dysfunction and a high risk of mortality. A_1AR antagonists have previously been investigated to improve renal function in patients with acute heart failure. However, they have not transitioned into the clinic due to dose-limiting on-target adverse effects. A_1AR negative allosteric modulators (NAMs), which target a topographically distinct A_1AR site, can fine-tune A_1AR inhibition with high subtype selectivity and spatiotemporal specificity, thus representing a novel pharmacological strategy for the treatment of heart failure and associated co-morbidities. This study aims to identify novel A_1AR NAMs using a combination of computational, chemical and pharmacological approaches. The optimized A_1AR model (based on the A_{2A}AR crystal structure; PDB ID: 3QAK) was subjected to a prospective virtual ligand screening (VLS) of a filtered ZINC library of 4.6 million drug-like compounds. The ability of top-ranking compounds to modulate the binding (of [^{3}H]DPCPX) and function (inhibition of forskolin-stimulated cAMP accumulation, ERK1/2 phosphorylation, intracellular calcium mobilisation, and G protein-coupled receptor kinase 2 (GRK2) recruitment) of the agonist NECA was assessed in 3xHA-A_1AR FlpINCHO cells and physiologically relevant native cells (rat neonatal ventricular cardiomyocytes (NVCMs) and mouse cortical neurons (CNs)). Functional interaction studies revealed ZINC439966, ZINC20220354, ZINC24760387, and ZINC8923830 were NAMs, while ZINC7755186 and ZINC7985611 were competitive antagonists. The highest affinity compounds were ZINC7985611, ZINC20220354, ZINC8923830 with an affinity ranging from approximately 10 nM – 400 nM. A_1AR NAM activity of the lead VLS compounds, ZINC20220354 and ZINC8923830 was relative consistent across a range of pathways, causing a significant, approximate 4- to 10-fold decrease, in NECA potency in recombinant cells, NVCMs and CNs. ZINC20220354 had significant A_1AR selectivity relative to the A_3AR and A_{2B}AR, whereas ZINC8923830 was A_1AR selective (approximately 30 -100 fold compared to the other AR subtypes). Collectively, this study describes first-in-class A_1AR NAMs with relatively high affinity and subtype selectivity, providing a novel approach to improve renal function in heart failure patients in the absence of the on-target adverse effects commonly observed with A_1AR antagonists.

304 Structural mechanism of partial agonists and antagonists of PPARgamma for use as antidiabetics

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Introduction. Synthetic full agonists of PPARγ have been prescribed for the treatment of diabetes due to their ability to regulate glucose homeostasis and insulin sensitization. While the use of full agonists of PPARγ has been hampered due to severe side effects, partial agonists and antagonists have shown promise due to their decreased incidence of such side effects in preclinical models. No kinetic information has been forthcoming in regard to the mechanism of full versus partial agonism of PPARγ to date and little structural and dynamic information is available which can shed light on the mechanistic difference between full and partial agonists as well as antagonists.
Aims. Determine the structural mechanism of partial agonists and antagonists of PPARγ.
Methods. We have used X-ray crystallography, cellular assays, Hydrogen Deuterium Exchange (HDX), and Surface Plasmon Resonance (SPR) to probe the mechanism of several PPARγ partial agonists and antagonists.
Results and Discussion. Our findings demonstrate that not only do partial agonists and antagonists act through distinct transcriptional mechanisms, they also demonstrate differences in structure, dynamics, and kinetics as compared to full agonists.

"PPARgamma in Complex with an Antagonist and Inverse Agonist: A Tumble and Trap Mechanism of the Activation Helix." Frkic R et al (2018), iScience (in press).

305 Innovation in the treatment of depression: new insights into neuroimmune mechanisms of depression

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Major Depressive Disorder (MDD) is a disabling disorder, with current antidepressant treatment achieving remission in only around 30% of individuals (Trivedi et al, 2006). Recent meta-analyses have found an association between adult MDD and elevated circulating levels of pro-inflammatory markers (Dowlati et al, 2010; Kohler et al, 2017). Furthermore, there is evidence that reduction of depressive symptoms is associated with lowering of inflammatory marker levels in a subgroup of individuals with raised levels of inflammation at baseline (Raison et al, 2013). A number of novel agents, targeting inflammation / immune dysregulation, are now under investigation as potential treatments for MDD. This presentation will discuss some current studies using these novel agents to treat MDD, including some studies at the University of Adelaide. A number of these novel agents have shown promise as an adjunct to antidepressant treatment. Further research is continuing into the potential use of these agents, in particular as augmentation to current antidepressants.

Dowlati Y et al (2010) Biol Psychiatry 67(5):446-57
Kohler CA et al (2017) Acta Psychiatr Scand 135(5):373-87
Raison CL et al (2013) JAMA Psychiatry 70(1):31-41
Trivedi MH et al (2006) Am J Psychiatry 163(1):28-40

306 Pharmacogenomics of antidepressants – current recommendations and pitfalls in clinical application

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In treatment of depressive disorders, the use of antidepressant medication plays a major role in improving symptom remission and patient wellbeing. However, many patients with major depressive disorder (MDD) do not benefit from their prescribed medication or experience adverse side effects affecting compliance and remission rates. Enzymes involved in the pharmacokinetics of antidepressants are well studied and the analysis of *CYP2D6* and *CYP2C19* genetic variants currently forms the backbone of pharmacogenetic investigations offered in different medical laboratories. Moreover, genetic variants in genes encoding for medication targets have shown increasing evidence of their role in antidepressant treatment response and potential relevance for pharmacogenetic testing. As more and more laboratories are offering pharmacogenetic testing, it has become increasingly important to take a close look at currently used decision-making tools and interpretation of results. The key question will be the degree to which these decision-making tools agree on detected genotypes, subsequently predicted phenotypes, and resulting medication recommendations as standard guidelines are currently missing.
Thus, this presentation will summarise the recent knowledge and recommendations in the field of pharmacogenetic testing regarding the treatment of MDD and highlight benefits and current pitfalls in clinical practice.

307 Ketamine for treatment resistant depression

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Clinical pharmacologists played a major role in the FDA's approval of the NMDA antagonist ketamine in 1970 through a pivotal clinical trial conducted by Edward Domino and colleagues in 1965. Although it has TGA approval for anaesthesia and accepted use for procedural sedation and analgesia, it was not until a decade ago that it was being trialled for treatment-resistant depression but at a low fixed dose impractical 40 min IV infusion. This talk will provide background knowledge of ketamine's chemistry, pharmacokinetics and metabolism and supposed multi-target interactions. In collaboration with several academic psychiatrists, we conducted a dose titration, route (IV, IM, SC) and active placebo study to identify the optimal dosing regimen. The dose is substantially lower than for anaesthesia (0.5-0.75 mg/kg versus > 2 mg/kg), response is rapid within 4 hours and the best mode of administration was subcutaneous (Loo et al 2016). How ketamine works at such low doses involves not only NMDA but also AMPA and mTOR mechanisms and the contribution of stereoselective active metabolites remains to be firmly established. Although short-term studies are promising, a significant number of patients fail to respond, which may have a pharmacogenomic contribution, or are affected by adverse psychotomimetic effects requiring ketamine cessation. Several long-term effects remain to be firmly documented (Short et al 2018). The dose required to maintain remission after an initial response and for how long is important to determine, given the considerable off-label use by inexperienced practitioners.

Domino EJ et al (1965). Clin Pharmacol Ther 6: 279-291
Loo CK et al (2016). Acta Psychiatr Scand 134: 58-56
Short B et al (2018). Lancet Psychiatr 5: 65-78

308 Role of pharmacometrics and precision medicine in the treatment of depression

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Therapeutic Drug Monitoring (TDM) has undoubtedly improved patient care across a variety of therapeutic areas ranging from the treatment of bacterial infections through to anticonvulsant therapy. Indeed, the field of neuropsychopharmacology has a long-standing place in TDM, beginning with the use of lithium and later with tricyclic antidepressants where TDM is routine. Recently, updated consensus guidelines have been published on TDM in neuropsychopharmacology in general (Hiemke et al. 2018) and antidepressants specifically (Baumann et al. 2018). These guidelines have focussed more on the evidence for the TDM of specific pharmacotherapies, and centred upon the broad concept of altering a dosage to achieve a concentration somewhere within a therapeutic range, which is often achieved using of nomograms and the like. The concept of Target Concentration Intervention (TCI) offers more specificity in dose adjustment and the potential for highly individualised therapy through Bayesian forecasting (Holford et al. 1999). However, TCI requires the development of a model of the pharmacokinetics / pharmacodynamics of the drug, and a tool for its implementation. The data on ketamine from Loo et al. (2016) involving dose titration (0.1-0.5 mg/kg) across multiple routes of administration (IV, IM, SC) were modelled to develop a population pharmacokinetic-pharmacodynamic model for ketamine in treatment-refractory depression. This talk will highlight the role that pharmacokinetic-pharmacodynamic modelling and simulation (pharmacometrics) may play in better characterising the concentration-response relationship for ketamine in the treatment of depression and how this can be used to inform dosing algorithms and applied to TCI.

Baumann P et al. (2005) Dialogues Clin Neurosci 7:231-247
Hiemke C et al. (2018) Pharmacopsychiatry 51: 9–62
Holford N (1999) Br J Clin Pharmacol 48: 9–13
Loo CK et al. (2016) Acta Psychiatr Scand 134: 58-56

309 UGT2B11 and UGT2B28 Have Catalytic and Non-catalytic Functions and are Dominant Negative UGT Regulators

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Introduction. UDP-glucuronosyltransferases (UGTs) conjugate and inactivate myriad endo- and xenobiotics. UGT2B11 and UGT2B28 both have extremely low activities with traditional UGT substrates including steroids. Nonetheless, UGT2B11 and 2B28 expression are strongly regulated by steroids in breast and prostate cancer, and UGT2B28 is linked to prostate cancer progression, suggesting they are physiologically relevant for steroid metabolism. We are interested in how the functions of UGTs, including UGT2B11 and 2B28 may be diversified by alternative splicing and dimerization.
Aims. To define the activities, interactions, and cellular functions of full length and alternatively spliced truncated forms of UGT2B11 and UGT2B28, and hence better understand their functional significance in cancer cells.
Methods. UGT2B11 and UGT2B28 mRNA variants were cloned and expressed. UGT interactions were tested by co-immunoprecipitation. Glucuronidation was assayed by HPLC. Cell proliferation was assessed using crystal violet assays.
Results. UGT2B11 and 2B28 splice variants were cloned and shown to encode C-terminally truncated proteins that lack part of the sugar-binding domain. Truncated UGT2B11 and 2B28 proteins dimerized with their full length cognate forms, as well as with other UGT2B enzymes. Co-expression studies showed that both truncated and full-length UGT2B11 and 2B28 proteins robustly inhibit the activities of other UGTs including UGT2B7, 2B15, and 2B17. Expression of full length but not truncated UGT2B11 and 2B28 inhibited steroid-independent growth of HEK293T cells.
Discussion. We propose that UGT2B11 and UGT2B28 proteins have both catalytic and non-catalytic functions. Their catalytic activities remain poorly defined but our observation that full-length, but not truncated, UGT2B11 and 2B28 strongly inhibit cell growth suggests that they have novel substrates that are important in cellular metabolism. Their non-catalytic activities are mediated via hetero-dimerization with other UGTs and subsequent inhibition of glucuronidation. The ability of UGT2B11 and 2B28 to inhibit androgen-metabolizing UGT2B15 and 2B17 may explain their hitherto opaque functions in prostate cancer progression. Finally, the ratio of full length and truncated UGT2B11 and 2B28 variants expressed in cells may determine the ratio of their catalytic and non-catalytic (dominant negative) functions and hence cellular responses. Consistent with this, we have found that UGT2B28 splice variants are elevated in prostate cancer cells where they might provide a growth advantage by inhibiting androgen-metabolizing UGTs.

310 Enabling drug development decisions through Model Informed Drug Development

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The Food and Drug Administration has proposed to streamline regulatory processes to increase the efficiency of drug approvals. Central to these processes is the use of model informed drug development (MIDD) to inform key development and commercial decisions and the use of natural history databases to support drug development, in particular using model-based meta analysis (MBMA). The premise is to leverage established knowledge about the drug (or the drug class) and the disease, as well as previous experience with the drug (or drugs of the same class or mechanism of action) to quantitatively guide the development of the drug.

The purpose of this presentation is to illustrate the role of MIDD to support drug development decisions. The theme is developed using published examples. The application of MBMA to characterize the relationship between dose and efficacy among biologic disease modifying antirheumatic drugs revealed a consistent relationship across drug classes and supported comparison of drugs and regimens that had never been studied together in the same trial. Finally, the role of modeling and simulation to establish the relationship between exposure to a chemotherapeutic agent and its safety/efficacy, support dosing regimens and inform the drug label will be profiled.

311 Functional diversity of UDP-glucuronosyltransferase enzymes and novel modulation of nuclear receptor signaling

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Introduction. Nuclear receptors (NR) such as estrogen receptor (ER) and androgen receptor (AR) are steroid ligand-dependent transcription factors that regulate gene expression programs critical for prostate and breast cancer growth. The UDP-glucuronosyltransferase (UGT) enzymes conjugate a diverse range of xenobiotic and endobiotic molecules with sugars, rendering them inactive and promoting their elimination. Seven members of the UGT2B subfamily are expressed in steroid target tissues and conjugate diverse steroids; altered expression/function of several members (particularly UGT2B15 and 2B17 which conjugate androgens such as DHT) has been correlated with prostate and breast cancer risk, progression and/or treatment response. Whilst this correlation is presumed to involve modulation of steroid levels, there is also evidence that UGT2Bs may be involved in steroid-independent NR function.
Aims. 1) To define how androgen-metabolizing UGT2B enzymes alter AR function. 2) To identify alternatively spliced UGT2B variants and characterize their activities and ability to modulate AR function.
Methods. AR function was assessed by luciferase reporter assays. Interactions between UGTs and AR were assessed by co-immunoprecipitation. Alternative splicing of UGT2B genes was assessed by PCR; novel variant cDNAs were cloned and expressed alone or in combination with wildtype UGTs. Glucuronidation activities were assessed by HPLC.
Results. Co-expression of AR with UGT2B15 or 2B17 inhibited AR-transcriptional activity in the presence of DHT or R1881 (a non-metabolizable ligand). Both UGTs physically interacted with AR and a constitutively active truncated AR. We identified novel intergenic alternative splicing of UGT2B15 and 2B17 in breast, prostate, and/or liver cells generating chimeric UGT transcripts. These encoded C-terminally truncated UGTs that were inactive and inhibited wildtype UGTs via heterotypic interactions. The inactive UGT variants also inhibited AR activity via direct interaction.
Discussion. We have defined two mechanisms by which UGT2B15 and 2B17 can alter AR function: 1) by altering androgen levels (pre-receptor modulation); 2) by interacting with AR (direct receptor modulation). This work defines a novel non-metabolic function for UGTs in cancer. Production of UGT2B splice variants might be specifically regulated during cancer progression and the potent dominant-negative functions of UGT variants could have profound effects on both steroid-dependent and -independent AR function, as well as on UGT-mediated drug clearance pathways.

312 Population pharmacokinetics of colistin in paediatric patients: implications for selection of dosage regimens

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Introduction. Intravenous colistin is widely used to treat infections in paediatric patients. Unfortunately, there is a paucity of pharmacological information to guide selection of dosage regimens. The daily dose recommended by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) is the same body weight-based dose traditionally used in adults.
Aims. The aim was to increase understanding of the patient factors that influence the plasma concentration of colistin, and assess the likely appropriateness of the FDA and EMA dosage recommendations.
Methods. Five patients with median age 1.75 (range 0.1-6.25) years, weight 10.7 (2.9-21.5) kg and creatinine clearance 179 (44-384) mL/min/1.73m^2 received 8-hourly intravenous infusions of colistimethate. Median daily dose was 0.21 (0.20-0.21) million IU/kg, equivalent to 6.8 (6.5-6.9) mg colistin base activity per kg/day. Plasma concentrations of colistimethate and formed colistin were subjected to population pharmacokinetic modelling to explore patient factors influencing the concentrations of colistin.
Results. Median average steady-state plasma concentration of colistin ($C_{ss,avg}$) was 0.88 mg/L, with the individual values ranging widely (0.41-3.50 mg/L), although all patients received the same body weight-based daily dose. Despite that the daily dose was ~33% above the upper limit of the FDA and EMA recommended dose range, only 2 patients achieved $C_{ss,avg}$ ≥2 mg/L; the remaining 3 patients had $C_{ss,avg}$ <1 mg/L. The pharmacokinetic covariate analysis revealed that clearances of colistimethate and colistin were related with creatinine clearance.
Discussion. The FDA and EMA dosage recommendations may be suboptimal for many paediatric patients. Renal function is an important determinant of dosing in these patients.

313 Extracellular nanovesicle derived cytochrome P450 3A4 protein expression and activity

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Introduction. Cytochrome P450 (CYP) 3A4 is the drug metabolising enzyme of greatest clinical importance as it plays a major role in the metabolism and elimination of more than 30% of all clinically used small molecule drugs. Variability in CYP3A4 activity is primarily driven by differences in expression that are poorly described by pharmacogenetics. In recent years extracellular nanovesicles (exosomes) have revolutionised the diagnosis of multiple diseases. Exosomes contain cargo including proteins derived from their origin, and hence reflect the functional state of that organ.
Aims. This study sought to evaluate the expression and *ex vivo* activity of CYP3A4 protein isolated from circulating exosomes. Correlations with *in vivo* midazolam clearance were also evaluated.
Methods. Plasma derived exosomes were characterised by transmission electron microscopy, nanoparticle tracking analysis and identification of verified protein markers. CYP3A4 protein expression in plasma derived exosomes was quantified by ELISA normalized to the optical density of GAPDH. The *ex vivo* activity of exosome derived CYP3A4 protein was examined using verified assay conditions for the assessment of 1-hydroxy midazolam formation kinetics.
Results. Kinetic data for the NADPH-dependent oxidation of midazolam to 1-hydroxy midazolam by exosomes and HLMs were well described by the Michaelis-Menten equation. Mean kinetic parameters (K_m, V_{max} and CL_{int}) describing 1-hydroxy midazolam formation by exosomes were 6.5 µM, 31.1 pmol/min/mg, and 4.8 µL/min/mg. By comparison, kinetic parameters for the HLM catalysed reaction were 4.4 µM, 2895 pmol/min/mg, and 658 µL/min/mg. Mean (95% CI) exosome derived CYP3A4 protein expression in a cohort of 6 healthy males pre- and post- rifampicin dosing was 2.4 (2.0 - 2.8) and 4.2 (2.1 - 6.5) ng/mL. R^2 values for correlations of exosome derived CYP3A4 protein expression and *ex vivo* CYP3A4 activity with midazolam CL/F were 0.905 and 0.832, respectively.
Discussion. Strong concordance was observed for exosome-derived CYP3A4 protein expression and *ex vivo* activity with midazolam CL/F. The significance being that CYP3A4 is the drug-metabolizing enzyme of greatest clinical importance and variability in CYP3A4 activity is poorly described by existing precision dosing strategies.

314 Deregulation of ADME genes that are involved in drug absorption, distribution, metabolism, and excretion in hepatocellular carcinoma

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Introduction. ADME genes are a group of genes that are involved in Drug Absorption, Distribution, Metabolism, and Excretion. ADME genes are abundantly expressed in liver, and the combined effects of ADME genes determine the hepatic drug metabolism capacity. The expression of ADME genes is known to be affected by various types of liver diseases; however their global dysregulation has not been assessed in liver cancer.
Aims. To define the ADME genes which are deregulated in Hepatocellular Carcinoma.
Methods. RNA-seq and miRNA-seq data from The Cancer Genome Atlas (TCGA) hepatocellular carcinoma (HCC) sample set (421 samples including 50 paired HCC and adjacent non-cancerous liver) were downloaded from the Genomic Data Commons (GDC) Data Portal (https://portal.gdc.cancer.gov/) and subjected to differential gene expression analysis using DESeq2 program and Spearman's ranking correlation analysis using GraphPad Prism.
Results. Out of 298 defined ADME genes, 233 (80%) were expressed in HCC. Of these genes, 58 (24%) were downregulated and only 15 (6%) were upregulated. Most dysregulated genes belonged to Phase I (CYPs, ADHs, ALDHs) and Phase II (GSTs, SULT, UGTs) drug metabolizing enzymes, and drug transporters (ABC and SLC transporters). Of the 28 genes defined as the core ADME set, 14 (50%) were significantly downregulated. Several transcription factors (TFs) that were shown to be positively (PXR, PPARA, CAR) or negatively (PPARD and PPARG) correlated to ADME genes were also dysregulated in HCC. Finally, most miRNAs known to regulate ADME genes were upregulated in HCC.
Discussion. This study reports for the first time the complete list of ADME genes that are dysregulated in HCC and suggests that this is in part due to altered levels of specific TFs and miRNAs. The abundant expression of genes associated with drug clearance in HCC may contribute to the failure of many therapeutic regimens in clinical trials. In contrast, the relative downregulation of CYP3A4 and UGT1A9 that are responsible for metabolism of sorafenib and regorafenib may explain their efficacy for treating HCC. The correlations of ADME genes with each other and with TFs provide compelling evidence for transcriptional coregulation of ADME genes in response to stimuli such as drugs.

315 PK Graph: a new freely available pharmacokinetic simulation application for medical education

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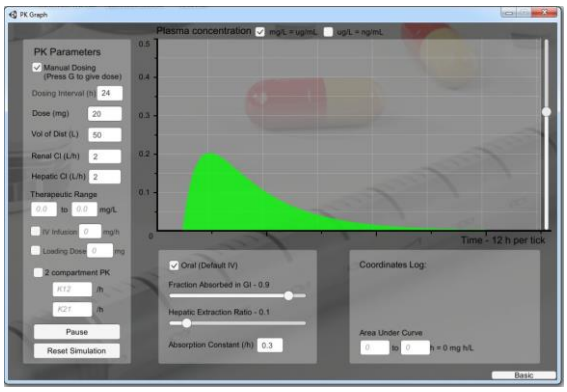

Introduction. Many pharmacology departments use pharmacokinetic simulations to teach students about drug disposition. Unfortunately the cost of using licensed software is frequently prohibitive and departments are also keen to reduce costs or divert funds to research activities.
Aims. We set out to create a new pharmacokinetic simulation program to address a current instructional need with both flexibility and low cost.
Methods. The freely available (for educators) game development platform Unity (https://unity3d.com/) and textbook PK formulas were used to developed an application able to model both introductory situations and advanced clinically relevant circumstances.
Results. The application is capable of simulating both oral and IV dosing situations including repeat dosing. It has a basic and advanced mode (pictured). In the advanced mode the student can set the microconstants for a drug with 2-compartment pharmacokinetics and thus learn about more complex models of drug disposition.
Discussion. This software will be trialled at Sydney Medical School in 2019 with a series of exercises for students to work through. Drugs with special pharmacokinetic properties such as gentamicin and digoxin will be featured. The simulator will be able to exemplify the effects of renal insufficiency, repeat dosing and the selection of the most parsimonious model (as in the case of digoxin where a 2-compartment model provides the most accurate results). It is hoped that other institutions will take up our offer of free access to the software and code therein and let us know about the results. The application is freely available as is at the following link: http://bit.ly/PK-graph

316 Deprescribing in the pharmacy curriculum: feasibility and acceptability of a novel deprescribing workshop

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Introduction. Given that health professionals, including pharmacists, are increasingly expected to engage and conduct deprescribing as part of their clinical practice, it is important to introduce deprescribing activities into curriculum.
Aims. To assess the impact of a novel deprescribing workshop on improving the knowledge, skills and attitudes towards deprescribing among undergraduate pharmacy students.
Methods. Participants were final year students enrolled in the Bachelor of Pharmacy Programme at The University of Sydney, Australia. The workshop involved case scenarios and discussion points about deprescribing concepts to provide students with knowledge and skills, and a matched assessment, to help students apply their skills. The modified deprescribing survey was adapted from a validated tool, assessing patient safety attitudes among pharmacy students. The deprescribing survey included 8-items that assess the role of pharmacists in the deprescribing process and knowledge using the five-point Likert-type scale. The items were reviewed and piloted by all investigators for content validity. The deprescribing survey was administered before and after the workshop and assessment to compare changes in student learning and attitudes.
Results. Of 206 students enrolled, 192 (93.2%) completed the survey, with a mean age 22.5 years (SD:3.1) and 64.1% were female students. At baseline, 91.0% of students perceived that teaching deprescribing is an appropriate use of time in pharmacy programmes at university. Eighty-nine percent of pharmacy students stated that university curriculum should include deprescribing concepts. Following workshop and assessment task (comparative sample, n=160 (77.8%) matched student responses), students reported greater confidence in their ability to identify patients who may benefit from deprescribing medications (45.0% vs 58.0%; Z=6.65; p<0.0001). Moreover, peer-led education, such as from pharmacist colleagues or fellow students was perceived as an effective platform for developing better understanding of deprescribing concepts (77.9% vs 89.9%; Z=3.19; p=0.001).
Discussion. Our results suggest that incorporating a novel deprescribing workshop into pharmacy curricula is an effective intervention for developing positive attitudes and necessary skills towards deprescribing concepts among students.

317 Structural insights into the activation of the adenosine A_1 receptor

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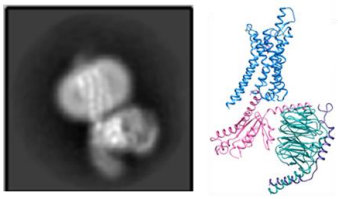

Introduction. In recent years there has been an exponential growth in the amount of determined inactive GPCR structures, however there still remains a dearth of active-state, G-protein-bound, GPCRs. To date, all solved active-GPCRs have been coupled to the stimulatory Gs protein. The class A adenosine A1 receptor (A_1R) is a GPCR that preferentially couples to the inhibitory Gi/o family of heterotrimeric G-proteins, has been implicated in numerous diseases, yet remains poorly targeted.
Aims. We sought to solve an active-state structure of the A_1R bound to the Gαi2 inhibitory G protein.
Methods. Generation of a dominant negative Gαi2 enabled formation of a stabilized ternary complex of the adenosine bound A_1R with Gi2, which was purified to homogeneity and subjected to Volta phase plate cryo-electron microscopy. that was purified to
Results. We were successful in solving a 3.6 Å structure of the human A_1R-Gi2 ternary complex. Compared to inactive A_1R, there is contraction at the extracellular surface in the wide orthosteric binding site that is mediated via movement of transmembrane domains 1 and 2. At the intracellular surface, the G-protein engages the A_1R primarily via amino acids in the C-terminus of the Gαi α5 helix, concomitant with a 10.5 Å outward movement of A_1R transmembrane domain 6. Comparison with the agonist-bound β_2 adrenergic receptor·Gs-protein complex reveals distinct orientations for each G-protein subtype upon engagement with its receptor
Discussion. This active A_1R structure provides novel molecular insights into receptor/G protein selectivity.

318 Exploring subtype differences in α1-adrenoceptor ligand residence time

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Introduction. Traditional drug discovery approaches rely on marked differences in equilibrium-based affinity values however, the recognition of the importance of incorporating kinetic considerations, such as residence time, is growing. There is limited information on ligand binding kinetics for α_1-adrenoceptors (ARs), however we have previously observed marked differences in [^{3}H]-prazosin dissociation across the subtypes, with α_{1B}AR eight-fold slower than α_{1A}AR. This may mediate the observed differences in intracellular responses induced by adrenergic compounds. The structural mediators of these effects remain unknown, with very few binding site residues differing between the fairly conserved α_1AR subtypes.
Aims. Investigate the contribution of non-conserved residues to the ligand binding kinetics of α_1-ARs.
Methods. In silico methods were used to identify non-conserved residues within the possible binding trajectory but beyond the orthosteric site of the α_{1A}AR. Site-directed mutagenesis generated α_{1A}AR mutants, which exchanged α_{1A} residues for those of the α_{1B}, were characterised using competition, saturation, competitive association and dissociation [^{3}H]-prazosin radioligand binding assays in addition to functional Ca^{2+} signalling studies.
Results. α_{1A} residues F86$^{2.64}$, A189$^{5.43}$, M292$^{6.55}$ and T174^{ECL2} were investigated. T174K mutagenesis resulted in no observable binding or signalling. Competition, saturation and Ca^{2+} signalling showed no change in [^{3}H]-prazosin and noradrenaline (NA) affinity or potency relative to wild type for all other mutants (P>0.05). In contrast, [^{3}H]-prazosin dissociation was shown to be substantially slowed by the M292L mutation but increased by F86M relative to α_{1A} wild type (K_{off} (min^{-1}): α_{1A} 0.05±0.004, α_{1A} F86M 0.11±0.005, α_{1A} M292L 0.004±0.003). Further characterization of ligand binding kinetics using competitive association is ongoing with preliminary results indicating that the A189S mutation, in addition to M292L, slows NA binding kinetics.
Discussion. Residues contributing to the kinetic differences between α_{1A} and α_{1B} have been identified within TM5 and TM6 above the orthosteric pocket suggesting that subtype differences in ligand binding kinetics may be due to a complex interplay of several opposing contributions of non-conserved residues. Furthering the understanding of the structural basis of these effects has the potential to improve drug development and selectivity.

319 Probing the structural basis of $mGlu_5$ allosteric agonism and biased signalling

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Introduction. Metabotropic glutamate receptor subtype 5 ($mGlu_5$) has gained immense interest as a potential therapeutic target for a wide range of CNS disorders, with a recent focus on targeting non-conserved allosteric binding sites. As such, multiple $mGlu_5$ allosteric modulators have been identified that bind to a common allosteric binding site within the transmembrane domains. Recently we showed that chemically distinct $mGlu_5$ positive allosteric modulators (PAMs) are biased agonists relative to DHPG (Sengmany et al, 2017). Despite numerous $mGlu_5$ structural and mutagenesis studies, there remains a lack of understanding of the specific residues involved in $mGlu_5$ biased agonism.
Aims. To probe the structural determinants of $mGlu_5$ biased allosteric agonism.
Methods. A robust evaluation of biased agonism of four $mGlu_5$ PAMs from diverse chemical scaffolds (DPFE, VU0409551, VU29, VU0424465) at the three receptor endpoints (iCa^{2+} mobilisation, IP_1 accumulation and ERK1/2 phosphorylation) was conducted using $mGlu_5$ receptors containing single-point mutations within the common allosteric binding pocket. Transduction coefficients were calculated to allow comparison of allosteric agonism across multiple pathways, with statistical comparisons made relative to wild type (ANOVA, Dunnett's post-hoc analysis).
Results. $mGlu_5$ allosteric binding pocket mutations had differential effects on the ability of different $mGlu_5$ PAMs to activate multiple signalling pathways. A loss of Ca^{2+} agonism was evident for DPFE and VU0409551 for most mutants, whereas IP_1 and ERK pathways were still activated, albeit with a reduced maximal response. Bias analysis was complicated by this loss of agonism but revealed that, relative to DHPG, bias profiles between Ca^{2+} mobilisation and IP_1/ERK pathways remained similar to wild-type. F787A reversed the bias between IP_1 and ERK pathways for most ligands, and a loss of bias between IP_1 and ERK was also evident for A809V and W784A mutants.
Discussion. These data provide further insight into the structural requirements for allosteric agonism across multiple $mGlu_5$-mediated signalling pathways. An understanding of biased agonism at the structural level will provide the foundation for rational structure-based design of biased allosteric ligands for the treatment of neurological disorders.

Sengmany et al (2017) Neuropharm 115:60-72

320 The Dynamics of Ligand-GLP-1R-G protein coupling and its contribution to biased agonism

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Introduction. Biased agonism is now a well-accepted concept in G protein-coupled receptor (GPCR) research and is recognized as an avenue for developing novel therapeutic strategies. Although many studies have focused on investigating biased behavior of GPCRs, little is known regarding the mechanistic basis of how differences in ligand-receptor interaction lead to distinct effector engagement and how this in turn contributes to distinct cellular signalling profiles. Here, we have ultilised the cognate ligand GLP-1 and a series of biased agonists to examine the kinetics of ligand-receptor interaction, receptor-G protein engagement, receptor-mediated G protein activation as well as measures of downstream signaling.
Aims. Investigating the contribution of ligand binding kinetics and dynamics of receptor/G protein coupling in GLP-1R biased agonism.
Methods. Ligand binding kinetics was measured by NanoBRET technology using plasma membrane purified from HEK293A cells overexpressing Nluc-hGLP-1R. Ligand induced G protein activation was measured using NanoBIT luciferase complementation assay. Second messengers were measured as mentioned previously (Koole et al 2015).
Results. Our results suggest that the kinetics of ligand-receptor engagement directly regulates the efficiency of receptor-G protein coupling. In addition, differential G protein-coupling properties (including differential subtype coupling and/or differential conformational transitions within the same G protein) induced by GLP-1R agonists also contribute to their distinct biased profiles.
Conclusion. This study furthers our understanding of the molecular events that couple ligand binding to intracellular signalling.

Koole, C et al (2015) J Pharmacol Exp Ther 353(1):52-63

321 The receptor-effector interactions underpinning adenosine A_{2B} receptor signal transduction

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Introduction. The adenosine A_{2B} receptor (A_{2B}AR) is a potential novel therapeutic target, particularly for cardiac fibrosis[1]. The A_{2B}AR preferentially couples to Gs proteins, however, it has also been suggested to couple to other G proteins and β-arrestins. Currently, there is a knowledge gap regarding a detailed understanding of the receptor-effector interactions, particularly G proteins, that stimulate therapeutically relevant A_{2B}AR signalling.
Aims. To establish the receptor and effector interactions underpinning A_{2B}AR signalling.
Methods. CRISPR/Cas9-gemone edited HEK293A cells with G protein or β-arrestin deletions were transiently transfected with the human A_{2B}AR. ERK1/2 phosphorylation (pERK1/2), cAMP accumulation and calcium mobilisation (Ca^{2+}_i) were performed as previously described[2] to evaluate A_{2B}AR interactions with downstream signalling effectors upon stimulation with the agonists, including NECA, BAY60-6583, VCP746, capadenoson.
Results. A_{2B}AR-mediated cAMP accumulation was abolished in CRISRP/Cas9 HEK239A cells lacking Gs proteins in time-course assays and concentration-response curves (p<0.05, One-way ANOVA Dunnett's *post hoc* test). The deletion of Gi/o and Gs proteins altered the time-course of pERK1/2. A_{2B}AR-mediated Ca^{2+}_i was significantly reduced in cells lacking Gs, Gi/o or Gq/11 (p<0.05, One-way ANOVA Dunnett's *post hoc* test).
Discussion. Understanding the interactions that underpin A_{2B}AR signal transduction is important for the development of A_{2B}AR therapeutics for use in different disease contexts. Collectively, our results suggest A_{2B}AR-mediated cAMP accumulation is exclusively mediated by Gs proteins, whereas Ca^{2+}_i and pERK1/2 appear to involve pleotropic coupling to additional G proteins. Future studies will further interrogate the role of Gq/11 and Gi/o proteins in A_{2B}AR-mediated signalling, particularly Ca^{2+}_i and pERK1/2.
1. Vecchio E A et al (2017) Front Pharmacol 8:243
2. Vecchio E A et al (2016) Biochem Pharmacol 117:46-56
3. Baltos J-A et al (2017) Biochem Pharmcol 135:79-89

322 Dysregulation of the actin cytoskeleton by ω-3 tolyl-urea fatty acid - a novel inhibitor of breast cancer cell migration

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Introduction. Metastasis, in which primary tumour cells migrate to distant tissues and form secondary tumours, is the major life-threatening characteristic of malignant tumours. Dietary ω-3 polyunsaturated fatty acids (PUFAs) are associated with decreased metastasis; this may be due in part to lipid metabolites. In this study, we characterized the mode of action of a novel ω-3 PUFA metabolite analogue termed ω-3 tolyl-urea (OTU) that inhibited migration and invasion by highly metastatic MDA-MB-231 breast cancer cells.
Methods. Mass-spectrometry-based proteomics and Ingenuity Pathway Analysis were used for protein expression profiling and bioinformatics analysis in OTU-treated cells. Cytoskeletal fractionation, phalloidin staining and confocal microscopy, G-Lisa and immunoblotting were used to characterize the actin cytoskeleton.
Results. OTU (10 µM) inhibited invasion (52±6% of control) and migration (52±1% of control) by MDA-MB-231 cells. From proteomics analysis the expression of 423 proteins was altered by a factor >2 by OTU; the actin cytoskeleton was a major down-regulated process. OTU (10 µM) decreased the content of filamentous actin (F-actin) to 66±5% of control (P<0.05). This was corroborated by phalloidin staining and confocal imaging, which showed a decrease in actin-rich filopodia in OTU-treated cells. Wnt/planar cell polarity (PCP) signalling, the master regulator of the actin cytoskeleton, was inhibited by OTU treatment. Significant inhibition (P<0.05) of RhoA activity, and the expression of phospho-Dvl2, phospho-Limk and phospho-Cofilin, the major components of the Wnt/PCP pathway, to 62±4%, 59±9%, 64±8% and 69±5% of respective control, was noted. In nude mice carrying MDA-MB-231 cell xenografts, OTU as its ethyl ester prodrug, at a dose of 10 mg/kg i.p. once daily, 6 days/week for 38 days, decreased the number of abdominal micrometastases (0.6±0.6 per mouse relative to 5.5±2.8 in control; n=10 per group, P<0.01).
Conclusion. OTU is the first member of a new class of anti-metastatic agents based on a ω-3 polyunsaturated fatty acid metabolite that impairs actin polymerization in MDA-MB-231 breast cancer cells by dysregulating pro-migratory Wnt/PCP signalling.

323 Dimethylarginine dimethylaminohydrolase-1 (DDAH1) inhibition as a novel therapeutic strategy in cancer types associated with excessive nitric oxide synthesis

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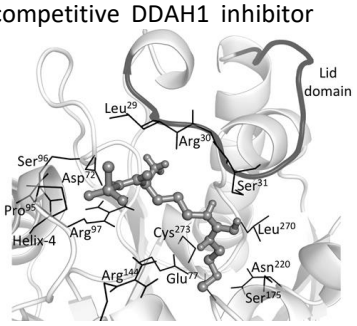
Introduction. Excessive synthesis of nitric oxide (NO) favours the onset and progression of several cancer types. The enzyme dimethylarginine dimethylaminohydrolase-1 (DDAH1) modulates NO by metabolizing the endogenous inhibitors of the NO synthase (NOS) enzymes, asymmetrically methylated arginines. ZST316 is the most potent competitive DDAH1 inhibitor developed to date (figure) and represents a novel therapeutic approach in the treatment of aggressive cancers, such as triple negative breast cancer (TNBC) and prostate cancer (PCa).



Aims. To validate DDAH1 as an appropriate molecular target for the treatment of cancer and to identify ZST316 as a novel anticancer agent.
Methods. Levels of DDAH1 mRNA and protein expression were measured in TNBC, PCa, and *VeraVec*™ endothelial cell lines by RT-PCR and Western blot. The ability of ZST316 to inhibit angiogenesis and vasculogenic mimicry (VM) was quantified using the Matrigel® tube formation assay.
Results. Expression of DDAH1 was elevated in both TNBC and PCa cell lines, as well as *VeraVec*™ cells. Incubation of cells with 100 µmol/L ZST316 resulted in a significant reduction in the number of tubes formed in the tube formation assay, both in a model of angiogenesis (*VeraVec*™ cells, 26% reduction) and of VM (MDA 231 cells, 33.5% reduction).
Discussion. DDAH1 inhibition by ZST316 represents a novel strategy to suppress angiogenesis and VM in TNBC and PCa.

324 Systemic inflammation is associated with altered drug utilization in metastatic colorectal cancer patients

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Introduction. Systemic inflammation is found in 25% of metastatic colorectal cancer (mCRC) patients and is associated with a 50% reduction in overall survival. However, the clinical implications on drug utilization of systemic inflammation remain unknown.
Aims. This study aimed to investigate the effect of systemic inflammation on quality use of medicine and outcomes using a linked dataset of mCRC patients.
Methods. Records from Queensland's CHARM oncology prescribing database were linked to external QLD health data collections (blood counts, death records) from four hospital sites between 2009 and 2014. Statistical analysis was used to investigate validated inflammatory biomarkers and the prediction of drug utilisation and survival.
Results. 25% of the 487 patients with mCRC presented with elevated systemic inflammatory markers. Patients with high inflammation recorded a reduced overall survival (OS) compared with those with low inflammation (9.9 vs 23.8 months, $p < 0.0001$). As a cohort, patients with high inflammation received significantly less cycles of therapy within the first line, reduced first line duration and less lines of therapy overall. Such patients were also less likely to receive targeted biologics in the first line setting compared to patients with low inflammatory markers (27% vs 39%, $p = 0.07$). Patients with high inflammation receiving mono- and doublet therapy received less cycles, reduced first-line duration and less lines of therapy overall, while patients receiving triplet therapy recorded no differences in first-line drug dosing and scheduling. However, all three treatment groups recorded a significantly reduced OS for mCRC patients with high inflammation compared to patients with low inflammation.
Discussion. The use of this novel linked-dataset has further substantiated the reduced survival outcomes in mCRC patients with high inflammation and highlighted their altered drug utilization. This data enhances our understanding of current treatments that can be optimised to improve survival for this high risk group.

325 Adherence and persistence to cholinesterase inhibitors: a longitudinal cohort study using dispensing records

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Introduction. Real-world estimates of adherence and persistence with acetylcholinesterase inhibitors (AChEIs) vary widely between countries. In Australia, response to the medication needs to be assessed at six months before the decision is made to continue the treatment.
Aims. To examine adherence and persistence with AChEIs in Australia.
Methods. This was a retrospective cohort analysis using medication dispensing information from 10% random sample of the Australian Pharmaceutical Benefits Scheme (PBS). The cohort was all people aged 65 or older who had their first dispensing of any AChEI (no previous dispensing within three years) between 01 Jan 2009 and 30 Jun 2017. Persistence was defined as the time from first AChEI dispensing until a gap of 60 or more days between estimated medication depletion and subsequent refill of an AChEI prescription, as each supply generally lasts 30 days. People who were dispensed AChEI only once before they discontinued were considered initially non-persistent. For those who were initially persistent, Kaplan-Meier curves were used to examine longer-term persistence. Adherence was assessed using the proportion of days covered (PDC) during the time the person was considered persistent with the AChEI.
Results. 9833 people initiated an AChEI within the study period. Of these, 1248 (12.7%) discontinued after being dispensed an AChEI once only. Of the 8585 people who refilled their AChEI supply at least once, 4988 (67.2%) discontinued over a mean follow-up period of 3.1 years. The median length of persistence of those supplied AChEI more than once was 718 days (IQR: 172-1932 days); 2281 (26.7%) discontinued within 6 months and 3306 (39.4%) discontinued within one year. The median PDC of those initially persistent was 0.96 (Interquartile range: 0.89-1.00), 7564 people (88.1%) had a PDC of 0.80 or higher.
Discussion. One in three people living with Alzheimer's disease who initiated an AChEI received only one prescription or persisted for 6 months or less. Of those who were dispensed a second prescription, 60% were persistent beyond one year. Adherence was generally high.

326 Deprescribing in older inpatients: development of consumer information leaflets

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Introduction. Communication of deprescribing decisions made in hospital with older inpatients and their carers at transitions of care may assist in reducing polypharmacy.
Aims. To develop information leaflets for older inpatients and/or their carers for deprescribing of antipsychotics, benzodiazepines and proton pump inhibitors (PPIs).
Methods. An iterative mixed-methods approach involving face-to-face user-testing and semi-structured interviews was performed over four rounds with consumers and hospital health professionals. Thirty-seven consumers aged 65 years or older, admitted to hospital in the previous five years, and taking at least one regular medicine (not the medicine being tested), or their carers, completed user-testing. Hospital health professionals included seven pharmacists and five doctors. The antipsychotic leaflet was tested in round 1 (consumers, n=10) and round 2 (consumers, n=9 and health professionals, n=5). The benzodiazepine leaflet (consumers, n=9 and health professionals, n=9) and PPI leaflet (consumers, n=9) were tested in round 3. Findings from round 3 informed the final design of all leaflets. Consumer user-testing involved 12-13 questions to evaluate their ability to locate and understand information in the leaflet. Usability by health professionals was assessed using the System Usability Scale (SUS). Interview transcripts were thematically analysed.
Results. At least 80% of consumers identified and understood the leaflet user-testing questions, including 9/12 in round 1, 10/12 in round 2, 12/13 (benzodiazepine) and 11/12 (PPI) in round 3. The SUS scores obtained from health professionals were 91.0±3.8 for the antipsychotic leaflet and 86.4±6.6 for the benzodiazepine leaflet, indicating excellent usability.
Discussion. The feasibility and effectiveness of the consumer information leaflets to support deprescribing at transitions of care should be explored in clinical practice.

327 Prevalence and incidence of prescription opioid analgesic use in Australia

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Introduction. Opioid use has increased rapidly in Australia and internationally over the past two decades and has been associated with parallel increases in opioid-related morbidity and mortality, including dependence, hospitalisations and overdose. Following recent declines in annual prescribing rates in the US, it is unclear whether prevalence and incidence of opioid use has changed in Australia in recent years.
Aims. To determine the prevalence and incidence of prescription opioid analgesic use in Australia and compare the characteristics of people with and without cancer initiating prescription opioid analgesics.
Methods. A retrospective population-based study was conducted using the random 10% sample of adults who were dispensed prescription opioid analgesics in Australia between July 2013 and June 2017 through the Pharmaceutical Benefits Scheme. Poisson regression was used to calculate rate ratios (RR) for opioid prevalence and incidence. Characteristics of people initiating opioids including type of opioid initiated, total oral morphine equivalents dispensed, prescriber speciality, medical comorbidities, past analgesic and benzodiazepine use were compared for people with and without cancer.
Results. Opioid prevalence increased (RR=1.006 [95%CI 1.006-1.007]), while incidence decreased (RR=0.977 [95%CI 0.976-0.978]) from 2013/2014 to 2016/2017. There were between 287,677 to 307,772 prevalent users each year. In total, 769,334 adults initiated opioids between 2013/2014-2016/2017 and half of these initiations were by general practitioners. Initiation with a strong opioid occurred in 55.8% of those with cancer and 28.2% of those without cancer.
Discussion. Rates of opioid use have remained high since 2013, with approximately 3 million adults using opioids and over 1.9 million adults initiating opioids each year in Australia. Between 2013-2017, opioid prevalence has slightly increased but incidence has decreased. People without cancer account for the majority of opioid use and are more likely to be initiated on short-acting and weak opioids. Initiation of strong opioids has increased over time, reinforcing concerns about increased use and harms associated with strong opioids in the community.

328 MedicineInsight: National General Practice Prescribing Data

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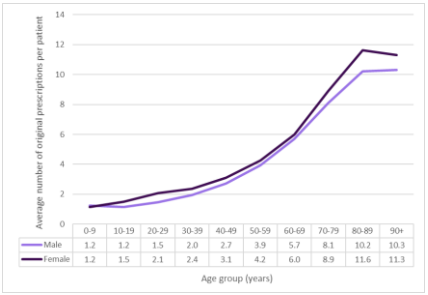
MedicineInsight is an Australia-wide real world general practice dataset extracting longitudinal, de-identified practice data from the clinical information systems of participating practices. With funding from the Australian Government Department of Health, a General Practice Insights Report providing national level data from a nationally weighted sample of general practices with quality data is available.
Aims. To present a cross-sectional analysis of national General Practice activities from 1 July 2016 to 30 June 2017, including medicines prescribed, patient risk factors and conditions and pathology test results.
Methods. Using a multi-step iterative selection process, a cohort of 475 general practices, 2.2 million patients and 10.4 million encounters with high quality data were identified representing 5.9% of practices across Australia. The data were weighted to a national level using national MBS billing data.
Results. There were similar age-specific patterns of prescribing for both males and females, with prescribing rates for original prescriptions higher for females across all age groups. The most common medicines prescribed (including original and repeat prescriptions) by ATC level 3 class were antidepressants (N06A), lipid-modifying agents (C10A), drugs for gastro-oesophageal reflux (A02B), similar to national PBS data for medicines dispensed. The most frequent chronic conditions ever recorded from coded and free-text fields were hypertension, depression, asthma and anxiety. This is similar to national health survey data.



Discussion. Initial data from MedicineInsight show similar patterns of prescribing and patient conditions to other sources of data. A second report is in preparation with ongoing refinement of the cohort selection and weighting strategy. MedicineInsight data are available for research with work ongoing to link the dataset to national PBS and MBS data.

329 Treatment initiation for Type 2 Diabetes in Australia: are the guidelines being followed?

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Introduction. Australian guidelines recommend metformin monotherapy for the initial treatment of Type 2 Diabetes (T2D) but in contrast to international guidelines, do not discuss initial treatment with combination therapy
Aim. To determine the patterns and predictors of treatment initiation for T2D, and whether treatment initiation is consistent with Australian clinical practice guidelines.
Methods. Individuals aged 18-99 years initiating treatments for T2D between July 2013 and November 2017 were identified from a 10% random national sample of pharmacy dispensing data. Individuals initiating insulin were excluded. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the predictors of initiating non-metformin monotherapy and combination therapy compared to metformin monotherapy. Predictors included age, sex, year of initiation and comorbidities.
Results. 62,976 individuals initiated T2D medications, (54.8% women, mean age was 53.5 years). Overall, 87.8% initiated metformin monotherapy, 5.4% initiated non-metformin monotherapy and 6.7% initiated combination therapy. Age ≥70 versus <30 years was associated with initiating non-metformin monotherapy (OR 7.58 [95%CI 6.05-9.49]) and combination therapy (OR 2.53 [95%CI 2.09-3.05]) compared to metformin monotherapy. Women were less likely to initiate non-metformin monotherapy (OR 0.83 [95%CI 0.77-0.89]) and combination therapy (OR 0.59 [95%CI 0.55-0.63]). Congestive heart failure (OR 1.38 [95%CI 1.20-1.59]) and cerebrovascular disease (OR 1.43 [95%CI 1.27-1.61]) were associated with having higher odds of initiating on combination therapy. Having a higher number of comorbidities was associated with higher odds of initiating non-metformin monotherapy but lower odds of initiating combination therapy.
Discussion. Treatment initiation for T2D in Australia is largely consistent with current clinical practice guidelines. Individuals who are prescribed combination therapy are more likely to be older, male and to have ≤3 comorbidities whereas people who are prescribed non-metformin monotherapy are more likely to be older, male and to have a greater number of comorbidities.

330 The 'Talking about deprescribing' resource to facilitate conversations about medicines discontinuation in residential aged care

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Introduction. Deprescribing refers to the stepwise reduction of unnecessary or potentially inappropriate medicines after consideration of therapeutic goals, benefits and risks. Up to 41% of residents of aged care services report an intrinsic desire to stop one or more of their medicines, and 79% are interested in doing so if their doctor says it is possible (Kalogianis et al, 2016). Clinicians report feeling uncertain in relation to how best to start conversations about deprescribing (Turner et al, 2016).
Aims. To develop a resource to assist general practitioners (GPs), nurses and pharmacists proactively discuss the topic of medicines discontinuation with residents of aged care services.
Methods. Sample phrases were developed in focus groups of 'deprescribing champions' comprising aged care nurses, pharmacists, geriatricians and GPs. Sample conversations were scripted, role-played by actors and video recorded. The video-recorded scenes were pilot tested for face validity in focus groups of aged care nurses and residents in regional and rural Victoria. These informed final production of a 10-minute video called 'Talking about deprescribing'.
Results. The 'Talking about deprescribing' concept was well received in the pilot testing phase. Constructive feedback was given, including suggestions for enhancing the scenarios and clarification of the educational needs of aged care nurses and their preferred format. 'Talking about deprescribing' will be screened during the presentation.
Discussion. This resource may assist GPs, nurses and pharmacists working in the aged care setting to initiate conversations about deprescribing with residents and their families, and other health care professionals.

Kalogianis M et al (2016) Res Soc Adm Pharm. 12(5):784-8.
Turner JP et al (2016) BMJ Open. 6(3):e009781.

331 Mechanism of human mast cell activation by polymyxins

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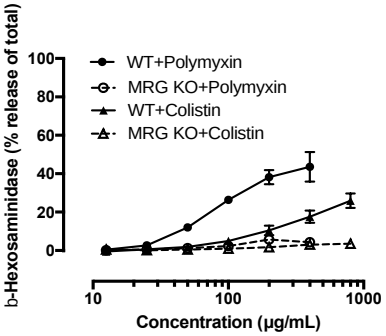
Introduction. Polymyxins are known to cause hypersensitivity in patients through release of mediators from mast cells. However, the receptor or pathway involved has not been well characterised. Mast cells have important roles in allergic and inflammatory diseases and can be activated via the classical IgE-dependent pathway and by IgE-independent mechanisms. Recently, the MAS-related G protein-coupled receptor X2 (MRGPRX2) has been shown to mediate IgE-independent mast cell activation to a diverse range of commonly polybasic stimuli. As polymyxins are polybasic compounds, they have been hypothesised to activate mast cells through MRGPRX2.



Aims. To investigate the role of MRGPRX2 in the activation of human mast cells by two clinically used polymyxins, polymyxin B and colistin.

Methods. The FcεRI and MRGPRX2 expressing LAD2 human mast cell line was used to characterise the degranulation, calcium mobilization, cytokine release and cell viability following treatment with polymyxin B and colistin. CRISPR knock-down MRGPRX2 LAD2 cells were used to investigate the dependence of MRGPRX2 to the actions of the polymyxins.

Results. Polymyxin B and colistin caused degranulation, calcium mobilization and cytokine production, with no cytotoxicity, in LAD2 cells in a concentration-dependent manner. As shown in the graph, colistin was less active than polymyxin B in eliciting degranulation. In contrast, the two drugs had equivalent activity in calcium mobilization and cytokine production. Responses were ablated by greater than 90% in the MRGPRX2 knock-down cells.

Discussion. Polymyxin B and colistin activation of mast cells was shown to be mediated through MRGPRX2 and might contribute to patient hypersensitivity to these drugs. Better understanding the difference in mast cell degranulation induced by polymyxin B and colistin might enable more selective use of these drugs in clinical situation.

332 Influenza A virus infection during pregnancy induces severe maternal vascular dysfunction and fetal growth restriction

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Introduction: Influenza A virus (IAV) infection during pregnancy can be life-threatening to both mother and child. IAV-infected pregnant women have a higher incidence of acute respiratory distress syndrome, pneumonia and heart failure. Although IAV is not vertically transmitted to the fetus, the risks to the offspring include preterm birth, congenital malformations, growth restriction, and long-term chronic immune and cognitive diseases later in life. How IAV infection in pregnancy causes these adverse fetal effects *in utero* is unknown.

Aims: To characterise the lung, systemic, placental and vascular inflammation following IAV-infection in pregnancy.

Methods: Eight-to-twelve-week old time-mated pregnant (E12 gestation) and non-pregnant C57BL/6 female mice were intranasally infected with the H3N2 IAV strain (HKx31; 10^4 PFU) or PBS for tissue analysis 3 days post-infection. Maternal and pup weights were recorded. Inflammation and viral mRNA expression in lungs, aorta and placenta were determined by qPCR or flow cytometry. Thoracic aorta reactivity was assessed with myography and endothelium-dependent and independent smooth muscle relaxation determined using Ach and sodium nitroprusside, respectively.

Results: IAV infection during pregnancy resulted in exacerbated systemic inflammation characterised by a significant increase in blood neutrophils and circulating cell-free fetal DNA compared to non-pregnant IAV-infected mice. Pup weights were significantly reduced with IAV-infection, however, there was no evidence of pro-inflammatory cytokine expression in the placentas. IAV infection during pregnancy caused a significant ~60-70% impairment in maximal relaxation of the thoracic aorta. This was associated with significantly elevated levels of pro-inflammatory cytokines and influenza viral mRNA. No impairment in vascular function was observed in non-pregnant IAV-infected mice.

Discussion: This study is the first to demonstrate that IAV infection disseminates into the maternal aorta causing severe impairment of vascular function that occurs selectively in pregnancy. This impairment in vascular function is likely to reduce the blood flow to the placenta and offspring resulting in fetal growth restriction. Therapies restoring vascular function are an exciting and novel strategy for the management of IAV infection during pregnancy.

333 Apocynin prevents hydrogen peroxide-induced atrophy in C2C12 skeletal muscle myotubes

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Introduction. Chronic obstructive pulmonary disease (COPD) is a major global health burden and is currently the 4th leading cause of preventable deaths worldwide. Comorbidities are major drivers of morbidity and mortality associated with COPD and are thought to be caused by increased oxidative stress. In fact, patients with COPD have elevated levels of the oxidant hydrogen peroxide (H_2O_2) in their exhaled breath condensate and this is further increased during pathogen-induced exacerbations. Skeletal muscle wasting (SMW) is a highly debilitating comorbidity seen in approximately 40% of individuals with COPD. Despite its prevalence, there are currently no effective pharmacological therapies available to treat SMW in COPD, highlighting the need for a targeted drug approach.
Aims. To investigate whether H_2O_2 induces skeletal muscle atrophy, and to determine if prophylactic treatment with the antioxidant apocynin reduces skeletal muscle atrophy in response to H_2O_2.
Methods. C2C12 murine skeletal muscle cells were cultured into myoblasts, differentiated into myotubes, and exposed to H_2O_2 (0-500 µM) for 24 hours, in the presence or absence of prophylactic apocynin (500 nM). Cell supernatant and lysate were then collected for ELISA and qPCR analysis respectively, and immunostaining was performed on the resulting myotubes to determine myotube diameter as a measure of atrophy.
Results. Myotube diameter was significantly reduced following H_2O_2 exposure (~36% reduction, $P<0.0001$), which was prevented by apocynin treatment. Furthermore, H_2O_2 significantly increased pro-inflammatory IL-6 gene (4 fold) and protein (3 fold) expression, NOX2 (oxidative stress enzyme) gene expression (6 fold), and atrogin-1 and myostatin (atrophic factor) gene expression (2 fold, all $P<0.05$). Conversely, H_2O_2 significantly decreased IGF1 (hypertrophic factor) gene and protein expression (50% reduction, $P<0.05$). Prophylactic treatment with apocynin inhibited H_2O_2-induced increases in NOX2 gene expression and prevented the reduction in IGF1 gene and protein expression.
Discussion. H_2O_2 reduced C2C12 myotube diameter via upregulation of atrophic gene expression and downregulation of hypertrophic gene expression, leading to an overall shift towards atrophy. Moreover, apocynin mitigated H_2O_2-induced skeletal muscle atrophy by reducing NOX2 expression and preserving hypertrophic signalling, suggesting that it may be an effective therapy for the treatment of oxidative stress-induced SWM in COPD.

334 Stiff cellular microenvironments promote the differentiation of human lung lipofibroblasts into (myo)fibroblasts

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Introduction. The aberrant accumulation of myofibroblasts and the excessive deposition of extracellular matrix (ECM) are the key histopathological features of idiopathic pulmonary fibrosis (IPF). The suggested sources of myofibroblasts include, epithelial mesenchymal transition-derived fibroblasts, resident lung fibroblasts and lipofibroblasts. Lipofibroblasts are lipid-droplet containing cells that have a key role in maintaining homeostasis of the lung by facilitating alveolar maturation and surfactant production. However, IPF patients have stiffer lungs, impaired re-epithelialization process and augmented differentiation of fibroblasts to myofibroblast.
Aim. To characterize the phenotype of human lung fibroblasts in soft and stiff *in vitro* culture models.
Methods. Fibroblasts derived from IPF patients and non-diseased controls were established in three settings: 2D stiff (conventional) cultures established on uncoated flat-bottom plates (~3GPa stiffness); 2D soft (~350 Pa stiffness) cultures generated on 24-well plates coated with rat tail collagen type-1 solution or Cytosoft® 6-well plates (2kPa stiffness; 3D soft cultures (spheroids; ~400Pa stiffness) generated on polyhydroxyethylmethacrylate coated ultra-low attachment round-bottom 96-well plates by centrifugation at 1,000g for 10 minutes. ECM components, myofibroblast and lipofibroblast marker expression in the three culture settings were analyzed by RT-qPCR and western blotting. The level of neutral lipid in 2D stiff, 2D soft and 3D soft fibroblasts was measured with HCS LipidTOX™ lipid stain.
Results. In 2D soft and 3D soft cultures, there was a striking downregulation of the myofibroblast marker, alpha smooth muscle actin (α-SMA). In contrast, the expression of lipofibroblast markers, such as adipose differentiation-related protein (ADRP) and CEBPA, were upregulated in soft settings. The level of lipid-droplet inclusions that mark the formation lipofibroblasts was increased in soft settings. Interestingly, transforming growth factor-β1 (TGF-β1) promoted the differentiation of lipofibroblast into myofibroblasts in stiff, but not in soft microenvironments. Furthermore, serially passaging (myo)fibroblasts on Cytosoft® 6-well plates (2D soft) for three successive weeks resulted in progressive induction of lipogenic markers with the passage number, and TGF-β1 induction of myogenic markers decreased.
Conclusion. Softening the cellular microenvironment may promote the lipofibroblast phenotype in IPF, facilitating alveolar repair and regeneration.

335 Differential expression of thromboxane synthase in human colon; upregulation in Crohn's disease and with age

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Introduction. Thromboxane A2 (TxA_2), a well-known vasoconstrictive and pro-coagulant eicosanoid, also found to have pro-inflammatory properties. Overexpressed thromboxane synthase (TxS) is shown in the active state of inflammatory bowel disease (IBD), consisting of ulcerative colitis (UC) and Crohn's disease (CD). However, it is unclear how TxS is involved in different inflammatory colonic diseases, including UC, CD as well as acute diverticulitis disease (DD).
Aims. Our study aims to localise cellular expression of TxS in the human colon and determine the differences in TxS expression level between UC, CD and DD, matched with their respective gender-, region- and age-matched controls. We also aim to explore the effects of selective inhibition of TxS by ozagrel in an *ex vivo* human colitis model.
Methods. Immunofluorescent double-labelling of anti-TxS antibody (Ab187176, Abcam) with various cell marker antibodies were used to localise TxS in the control human colon sections. The TxS immunoreactivity (TxS-IR) on CD, UC and DD tissues were compared to their matched control tissues and quantitively analysed by ImageJ. For the colitis model, fresh human colonic strips were incubated with TNF-α and IL-1β (10 ng/ml) for 16 hrs to induce crypt damage (Diezmos et al. 2018). Ozagrel (10 µM) was added to determine its protective effect against inflammation.
Results. In the human colonic mucosa, the majority of TxS-IR is co-localised with IBA-1 positive macrophages and microglia, while a small population also resided within MUM-1 positive plasma cells. There was an age-related increased in TxS-IR (correlation coefficient r = 0.60, n = 18, $P = 0.0084$), and the TxS level was 1.7-fold higher in the mucosal region of sigmoid colon (n = 12) than that of ascending colon (n = 6, $P = 0.048$ Mann-Whitney test). TxS-IR was significantly elevated in the colonic mucosa of CD patients compared to its corresponding control (n = 6 for both groups, $P = 0.0043$). UC and DD showed no differential TxS expression level compared to control. In the colitis model (n = 5), ozagrel significantly inhibited TxA_2 production, resulting in a slight improvement over tissue integrity.
Discussion. The discrepancy in TxS expression between the inflammatory colonic diseases could suggest a difference in their pathogenesis. The localisation of TxS in macrophages indicates that of an inflammatory function. Furthermore, TxS inhibition could potentially be a viable therapeutic option in intestinal inflammation, especially for CD patients.
Diezmos EF et al., Front Pharmacol. 2018; 9:865. doi: 10.3389/fphar.2018.00865

336 Serelaxin enhances the renoprotective effects of human amnion epithelial cell-derived exosomes in a murine model of obstructive nephropathy.

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Introduction. Fibrosis is a common feature of chronic kidney diseases and its inability to resolve causes severe organ dysfunction and end-organ failure. The limited anti-fibrotic efficacy of currently-available therapeutics suggests a need for alternative treatments/combination strategies. We recently demonstrated that the anti-fibrotic hormone-based drug, serelaxin, could enhance the therapeutic efficacy of human bone marrow-derived mesenchymal stem cells[1] and human amnion epithelial cells (hAECs) against obstructive nephropathy.
Aims. To determine whether the anti-fibrotic effects of serelaxin (RLX) could enhance the renoprotective effects of hAECs-derived exosomes (EXO; which offer manufacturing and regulatory advantages to using cell-based therapies) in a murine model of unilateral ureteric obstruction (UUO)-induced kidney disease.
Methods. 8-10 week-old male C57Bl/6J mice were subjected to UUO-induced renal injury. Sub-groups of mice (n=6/group) were then untreated (injury control group) or immediately treated with RLX (0.5mg/kg/d; via minipumps), EXO (25□g/mouse; via intrarenal (i.r) injection) or both combined. A separate sub-group i.r-administered with hAECs ($1x10^6$/mouse)+RLX were included for comparison. Sham-operated mice were used as non-injury controls. All mice were culled 7-days post-UUO, a time-point at which renal fibrosis is well-established in untreated mice. Various measures of renal inflammation, fibrosis and damage were then evaluated.
Results. While EXO alone were able to prevent measures of renal inflammation (by 55-60%), interstitial renal fibrosis (by ~48%), total renal collagen concentration (by ~21%) and renal damage (by ~75%) by 7 days post-UUO, all these measures were further prevented by the combined effects of EXO+RLX, which also had or trended to have improved therapeutic effects over the combined effects of hAECs+RLX. Of note, renal damage was completely prevented by the combined effects of EXO+RLX.
Discussion. RLX appeared to enhance the ability of EXO to prevent UUO-induced renal damage. Evaluating the therapeutic effects of this combination strategy will be key to translating its potential as a therapy of the future.
[1]Huuskes BM et al., 2015 FASEB J 29:540-553.

337 Mechanisms underlying purinergic P2X7 receptor antagonism in maintaining urothelial barrier function against acrolein-induced inflammation and cytotoxicity

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Introduction. Damaged urothelium is a significant characteristic of cystitic bladders. Our recent study has shown that P2X7R antagonism protects the urothelium from acrolein-induced damage and apoptosis and preserves mucosal contractility in the *ex vivo* model of the whole porcine bladder (Taidi et al., 2018).
Aim. To explore the mechanisms underlying the protective effect of P2X7R antagonism against acrolein-induced urothelial inflammation and cell damage.
Methods. Urothelial cells from female porcine bladder were plated in permeable transwell inserts ($1x10^5$ cells/well) and cultured until a steady transepithelial electrical resistance (TEER) reading has reached. Cell damage was induced by incubating cells with the cytotoxic agent acrolein (50 µM), and the protective effect of P2X7R inhibition was determined by pre-treating cells with the P2X7R antagonist A804598. The P2X7R-dependent large pore forming property was investigated by the measurement of high concentrations of ATP- and Bz-ATP-evoked YO-PRO-1 uptake into cultured urothelial cells, in the presence or absence of P2X7R antagonists.
Results. Acrolein caused a 60% reduction in TEER values for up to 48 h ($P < 0.0001$, two-way ANOVA, compared to control), indicating persistent damage to the urothelial barrier. Pre-incubation of urothelial cells with A804598 (10 µM) reversed acrolein-induced TEER reduction to the level equivalent to the control ($P < 0.0001$, compared to the acrolein group). Both ATP (300 µM) and the selective P2XR7 agonist Bz-ATP (300 µM) induced a long-lasting increase of YO-PRO-1 fluorescence intensity ($P < 0.001$, two-way ANOVA), and this action was completely abolished following co-treatment with P2X7R antagonists, AZ11645373 (100 µM) and A-804598 (1 µM).
Discussion. This was the first report showing that acrolein can disrupt cell integrity and increase permeability in cultured urothelial cells, and the disruptive effect of acrolein was greatly attenuated or completely abolished by P2X7R antagonists. Our results suggest that P2X7R plays an important role in urinary inflammation and cell damage.
Taidi Z, et al. (2018). Neurourol Urodyn 37: S104-S106.