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Proton Pump Inhibitors - Robust Evidence of Public Health Hazard, or Just Plain Confounding?

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Proton pump inhibitors (PPIs) are very widely used in healthcare settings for prevention or treatment of upper gastrointestinal disease. The perceived safety of PPIs may have led prescribers and patients to accept extended durations and indications for such therapy without any major concerns. However, PPIs have been dogged with controversy recently, with a number of drug safety updates from the regulatory authorities that warn of potentially serious harm. These problems include reported associations between PPI use and increased risks of fracture, as well as Clostridium difficile infection (CDI). A potential drug interaction between clopidogrel and PPIs has also been hotly debated, with suggestions that use of certain PPIs can significantly elevate the risk of adverse cardiovascular events. However, proponents of PPIs argue the gastrointestinal benefits of PPIs are substantial and that the signals for harm stem from studies that are methodologically weak.
This presentation will discuss evidence from several recent systematic reviews of adverse effects, and provide an update on what is known (and not known) about the benefits and harms of PPIs.

Key References:

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Transporter-based drug-drug interactions – principles and examples

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Transporter-based drug interactions (DDI) can be complex and difficult to predict due to their tissue, cell, and cell-membrane specific localization. Transporter-based DDI will be clinically relevant for victim drugs with relatively narrow therapeutic window and where transporters are the rate-limiting step in their disposition (including tissue distribution). In my presentation, I will illustrate the concepts of rate-limiting step and "cloaked" DDI using data from animal and human studies. Supported by NIH (multiple grants), UWRAPT, Pfizer Inc.

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Role of organic cation transporters in mediating oral drug absorption

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Intestinal epithelium is a formidable barrier to oral drug absorption. The tightly packed columnar cells with narrow intercellular (paracellular) space further restricted by the presence of tight junctions, present a physical barrier to hydrophilic compounds, particularly those with net charge over the pH range within the intestinal lumen. This is because the hydrophilic and/or charged compounds cannot cross the lipid bilayer of cell membranes of the enterocytes, and cannot traverse the narrow paracellular space, restricted by the presence of tight junctions. Therefore, transporters play an important role in the oral absorption of hydrophilic charged compounds. Specifically, we have asked the question why certain hydrophilic cationic drugs have high bioavailability despite their hydrophobicity and net positive charge. Hence, we have investigated the mechanism of absorptive transport of the hydrophilic weak bases H2-receptor antagonists, ranitidine and famotidine, as well as the leading anti-diabetic drug, metformin, which is permanently charged at all physiologic pHs, in various cellular, tissue, and in vivo models. Our studies showed that organic cation transporters (OCTs), specifically OCT1, play an important role in oral absorption of these drugs. However, paracellular transport also contributes significantly to their intestinal absorption. Thus, our studies in the Caco-2 cell monolayers showed that almost 50% of ranitidine absorptive transport was transcellular and was mediated by cation-selective transporters, whereas remaining 50% of absorptive transport was paracellular. In contrast, almost 90% of metformin crosses Caco-2 cell monolayers in the absorptive direction via the paracellular route, although cation-selective transporters mediate apical uptake of the drug into the cells. A novel mechanism for intestinal absorption of metformin will be described that involves repeated cycling between enterocytes and intestinal lumen, mediated by multiple apical cation-selective transporters, and paracellular absorptive transport that is assisted by claudins in tight junctions of the intestinal epithelium.

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Role of transporters in mediating hepatobiliary clearance of drugs

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Hepatic transport proteins facilitate the uptake and excretion of many drugs, and are well-recognized sources of potential drug-drug interactions (DDIs). Inhibition of hepatic uptake and/or efflux transporters may alter drug exposure in the liver and/or the systemic circulation leading to changes in efficacy and/or toxicity. A technique coupling gamma scintigraphy with a customized oroenteric catheter and specialized clinical protocol was developed to accurately quantify hepatic uptake, exposure, and biliary clearance ($Cl_{biliary}$) of drugs in humans [Ghibellini et al (2007) Clin Pharmacol Ther 81:406-413]. Using this approach, the effect of ritonavir, a potent inhibitor of hepatic transport proteins, on the hepatobiliary disposition of ^{99m}Tc-mebrofenin was investigated in healthy volunteers. The efficient hepatic uptake of ^{99m}Tc-mebrofenin is mediated by OATP1B1 and OATP1B3; ^{99m}Tc-mebrofenin is rapidly excreted into bile unchanged by MRP2 [Ghibellini et al (2008) Pharm Res 25:1851-1860]. Ritonavir (multiple-dose: 2x300mg) significantly increased systemic ^{99m}Tc-mebrofenin exposure compared to control (4464±1861 vs. 1970±311 nCi*min/mL; mean±s.d.) without affecting overall hepatic exposure or biliary recovery. A semi-physiologically-based pharmacokinetic model was developed based on the blood, liver and bile data to elucidate potential sites/mechanisms of the ^{99m}Tc-mebrofenin-ritonavir DDI, and in vitro studies were conducted. A novel extrahepatic distribution compartment was required to characterize ^{99m}Tc-mebrofenin disposition in humans. Ritonavir inhibited ^{99m}Tc-mebrofenin accumulation in human sandwich-cultured hepatocytes (IC_{50}=3.46±1.53 μM). Despite ritonavir accumulation in hepatocytes, intracellular binding was extensive (97.4%), which limited interactions with MRP2-mediated biliary excretion. In vitro data supported conclusions from modeling/simulation that ritonavir inhibited ^{99m}Tc-mebrofenin hepatic uptake, but not biliary excretion, at clinically relevant concentrations. This integrated approach utilizing clinical and in vitro data, and modeling/simulation, emphasizes the importance of hepatic and extrahepatic drug distribution, assessment of inhibitory potential in relevant in vitro systems, and intracellular unbound concentrations to accurately predict the functional consequences of potential hepatic transport DDIs. *This research was supported by NIH GM41935.*

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Impact of BBB Transporters on Delivery and Efficacy of Molecularly-Targeted Agents in Brain Tumors
Prof William Elmquist, University of Minnesota, USA

The importance of the blood-brain barrier in preventing effective pharmacotherapy of brain tumors has been controversial. The controversy stems from the fact that vascular endothelial cell tight junctions are disrupted in the tumor, allowing some systemic drug delivery. P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP) efflux drugs from brain capillary endothelial cells into the blood. We tested the hypothesis that although the tight junctions are "leaky" in the core of glioblastomas, active efflux limits drug delivery to tumor-infiltrated normal brain and consequently, treatment efficacy. Malignant gliomas were induced by oncogene transfer into wild-type (WT) mice or mice deficient for Pgp and BCRP (knockout, KO). Glioma-bearing mice were orally dosed with dasatinib, a kinase inhibitor and dual BCRP/PgP substrate that is being currently tested in clinical trials. KO mice treated with dasatinib survived for twice as long as WT mice. Microdissection of the tumor core, invasive rim, and normal brain revealed 2- to 3-fold enhancement in dasatinib brain concentrations in KO mice relative to WT. Analysis of signaling showed that poor drug delivery correlated with the lack of inhibition of a dasatinib target, especially in normal brain. A majority of human glioma xenograft lines tested expressed BCRP or PgP and were sensitized to dasatinib by a dual BCRP/Pgp inhibitor, illustrating a second barrier to drug delivery intrinsic to the tumor itself. These data show that active efflux is a relevant obstacle to treating glioblastoma and provide a plausible mechanistic basis for the clinical failure of numerous drugs that are BCRP/Pgp substrates.

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Inflammation resolution mediators in tumour growth and metastasis: good, bad and indifferent
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Inflammation, an ever-present feature of solid tumours, is a component of the tumour microenvironment that impacts on growth and metastasis. Angiogenesis, extracellular matrix composition/turnover, tumour cell migration and tumour immunity are influenced by the inflammatory *milieu*. Inflammation incites are solution process that limits the duration and severity of the inflammatory response. Mediators of this inflammation resolution include annexin-1 and its N-terminal peptide, and a series of trihydroxy polyunsaturated fatty acid derivatives (lipoxins, protectins, resolvins and maresins). In certain tumour types infiltration with macrophages is associated with a negative prognosis. This association might reasonably be explained by an overall negative influence of the inflammation associated with the function of these tumour-associated macrophages (TAM). Tumours have been described as “wounds that do not heal”. Thus, inflammation-resolving, as well as pro-inflammatory mediators may accompany the persistent granulation tissue. Is the causal link between TAM and poor outcome explained by persistence of pro-inflammation or pro-resolution mediators? The evidence to address this issue is scant and a consistent picture has yet to emerge. Inflammation resolving mediators act directly on breast tumour epithelium to promote growth and metastatic processes (Yao et al 2008; Rondepierre et al, 2009; Khau et al, 2011). The importance of such direct influences as opposed to impacts on inflammation intensity/duration need to be defined in order to understand the potential of receptors for inflammation resolving mediators such as formyl peptide receptors (FPR), as targets for adjuvant therapy in treatment regimens for different solid tumours. Evaluation of the targeting potential of the FPR2 receptor subtype is further complicated by the diverse signaling evoked by pro-resolution ligands, annexin-1 and lipoxin A_4, compared to pro-inflammatory ligands, such as serum amyloid A.

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Exploring allosteric modulation and ligand-directed stimulus bias at the glucagon-like peptide-1 receptor

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The glucagon-like peptide-1 (GLP-1) receptor is a key regulator of insulin secretion and a major therapeutic target for the treatment of diabetes. However, GLP-1 receptor function is complex with multiple endogenous peptides that can interact with the receptor, including full length (1-37) and truncated (7-37) forms of GLP-1 that can each exist in amidated forms, and the related peptide oxyntomodulin. Furthermore, modified forms of GLP-1 or exogenous peptide mimetics such as exendin are currently used in clinical treatment of diabetes. Despite the prevalence of peptide-based GLP-1 receptor drugs, there is great interest from the pharmaceutical industry in the development of small molecules that can be administered orally. We have identified evidence of peptide and small molecule engendered signal bias at the GLP-1 receptor indicating that different ligands may ultilise distinct conformational rearrangements of the receptor to couple to second messenger pathways. In addition, small molecule ligands that bind to topographically distinct allosteric sites of this receptor can also promote bias in orthosteric ligand signalling, which can vary depending on the nature of the orthosteric ligand being assessed. We also demonstrate the hitherto-unappreciated potential for allosteric ligands to cause marked potentiation of previously "inert" metabolic products of GLP-1 peptides in both ex vivo and in vivo assays of insulin secretion. These studies open up new avenues for allosteric drug discovery highlighting the potential for distinct clinical efficacies depending on the properties of individual drugs. However, we also identify many challenges for how GLP-1 receptor targeted drugs are screened and developed.

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Physiological correlates of biased receptor signaling: relevance to opioid drug action in health and disease

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Opioid analgesics, such as morphine, mediate their therapeutics actions by activating the mu opioid receptor (MOR) which is a G protein-coupled receptor (GPCR). Unfortunately, opioids also mediate most of their side-effects, such as respiratory suppression, constipation and dependence, by actions at this receptor. Like most GPCRs, the MOR interacts with beta-arrestins. Beta-arrestins serve as scaffolding proteins which can, upon agonist stimulation, lead to receptor desensitization of G protein coupling. Interestingly, beta-arrestins can also mediate GPCR signaling to alternate pathways thereby introducing a point at which GPCRs can be directed to signal based on their recruitment of the G protein. The degree of interaction between the GPCR and the beta-arrestin can be influenced by the chemical composition of the ligand. Using beta-arrestin-2 KO mice, our laboratory has studied this protein's contributions to MOR-mediated biological responses. We have found that in the absence of beta-arrestin2, morphine analgesia is enhanced and tolerance is attenuated suggesting that beta-arrestin2 plays a role in desensitizing signal transduction leading to antinociception. Other morphine-mediated behavioral responses, including dependence (antagonist-induced withdrawal), respiratory suppression and constipation are attenuated in this animal model suggesting that Barrestin2 may play a facilitatory role in the signaling underlying these responses. In this presentation we will present data examining additional signaling roles mediated by individual beta-arrestins such as those contributing to resensitization and ubiquitination, as well present some early developments in our drug discovery efforts to generate MOR agonists that are biased against beta-arrestin recruitment. According to extensive studies in the beta-arrestin2 mouse models, such a strategy may allow for the treatment of pain with fewer side-effects then seen with traditional opioid therapies. Funding for this work has been sponsored in part by the National Institute on Drug Abuse: R01DA14600, R01DA18860, R03DA025158 to LMB and F31DA021952.

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Revealing the potential of GPCR bias signalling
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One of the key features of GPCRs is the ability of each receptor to couple to numerous down-stream signalling pathways. This flexible signalling capacity underlies the fact that any one GPCR subtype might regulate very different physiological responses in different cell types. However, despite understanding the broad signalling capacity of GPCRs, recent thinking in this area has simplified GPCR signalling into essentially two pathways. The first is the traditional GPCR signalling via heterotrimeric G-proteins mediating phosphoinositide hydrolysis and calcium mobilisation. The second is signalling that lies down-stream of receptor phosphorylation and arrestin recruitment. Although simple, considering GPCRs in the context of bimodal signalling has helped with regard to investigating the concept of stimulus bias. This concept proposes that a GPCR agonist may stimulate a receptor so that both arms of the bimodal signalling pathway are equally activated. Such a ligand would have no stimulus bias. However, if a ligand induced a receptor conformation that allowed for one arm of the bimodal signalling pathway to be activated in preference to another then this ligand would show stimulus bias. The power of this concept is that if we were to understand the signalling modality employed by any particular GPCR to deliver a particular physiological, or indeed therapeutic, outcome then we would be able to rationally design GPCR ligands to bias the signalling towards that physiological/therapeutic outcome. In this way we would increase clinical efficacy and potentially reduce on-target, but undesired, side affects. How do we test if this promise can be delivered. In this presentation approaches to address this issue by using animal models together with molecular pharmacology approaches will be discussed.

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Non-parametric population PK models and their use in individual patient care
Assoc Prof Michael Neely, University of Southern California, USA

Therapeutic drug monitoring in the 21st century can be much more sophisticated that simply comparing measured drug concentrations to a target range. In this session, attendees will be introduced to population modeling, and how it has advantages over traditional pharmacokinetic modeling that can improve patient care. We will discuss some of the differences between parametric and non-parametric population models, and show examples of how the latter are being used in practice to precisely control therapy in a flexible, powerful way. We will discuss some of the barriers to the use of such approach in routine care, as well as offer solutions to overcome many of those barriers.

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Emerging trends in dose individualisation in clinical practice
Assoc Prof Jennifer Martin, The University of Queensland

Dose individualisation aims to achieve maximal efficiency with lowest toxicity and in some circumstances, lowest cost. It is an old science that is being revisited due to cost constraints, the availability of new pharmacogenetic data, new pharmacokinetic assays, new pharmacometric models and software.

Dose individualisation is undertaken by consideration of an individual patient's demographic, anthropomomeric and comorbidity profiles. Pharmacogenetic information for the particular drug may be available. This data enables a priori drug choice and dose individualisation. After initiating therapy, the drug response is evaluated using pharmacokinetic measurements (usually blood or urine concentrations), pharmacodynamic variables and/or clinical outcomes, and dose regimen altered. This data can be added to pharmacometric models to further refine and update dosing predictions.

This presentation will discuss the clinical use of these methodologies and their emerging use to improve dose individualisation in clinical practice. Reasons for success and failure of drug treatment and methodologies to improve successful dosing will be discussed.

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Pharmacogenomics and drug safety

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The last decade has seen elucidation of many genes related to drug metabolism, drug transporters and pharmacodynamic factors of specific drugs that have the propensity to drive dose-dependent and pharmacologically predictable adverse drug reactions and drug interactions. Associations between immune response genes such those in the major histocompatibility complex (HLA) have shed light on the immunopathogenesis of serious adverse drug reactions such as drug hypersensitivity syndromes (DRESS/DIHS), Stevens-Johnson Syndrome and toxic epidermal necrolysis (SJS/TEN). The association between HLA-B*5701 and abacavir has been a notable discovery which has acted as a roadmap for T1 → T4 translation from discovery of an association between a gene and a drug toxicity through to clinical and laboratory translation, through to implementation of a screening test which has now been routinely used as part of guideline-based HIV clinical practice in the developed world to successfully essentially eliminate abacavir hypersensitivity. Whether a test will translate is driven by characteristics of the drug and the availability of therapeutic alternatives, attributes of the test itself, nature of the drug toxicity, having an environment or individual to champion the test, the ability to generate high levels of evidence to support the clinical utility and cost-effectiveness of the test, the development of appropriate laboratory support, infrastructure and quality assurance, and the design and implementation of appropriate clinical systems. More recent research has provided important structural and functional insights as to how drugs like abacavir and carbamazepine may specifically interact with HLA-B*5701 and HLA-B*1502 respectively to cause hypersensitivity and SJS/TEN. It is now feasible that these approaches examining how drugs specifically interact with HLA and other genes could be applied as part of a pre-clinical pharmacogenomic screening strategy to inform the design and development of safer drugs.

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Dose individualisation of dabigatran

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Dabigatran is a novel oral anticoagulant that has been marketed as an advance on warfarin, with apparent advantages including the capacity for 'fixed' rather than 'individualised' dosing, few pharmacokinetic drug interactions, and no need for routine laboratory coagulation monitoring. However, we believe that the variance in its pharmacokinetics, in relation to its renal clearance and to the oral availability of its prodrug, dabigatran etexilate, necessitate a greater degree of dose individualisation than has been promoted. Currently, determination of appropriate dose-rates by prescribers is largely on the basis of estimating the patient's glomerular filtration rate. However, it is unclear which glomerular filtration rate equation is best for guiding dabigatran etexilate dosing. Further, it is unclear how dosing should accommodate pharmacokinetic drug interactions, which is important as we have demonstrated that ~50% of patients discharged on dabigatran etexilate from our tertiary hospital institution have co-prescribed drugs that alter the oral availability of dabigatran etexilate. We propose that laboratory coagulation monitoring is a solution to these issues, and suggest targets for study and implementation.

Chin P et al (2012) Br J Clin Pharmacol (in press; DOI:10.1111/j.1365-2125.2012.04266.x)

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The patient journey towards the end of life -patient and family accounts of 'patient safety'

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Introduction. There is a dearth of research that is concerned specifically with end of life care and patient safety. Quality is driven by 'patient safety' mechanisms that prioritise the technical aspects of safety highlighting concepts such as 'incident reporting' and 'medical error' and the voices of dying patients and their families often go unheard.
Aim. This study explores patient safety towards the end of life and the potential of visual methods to facilitate mutual understanding between patients, families and clinicians to address patient safety. This paper will present video narratives of what patient safety means for dying patients and their families from their own perspectives.
Methods. Patient participants were recruited via referral from specialist clinicians in a metropolitan hospital. Drawing from a framework of indigenous research ethics, collaborative video methodologies provided a medium to sensitively explore the complexities of end of life care. Methods were emergent and incorporated patients, families and clinicians in reflexively responding to their filmed narratives. In keeping with the underlying philosophy of the research opportunity will be provided within this presentation for the conference audience to engage reflexively with themed video excerpts. Firstly, this will demonstrate a key component of the study facilitating understanding of methodology. Second, connecting with the audience in this way will allow them to affectively engage with the research contributing to stakeholder knowledge and literacy of patient safety issues towards the end of life and informing the thesis.
Findings. Video offered an accessible and profound medium for collaborative sense making providing a reflexive space of engagement for patients, families and those caring for them. This research study found that the field of patient safety does not presently address the needs of dying people. Habitual care patterns expose dying patients and their families to pervasive harms along with those healthcare workers caring for them. Visual methods provide a disruptive innovation that challenges these normative and habitual rhythms of inattentiveness to healthcare safeties.

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Towards a medicinewise Australia: the NPS approach to improving health literacy

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Health literacy can be defined as the ability to access, understand, evaluate and communicate information as a way to promote, maintain and improve health in a variety of settings across the course of a person's life.

To improve health literacy, it is necessary to engage effectively with both individuals and with systems.

The NPS approach involves:

- Finding ways to create awareness and promote curiosity amongst people in many different settings in order to start a conversation about the benefits and risks of medicines, medical tests and other medical options
- Finding ways to influence the influencers – opinion leaders and advocates in communities, the media and the health system
- Using messages and concepts that are simple and relevant to everyday life
- Working in partnership with expert intermediaries and others to promote and explain the importance of asking questions and seeking understanding about if, when and how to use medicines wisely
- Providing tools that help people put theory into action in a positive way
- Keeping the person and their carers at the centre and being wherever they are – in the community, in workplaces, in hospitals and other care settings, and at transitions of care in the health system.

Individuals seek and receive information about health choices from many sources, many of which are not evidence based. Health literacy develops iteratively over time and is enhanced when people are supported in relevant ways at times of particular information need. Navigating the health system and its myriad sources of information is a formidable task and health professionals have a critical role to play in helping people connect with reliable information and make good decisions wherever they are in their health journey. NPS information and tools can assist.

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The effect of ageing on paracetamol pharmacokinetics and toxicity in Fischer 344 rats

John Mach[1,2,3], Aniko Huizer- Pajkos[1,2], Himesha Vandebona[1,3], Victoria Cogger [3,4], Catriona McKenzie [5], David Le Couteur [3,4], Brett Jones[1,2,3,6], Sarah Hilmer[1,2,3]. Kolling Institute of Medical Research[1], Sydney, Sydney, NSW; Royal North Shore Hosp[2], Sydney, NSW; Sydney Medical School, Univ of Sydney, Sydney[3], NSW; ANZAC Research Institute, Concord Hosp[4], Sydney, NSW; Pathology Dept, Royal Prince Alfred Hosp[5], Sydney, NSW; Gastroenterology Dept, Royal North Shore Hosp[6], Sydney, NSW

Introduction. In paracetamol overdose, conjugation pathways that form non-toxic products are saturated, leading to the formation of a toxic metabolite N-acetyl-p-benzo-quinone imine(NAPQI) by Cytochrome P450 2E1(CYP2E1).With excessive NAPQI levels, NAD(P)H quinone oxidoreductase(NQO1) activity and glutathione stores are exhausted resulting in hepatotoxicity. The effect of ageing on the metabolism of toxic doses of paracetamol has not been well characterised.

Aims. To characterise the degree of hepatotoxicity, expression and activity of key proteins involved in hepatic metabolism of paracetamol after administration of toxic doses to young and old rats.

Methods. Young adult(6±1 months) and old(26±2 months) male Fisher 344 rats were injected ip with 800mg/kg paracetamol(young n=8, old n=5) or saline(young n=9, old n=9) four hours prior to euthanasia by ip injection of ketamine (75mg/kg)/xylazine(10mg/kg). Serum ALT and liver histology were assessed to indicate the degree of hepatotoxicity. Serum paracetamol, paracetamol glucuronide, paracetamol sulfate and creatinine were measured. CYP2E1 protein expression and activity, NQO1 activity, UGT1A6 mRNA expression and glutathione levels were measured in liver samples.

Results. Old paracetamol treated rats had significantly lower serum ALT and higher serum paracetamol and paracetamol glucuronide levels, compared to paracetamol treated young rats. Amongst saline treated, compared to young, old rats had significantly lower CYP2E1 activity(~2fold) and higher NQO1 activity(~4fold)($p<0.05$), with the same trend observed in animals treated with paracetamol. Hepatic glutathione did not differ with age, and was lower in animals treated with paracetamol than those with saline. There was no change in expression of UDP glucuronosyltransferase 1A6 mRNA with age or treatment. Compared to other groups, paracetamol treated old rats had higher serum creatinine($p<0.05$).

Discussion. Despite higher serum levels of paracetamol, older rats appear to have decreased susceptibility to paracetamol-induced hepatoxicity, which may be due to the reduced formation and enhanced metabolism of NAPQI. Older rats may be more susceptible to paracetamol-induced nephrotoxicity.

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Nox2β: A novel splice variant of Nox2 that promotes reactive oxygen species (ROS) production in macrophages

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Introduction: Nox2 oxidase is one isoform in a family of seven NADPH oxidases that generate reactive oxygen species (ROS) and thereby contribute to physiological and pathological processes including host defense, redox signaling, immune function and oxidative tissue damage.

Aims: To establish whether alternative mRNA splicing gives rise to functionally relevant splice variants of Nox2 that modulate ROS production.

Methods: Western blotting, RT-PCR, DNA sequencing, quantitative real-time PCR and L-012-enhanced chemiluminescence to measure ROS production, were performed on a variety of mouse tissues and primary macrophages, as well as the immortalized macrophage cell line (RAW264.7) and human alveolar macrophages.

Results and Discussion: Immunoscreening for the presence of truncated Nox2 proteins identified a 30-kDa protein in lung, and in peritoneal and alveolar macrophages from wild-type mice, and in atherosclerotic aortas from $APOE^{-/-}$ mice. RT-PCR analysis of mRNA from mouse macrophages, and from human alveolar macrophages, identified a truncated Nox2 transcript which, upon sequence analysis, was found to be a product of the 'exon skipping' mode of alternative splicing, lacking exons 4-10 of the Nox2 gene. The predicted protein is comparable in size to that identified by immunoscreening and contains two transmembrane helices and an extended cytosolic C-terminus with binding sites for NADPH and the Nox organiser protein p47phox. Importantly, selective siRNA-mediated knockdown of the transcript reduced expression of the 30-kDa protein in macrophages, and suppressed phorbol ester-stimulated ROS production by 50 %. Hence, this study provides the first evidence that Nox2 undergoes alternative mRNA splicing to yield a 30-kDa protein – herein termed Nox2β – that regulates NADPH oxidase activity and the oxidative burst in macrophages from mice and humans. The discovery of Nox2β paves the way for future examination of its role in physiological and pathological processes, in particular cardiovascular and lung diseases.

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Bitter taste receptor agonists are novel bronchodilators of small airways in mouse lung slices

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Introduction. Excessive contraction of airway smooth muscle in severe asthma, particularly in small airways, may be relatively insensitive to treatment with β_2-adrenoceptor agonists. Bitter taste receptor (TAS2R) agonists have been reported to elicit relaxation of human large airways (>2 mm diameter) and mouse trachea (Desphande et al. Nature Medicine 2010), but their effects on small airways have not yet been described.

Aims. To compare bronchodilator responses to the TAS2R agonists chloroquine (CQ) and saccharin (SACC) with the β-adrenoceptor agonist isoprenaline (ISO) in mouse trachea and in lung slice preparations in which small airway reactivity can be assessed *in sit.u*

Methods. Using Balb/C mice, isolated trachea were mounted under isometric conditions to measure changes in force and lung slices (~ 150 μm thick) were perfused to measure changes in small airway lumen area using phase-contrast microscopy image analysis. Dilator responses to TAS2R agonists or ISO were determined following precontraction with methacholine (MCh).

Results**.** CQ elicited complete relaxation in both mouse trachea (pEC_{50} 4.5 ± 0.1) and small airways (pEC_{50} 5.4 ± 0.1). SACC was 100-fold less potent than CQ. Despite its greater potency than the TAS2R agonists, ISO-mediated bronchodilation was incomplete, with maximum relaxation of only 54 ± 6 % and 73 ± 6 % in trachea and small airways respectively. The timecourse of small airway relaxation to maximally effective concentrations of CQ (100 μM) and ISO (10 μM) was similar. However, relaxation to CQ but not ISO was maintained with increasing MCh pre-contraction.

Conclusions. The TAS2R agonist CQ has greater dilator efficacy but lower potency than ISO in both large and small airways. Further studies are required in human small airways to confirm the potential of CQ relative to current bronchodilator therapies to improve clinical outcomes in patients with poorly controlled asthma.

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Screening potential skin sensitizers using high throughput direct peptide activity assay

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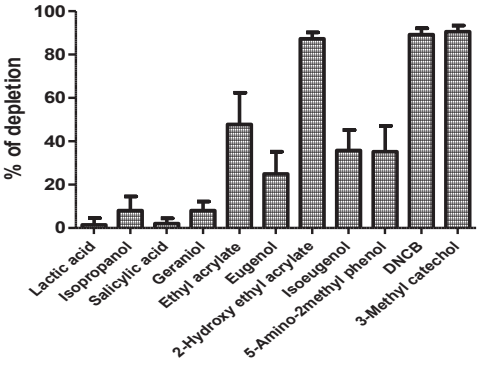
Introduction: Skin sensitizers and their metabolites are generally electrophilic and reactive towards nucleophilic sites on proteins. Among all amino acids, "Lysine and Cysteine" are relatively strong nucleophiles involved in hapten-protein interactions.

Aim: To develop an *in vitro* high-throughput LC-MS/MS method for measuring the depletion of a heptapeptide containing cysteine and lysine after incubation with a compound with unknown potential to cause skin sensitization reactions.



Method: Lactic acid, isopropanol, salicylic acid (non-sensitiser), geraniol, ethyl acrylate, eugenol (weak sensitiser), isoeugenol, 5-amino-2-methyl phenol (moderate sensitiser), 1-chloro-2,4-dinitrobenzene, and 3-methyl catechol (strong sensitiser) were incubated with a heptapeptide containing cysteine and lysine (Ac-NKKCDLF) at the ratio of 1 in 10 (peptide/test item). Following incubation at 37 °C, pH=7 (50 mM phosphate buffer) for 24h, the concentration of the peptide were measured using an optimised LC/MS/MS method. The mobile phase comprised 10 mM ammonium acetate buffer, pH=9.5 (A), and acetonitrile (B) on a Phenomenex; Gemeni C18 column. The detector (API 3200) settings and mobile phase gradient were optimised to generate the maximum response.

Results: The results shown in the figure (n=5) were significantly correlated (Spearman $r = -0.69$, $p < 0.05$) with published in vivo data (Natsch and Gfeller, 2008; Gerberrick et al, 2007) derived from the Local lymph node assay which is the gold standard method for assessment of skin sensitisation potential.

Discussion: This *in vitro* method has the potential to reduce animal use for assessment of skin sensitisation potential of novel compounds. Recent legislative changes by the European Union banning of animal use for testing of cosmetics and through enforcement of REACH regulations is driving the need for a cost-effective *in vitro* skin sensitization assay.

Gerberick, G F et al (2007) Toxicol Sci 97: 414-427.
Natsch, A and Gfeller, H (2008) Toxicol Sci 106: 464-478.

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The antimicrobial peptide LL-37 inhibits migration of the prostate cancer cells

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Introduction. Prostate cancer (PCa) is the most commonly diagnosed cancer in Australia. The progression of PCa is usually protracted but when metastases occur, disease progression is rapid and resistant to therapeutic intervention. The human cathelicidin-derived antimicrobial peptide, hCAP18/LL-37 is found in prostatic fluid in concentrations approximately 90-fold higher than the level found in blood plasma (90μg/ml vs1.2μg/ml). LL-37 has been implicated intumourigenesis and metastasis of lung, breast and ovarian cancer. However, the role of LL-37 in prostate cancer remains largely unexplored

Aims. To characterise the impact of LL-37 on PCa cell migration and the receptors involved.

Methods. The effect of LL-37 on the 2D and 3D migration of PC-3 and DU145 cell lines was assessed using a scrape wound closure assay and a modified Boyden chamber assay. The receptor dependence of the LL-37 effect was probed using agonists and antagonists of the formyl peptide receptor (FPR2), purinoceptors and the epidermal growth factor receptor (EGFR). Proliferation and cytotoxicity of LL-37 was assessed using Reazurin, a fluorescent indicator of mitochondria activity.

Results. LL-37 (0.1nM-10μM) concentration-dependently inhibited serum-induced wound closure by PC-3 cells. Migration of DU-145 cells into the scraped wound was not inhibited by LL-37 except at 10μM, which was cytotoxic. However, LL-37 (0.1 and 1μM) attenuated the migration of DU-145 cells in the Boyden chamber. The purinoceptor agonist, ATP (10-1000μM), but neither adenosine (1nM-10μM) nor UTP (10nM-100μM), inhibited wound closure by PC-3 cells ($P<0.01$). Furthermore, the relatively non-selective purinoceptor antagonist, suramin (60μM), attenuated the effect of only a high concentration of LL-37 (10μM).

Discussion. Our findings showed a novel anti-migratory effects of LL-37 and ATP. Impairment of PCa cell migration *in vivo* may engender an anti-metastatic effect of LL-37.

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Expression and Localisation of Pannexin-1 Hemichannels in Human Colon in Health and Disease

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Introduction. Pannexin-1 (Panx1) proteins function as channels for ATP release when coupled to purinergic receptors and have roles in many cellular processes such as blood flow regulation and immune function (Locovei et al, 2006; Pelegrin et al, 2006). However, there are limited studies investigating their potential role in the human intestine.

Aims. The aim of the present study was to characterise Panx1 expression and distribution in the human colon and evaluate its potential involvement in inflammatory bowel diseases (IBD).

Methods. Human colon samples were dissected into mucosa and muscle components, and evaluated separately for Panx1 gene expression by real-time PCR and protein expression by Western blotting. Immunohistochemistry was conducted to localise the cellular distribution of Panx1 in intact tissues.

Results. In ulcerative colitis (UC) muscle, Panx1 mRNA expression showed a 3.5-fold reduction compared to control muscle ($P=0.002$, n=9-10), but no change was seen in UC mucosa. In contrast, a significant reduction of Panx1 mRNA was observed in both Crohn's disease (CD) muscle and mucosa (2.7- and 1.8-fold reduction, respectively; $P<0.05$, n=6-11). There was a reduced Panx1 protein expression in CD muscle, but no change in CD mucosa, UC muscle or UC mucosa. In control, Panx1 immunoreactivity was localised to enteric ganglia, blood vessel endothelium, erythrocytes, epithelial cells and goblet cells. IBD samples showed a similar overall pattern of Panx1 staining, but significant Panx1 positive lymphocyte infiltrates were seen at the sites of inflammation. Furthermore, in UC myenteric ganglia, there was a significant reduction in Panx1 staining.

Discussion. The wide-spread presence of Panx1 in the colon suggests that they may play an important role in mediating gut function and changes to Panx1 distribution in disease suggest it may have a role in IBD pathophysiology.

Locovei S, Bao L, Dahl G (2006) Proc Natl Acad Sci USA 103: 7655-7659.
Pelegrin P, Surprenant A (2006) EMBO 25: 5071-5082.

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Neurokinin A potentiates spontaneous and purinergic evoked smooth muscle contraction and bladder afferent activity responses via activation of mouse bladder urothelial and detrusor NK2 receptors.

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Introduction. Neurokinin A (NKA) and its receptor, Tachykinin 2 (NK2), have been postulated to have an essential role in controlling normal bladder function. NK2 elicits contractions of human detrusor strips (Zeng et al, 1995) whilst it initiates a micturition reflex in Rats (Lecci et al, 1998). The nature of tachykinin induced bladder control, however, maintains poorly understood.

Aims. The aim of my study was to investigate the functional role of NKA and the NK2 receptor on mouse bladder function.

Methods. Mouse bladder sensory nerve and muscle activity were recorded using an in-vitro preparation which enables the simultaneous recordings of mouse afferent nerve firing and intravesical pressure. Bladders were continually perfused (30μl/min) with saline or NKA (10nM) and filled to a final pressure of 40mm/Hg every ten minutes. In separate experiments, the purinergic agonist $\alpha\beta$Me-ATP (30μM) was administered to partially distended (12mmHg) bladders in the absence and presence of NKA (3nM). Data were compared using Student's t-tests as appropriate.

Results. NKA elicits dose dependent detrusor contractions and an increase in afferent nerve activity which is completely blocked by the NK2 antagonist GR159897 (100nM) and Nifedipine (1μM). $\alpha\beta$Me-ATP evoked a profound increase in afferent discharge and a concurrent contraction which returned to baseline over 2-3min. In the presence of NKA (3nM), $\alpha\beta$Me-ATP contraction responses are significantly increased (peak contraction above baseline 22.56±3.19mmHg vs 16.34±2.62mmHg in controls, n=5, $p \leq 0.01$) which was blocked by GR159897 (100nM). Spontaneous detrusor contractions and afferent nerve activity are dramatically potentiated by intravesical NKA (10nM).

Discussion. Actions of NKA within the bladder are limited to the urothelium and smooth muscle with no independent effects of NKA on afferent nerve activity. The results suggest there is a functional link between activation of urothelial NK2 receptors and detrusor contraction, potentially through the release of urothelial factors such as ATP.

Lecci A, et al (1998) J Uro160:206-209
Zeng XP, et al (1995) J Uro 153:1688-1692

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Inflammatory biomarkers predict response and toxicity to FOLFOX in colorectal cancer patients.

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Introduction. FOLFOX is the first-line chemotherapy regimen used to treat metastatic colorectal cancer patients. Good response to FOLFOX significantly can extend patient survival by 2.5 years. However, there is large and unpredictable variability in drug response and toxicity between patients.

Aims. To identify novel biomarkers that predict FOLFOX response and toxicity in metastatic colorectal cancer patients.

Methods. Plasma proteomics (iTRAQ and SRM-MS) was used to identify and quantitate proteins in plasma samples collected from patients prior to and after treatment with FOLFOX. Protein expression was compared to neutrophil-lymphocyte NLR status (high > 5, low <5) as a surrogate marker of survival. Statistical analysis was conducted using the ratio of geometric means and ANOVA to identify proteins that are significantly up/down-regulated.

Results. Inflammatory proteins from various key pathways involved in carcinogenesis (survival, acute phase response, complement and coagulation) were significantly upregulated in patients with high NLR (lower survival). Whilst several proteins involved in the insulin-like growth factor system were significantly upregulated after 2 days of chemotherapy in patients with high NLR. These results are summarised in the following table.

Protein	Fold change (high vs low NLR)	P value
Complement factor H-related protein 2	3.15	0.003
Properdin	1.29	0.03
Protein S100-A9	3.56	0.02
Coagulation factor X	1.20	0.006
	Fold change (after vs before chemotherapy)	P value
Insulin-like growth factor binding protein 3	1.18	0.04
Insulin-like growth factor binding protein 4	1.18	0.03
Insulin-like growth factor binding protein 6	1.78	0.009

Discussion. Inflammation is crucial in driving carcinogenesis. This study has identified proteins that may be useful as biomarkers for response and toxicity and has the potential to offer a personalised approach to patient selection for treatment.

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What parents want to know about attention-deficit hyperactivity disorder (ADHD)- A qualitative investigation

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Introduction. Attention-deficit hyperactivity disorder (ADHD) is the most pervasive paediatric psychological condition. Stimulant medicines are used as first-line treatment, but are surrounded by controversy, placing parents in a difficult position to make treatment decisions. Parents must have access to comprehensive, relevant information about ADHD and it's treatments to empower them to make informed decisions on their child's behalf.
Aims. To explore the ADHD-related information sources, knowledge and information needs of parents with children affected by ADHD.
Methods. Focus groups (n=3) were conducted with 16 parents recruited from metropolitan Sydney areas by a market research company. Each focus group lasted 1-1.5 hours and was audio-recorded, transcribed verbatim and thematically content analysed.
Results. Most parents sourced verbal information from health-care professionals (HCPs) focused on ADHD medicines rather than the condition itself. Other sources of information were the internet followed by school staff and, lastly, pharmacists. Generally, parents had limited ADHD-related knowledge prior to their child's diagnosis and perceived ADHD medicines in a negative context. Parents reported improved knowledge after their child's diagnosis, but expressed dissatisfaction with information accessed which was often technical and not tailored to their child's needs. Parents expressed a desire for concise, tailored information about ADHD, its medicines, and changes to their child's medication needs with age. They requested increased availability of support groups and tools to assist them in sourcing information from HCPs during consultations, such as question prompt lists.
Discussion. Parents in this study appeared to have limited information about ADHD or its treatments, with current sources of information and support not meeting their needs. For ADHD medicines to be used safely and effectively, it is essential that future research focuses on providing parents with avenues to access relevant, reliable information and support in order to empower them to make the best decision for their child.

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The influence of disease and other factors on adherence

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Introduction. Poor adherence remains a common barrier to achieving optimal therapeutic outcomes. Common forms of poor adherence include delayed initiation, early discontinuation or non-persistence and various patterns of incorrect dosing including missed doses and drug holidays (Blaschke *et al*, 2012). Medication event monitoring system (MEMS) devices have been used to record exact times that a medication bottle is opened (Cramer *et al*, 1989). It has been reported that poor adherence appeared to share similarities across different disease states (Urquhart and De Klerk, 1998). However little is known about the independent influence of disease and other patient characteristics (factors) on adherence.
Aims. The aim of this project is to evaluate the independent influence of various factors, including disease, dosing regimen and patient characterstics, on adherence.
Methods. A literature search was conducted to retrieve adherence studies using MEMS devices. Studies were categorised into different therapeutic areas. Only the two most commonly studied therapeutic areas were selected. Data were extracted from each study and analysed using a model based meta-analysis technique. This technique provided an estimate of the combined and independent influence of each factor on adherence.
Results. The most commonly recorded adherence criterion was percentage of prescribed doses taken per day. The therapeutic areas chosen were HIV (23 studies) and hypertension (12 studies). The statistically significant factors were disease, age and dosing regimen. The indepentdent influences of each factor on adherence were: an increase in adherence of approximately 8% per 10 year increase of age, a 25% reduction from once to thrice daily dosing, and that HIV patients were 6% more adherent than patients with hypertension.
Discussion. This analysis provides the first evidence for the independent effects of various factors. It was found that the influence of disease while statistically significant was not clinically significant, however, and interestingly adherence improved with age.

Blaschke TF *et al*. (2012). Annu Rev Pharmacol Toxicol 52: 275-301.
Cramer JA *et al*. (1989). JAMA 261(22): 3273-3277.
Urquhart J and De Klerk E (1998). Stat Med 17(3): 251-267.

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Strategies used to support patients' adherence to medication: a national survey of community pharmacists

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Introduction: Adherence to medication is a key to achieving better clinical outcomes. Improving medication adherence requires a combination of strategies, ranging from educating patients to fostering behaviour change. Pharmacists are well placed to deliver adherence support services to patients, and have been shown to have a positive impact on patients' adherence.
Aim: To investigate the extent to which community pharmacists are currently providing strategies to identify and promote patients' adherence to medication in their practices.
Method: A sample of 2020 pharmacies was randomly selected from the lists of Australian community pharmacies, and mailed a survey on adherence, strategies used to identify non-adherence and promote adherence. Three follow-up reminders were sent to non-respondents after 2, 4 and 8 weeks to increase the response rate.
Result: Thirty two percent responded (n=627). Less than half (45%) estimated that 26-50% of their patients were non-adherent to medication. About 45% reported providing strategies to identify non-adherent patients. The most common strategy used to identify non-adherent patients (59%) was using dispensing records to determine how frequently patients collected repeat prescriptions. The majority of respondents reported that providing dose administration aid (97%), recommending a medication management review (74%) and simplifying patient's medication regimen (69%), were the most common strategies used to promote adherence. The median number of times that any strategy to promote patients' adherence was used in the previous 7 days, was 4 (IQR 2-10).
Discussion: Community pharmacists were found to address patient non-adherence to medications by using a variety of strategies to identify and promote adherence. However, it appeared that this service was provided infrequently. In conclusion community pharmacists have the opportunity to expand their scope of practice in supporting adherence to medication to improve patient benefits from pharmacotherapy.

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Beyond expectations? Do pharmacists perform clinical interventions when carrying out adherence support medication reviews?

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Introduction. In New Zealand, pharmacists are funded to provide adherence support via a service called Medication Use Review and Adherence Support (MUR). This is not intended to be a clinical review. However, improving adherence ignores a key role that pharmacists play in ensuring optimal prescribing and use of medications, patient safety, and improving patient health outcomes.
Aims. The aim of this study was to determine the types of interventions provided by pharmacists while conducting MURs and to identify those interventions that may be considered to be beyond adherence support.
Methods. A single district health board that funds MUR services was identified. Those pharmacies that provided MURs during the funded period (from 2007 – current) were invited to participate. All consultations that had been documented were scanned on site and the data extracted was categorised according to the Pharmaceutical Care Network Europe (PCNE) Classification Scheme for Drug Related Problems v 6.2.
Results. Consultation records for 353 individual patients were obtained. Of these patients, 56.4% were female and the median age was 73 years. A total of 886 drug related problems were identified and resulted in a total of 844 interventions. The most common intervention was "patient counselling" (20%) followed by "compliance packaging provided" (16%), however the third most common intervention was directed at the prescriber level "recommendation to change medication" (11%).
Discussion. In this study pharmacists were found to be performing beyond the expected and funded level of the MUR service, by providing clinical interventions. While the current specifications surrounding MUR services are patient focused, they are based on the assumption of "optimal" prescribing. The current service specification does not support pharmacists in their role of identifying medication appropriateness, effectiveness and promoting patient safety. (282)

Pharmaceutical Care Network Europe Foundation (2010) Classification for Drug Related Problems. http://www.pcne.org/sig/drp/documents/PCNE%20classification%20V6-2.pdf

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Asthma management in intellectual disability (ID) – identifying opportunities for the pharmacist

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Introduction. The inhalational route is the foundation of asthma treatment, however inhaler technique is complex, and optimal technique rarely achieved.[1] Australian data indicate 15% of persons with ID have asthma. [2] Due to physical and/or cognitive deficits, persons with ID may present a management challenge regarding inhaler use, however no evidence currently exists to inform health professionals in supporting this.

Aims. To describe the prevalence of asthma, asthma management practices and inhalational device use, in a community sample of Australian adults with ID.

Methods. Our retrospective audit combined electronic health records and hard copy records compiled by clinic doctors, to collect information on demographics, living situation, main health conditions and co-morbidities, and access to healthcare. Qualitative data were also collected.

Results. Among the 2254 adults who presented to the clinic, 134 (6%) had doctor diagnosed asthma. Of the 86% who were prescribed asthma medication, all was via the inhalational route. Less than 20% of clients were reported to have been referred to a respiratory specialist, and even less reported to have spirometry performed (3%), or asthma management Plans in place (9%). Nebuliser use was not necessarily consistent with current recommendations. Qualitative analysis revealed inhaler technique and adherence issues.

Discussion. People with ID and asthma are prescribed inhalers. In our study, key indicators of asthma management showed omission of recommended management strategies. In this complex area, there are opportunities for improved respiratory health outcomes for persons with ID, and for pharmacists, as medication experts, to play a more active role.

Lavorini F, Magnan A, Dubus JC, et al.(2008) Effect of incorrect use of dry powder inhalers on management of patient with asthma and COPD. Respir Med 4: 593-604.

AIHW (Australian Institute of Health and Welfare) (2008). Disability in Australia: intellectual disability. Bulletin no. 67. Cat. no. AUS 110. Canberra: AIHW.

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Effects of three different forms of inhaler technique education for health care professionals on patient asthma outcomes.

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Introduction. A high proportion of inhaler device users are not able to do so correctly. A co-ordinated/collaborative approach to asthma management and inhaler device use, could help to address this problem. However, enabling a co-ordinated/collaborative approach is difficult to achieve and estblishing evidence of its effect on patient outcomes is a challenge. The Collaborative Asthma Management in the Community (CAMCOM) study evaluated the impact of 3 models of interprofessional education on attitudes towards collaboration and patient health outcomes. This abstract focuses is on patient outcomes.

Aims. To evaluate the effect of three CAMCOM interventions on clinical asthma outcomes.

Methods. HCPs from three general practice networks were recruited into one of three groups (1, 2, and 3) receiving one of three models of inter professional education (joint setting group, online group and socio-cultural theory-based group, respectively). HCPs from a fourth general practice network, received no intervention and acted as a control. Following completion of an educational module, HCPs recruited people with asthma using inhaler devices and provided them with inhaler device education over a six month period (5 visits). Inhaler technique, asthma control, asthma quality of life, perceived control of asthma were evaluated over this time period.

Results. A total of 37 pharmacists, 13 general practitioners and 2 practice nurses recruited 312 patients with asthma. Linear mixed modelling with an autoregressive covariance indicated that there were significant differences between Groups 1, 2, 3 and control over time in terms of inhaler technique, asthma control, asthma quality of life and perceived control of asthma ($p<0.0001$ for all four outcomes).

Discussion. While the clinical content of continuing profession education sessions can be fixed, the impact of the interaction of health care professionals and the educational material can have a significant impact on the clinical outcomes for people with asthma.

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Community pharmacy as a health hub: meeting the needs of people with chronic conditions

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Introduction. Pharmacies are frequently visited by people with chronic conditions. There is limited information from the perspective of Australian consumer orientated and professional organisations of consumer needs within the context of community pharmacy.
Aim. To explore the viewpoints of health professional organisations, consumer groups and advocates with respect to the role of community pharmacy in supporting consumers/carers with long term condition(s).
Methods. Semi-structured interviews were conducted with 21 consumer and healthcare stakeholders between January-March 2012; representation was purposively sought from health priority areas defined by the Australian Government. Interviews were conducted face-to-face across the greater Brisbane area and by telephone for others. Interviews were analysed via thematic analysis.
Results. Stakeholders recognised a need for community pharmacy to become a "one-stop" healthcare destination to enable consumers to manage their medications and navigate the health system. Pharmacists were identified as having a "neutral" position and could further develop their health advocacy role. As consumers may not be aware of pharmacists breadth of expertise an improvement and extension of their current role was emphasised.
Discussion. There was a strong perception that community pharmacy practice should shift towards a more patient-centred approach as a health hub destination. This study adds support for pharmacists to collaborate further with consumer support organisations and other health providers to extend their role in chronic illness management.

The Australian Government DOHA (2006). National Chronic Disease Strategy.
Grant support: Australian Government Department of Health and Ageing, managed by The Pharmacy Guild of Australia.

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"Knowing how" is not enough: why people with asthma do not maintain correct inhaler technique over time.

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Introduction. Poor inhaler technique maintenance is a persistent problem contributing to suboptimal asthma control. Patient motivation can influence technique maintenance. Motivation is a complex and dynamic phenomenon yet important to understand due to its potential to influence health behaviours. There are no published accounts exploring motivation from the patient's perspective in relation to inhaler technique maintenance.
Aim. To explore factors that influence motivation for inhaler technique maintenance in people with asthma.
Methods. In-depth semi-structured interviews were conducted with a purposive sample of community dwelling people with asthma from Sydney who had participated in a preceding study involving repeated inhaler technique measures. The interview guide was based on theories of motivation and self-regulation. Interviews were recorded, transcribed verbatim and analysed for themes. Results were validated via independent cross-coding followed by discussion between three researchers. Nvivo 9 software aided data storage and organisation.
Results. 20 interviews were conducted (n=9 "Maintainers" and n=11 "Non-maintainers") ranging from 25-119 minutes. Multiple factors appeared to influence motivation for inhaler technique maintenance, however three core factors emerged that could sequentially differentiate those participants who maintained correct technique compared to those who did not. They were – 1. perceived threat of asthma, 2. perceived best method of self-management and 3. perceived confidence with self-management. Further, the potential for health care professionals to modify patient perceptions seemed to be strongest at points 2. and 3.
Discussion. In exploring patient motivation and inhaler technique maintenance, three core themes have been uncovered that can characterise those patients who are at greater risk of poor technique maintenance. Further investigation may lead to the development and validation of a potentially useful clinical tool to guide health care professionals in detecting these at risk patients.

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Is the process for approval of high cost drugs for off-formulary use leading to clinically appropriate outcomes?

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Introduction. Clinical outcome data for off-formulary high cost drug (HCD) use is scant, especially within Australia. Outcome data is important, as use of these drugs is expensive and clinical benefit or detriment is unknown at time of use. A retrospective review of approvals for off-formulary uses of HCD at Royal Brisbane and Women's Hospital from 2006-2011 was thus performed.
Aims. To analyse clinical outcomes from use of off-formulary drugs for pulmonary hypertension and compare with "expected" outcomes from the literature.
Methods. Data on patients treated for pulmonary hypertension, including brain natriuretic peptide, echocardiography, cardiac MRI, cardiac catheterisation, pulmonary function tests, six minute walk tests, quality of life, WHO functional class and hospital admissions, were collated from HCD approval records, hospital HCD committee meeting minutes, pharmacy dispensing records, patient medical records and pathology and imaging databases. Descriptive statistics on use, as well as clinical outcomes stratified by age, population group and underlying disease compared to "expected outcome" based on population literature for a matched group were undertaken.
Results. Forty-five patients were granted approval for sildenafil, four for iloprost and one each for epoprostenol, prostacycline and tadalafil over six years. We found that there was a poor correlation between clinical outcomes and outcomes expected by the clinical team. Further, there was a large amount of variation in outcomes within a population group.
Discussion. A better methodology for approving HCD is urgently needed. Specifically, the noted poor quality of data provided by the clinical team makes developing a methodology for future studies and regulation important. This data would assist decision-making around HCD use. It would also provide much needed evidence regarding the clinical outcomes and safety of use of newer pharmaceuticals.

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The performance of the Cockcroft-Gault, MDRD and CKD-EPI equations in predicting gentamicin clearance

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Introduction. Estimating glomerular filtration rate (GFR) is useful for adjusting doses of renally cleared drugs, such as gentamicin.
Aims. To identify the best creatinine-based GFR equation for predicting gentamicin clearance in the context of an isotope dilution mass spectrometry (IDMS)-aligned creatinine assay.
Methods. The bias and imprecision of the Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations for predicting gentamicin clearances, were assessed retrospectively in 255 patients treated with gentamicin during 2011-2012. The local creatinine assay was IDMS-aligned during this period. Gentamicin clearance was calculated using plasma concentrations following dosing with a one-compartment model.
Results. The CG equation had the highest percentage of estimates within 30% of the calculated gentamicin clearance (67%, $P < 0.001$) and lowest root mean square error (39 mL/min) compared with the other two equations, for the entire cohort. Setting a minimum serum creatinine of 60 μmol/L did not improve the performances of the equations. Correction for individual body surface area improved the performances of the MDRD and CKD-EPI equations in the subgroup with body mass indices (BMI) < 18.5 or ≥ 30 kg/m2, but not those with BMI 18.5-30 kg/m2. The GFR equations performed better in patients with gentamicin clearance < 90 mL/min than those with gentamicin clearance ≥ 90 mL/min; in this latter subgroup, the GFR equations increasingly underestimated gentamicin clearance as gentamicin clearance increased.
Discussion. The Cockcroft-Gault equation provided the best estimate of gentamicin clearance. The CKD-EPI and MDRD equations should be corrected for individual body surface area at the extremes of body size, if used for guiding gentamicin therapy. The GFR equations performed poorly in patients with gentamicin clearance ≥ 90 mL/min.

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Describing the use of antibiotics in acute exacerbations of chronic obstructive pulmonary disease (COPD)

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Introduction. COPD is a major cause of mortality and significant economic burden within Australia. Clear evidence based guidelines have been developed[1], however, Australian hospitals have a history of utilising antibiotic regimes that differ from guideline directed therapy[2].
Aims. To quantify the extent of non-guideline based antibiotic prescribing for patients with acute exacerbations of COPD and identify barriers preventing guideline based antibiotic prescribing.
Methods. A retrospective review of all patients admitted to the Princess Alexandra Hospital (PAH) with a diagnosis of acute exacerbation of COPD over a 6 month period. Patients were excluded if they received non-invasive ventilation, had evidence of pneumonia or sepsis, had an altered level of consciousness or were not for active treatment. Patients were considered in two groups with respect to the antibiotic therapy prescribed: 1) according to guidelines[1] or 2) not according to guidelines.
Results. Of 84 eligible admissions, 6.8% were prescribed antibiotics according to guidelines in the emergency department, while this was the case for 18.3% while inpatients. Patient characteristics did not show a statistically significant difference. The only difference in presenting feature was a significantly higher white cell count in the group prescribed according to guidelines. There was no difference in length of stay or readmission rates at 3 months. There were no complications attributed to prescribed antibiotics and no antibiotic failures.
Discussion. There are clear guidelines that are available in Australia for treating an acute exacerbation of COPD. However, in this tertiary hospital setting, these guidelines are rarely followed. From the data collected, there was no indication of clinical superiority of non-guideline based therapy in terms of duration of hospital admission or readmission rates. Given that there were no apparent benefits of non-guideline based antibiotics, it is difficult to justify.

McKenzie DK, Abramson M, Crockett AJ, Glasgow N, Jenkins S, McDonald C et al. (2011) The COPD-X Plan: Australian and New Zealand Guidelines for the management of Chronic Obstructive Pulmonary Disease. Queensland: The Australian Lung Foundation.
Radford J, Cardiff L, Pillans P, Fielding D, Looke D. (1999) Drug usage evaluation of antimicrobial therapy for community-acquired pneumonia. Australian Journal of Hospital Pharmacy; 29:317–320.

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Is Cytochrome P450 2C19 genotyping cost-effective for guiding clopidogrel treatment in Australia?

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Introduction. Cytochrome P450 (CYP) 2C19 genotype has been association with inter-individual variability in the therapeutic effect of the anti-platelet agent clopidogrel. The association is thought to be greatest for individuals undergoing percutaneous coronary intervention (PCI).
Aims. To assess the effectiveness and cost-effectiveness of genotyping CYP2C19 to guide selection of clopidogrel or ticagrelor for individuals with acute coronary syndrome (ACS) and planned PCI.
Methods. A decision-analytic Markov model was designed to enable simulation of the costs, events, and changes in quality-of-life over the lifetime of patients following ACS. Clinical events were derived from the genetic substudy (n=10,285) of the PLATO trial comparing ticagrelor and clopidogrel. Three treatment strategies were assessed: (1) use of clopidogrel for all individuals ('clopidogrel strategy'), (2) use of clopidogrel for individuals without a CYP2C19 loss-of-function (LoF) allele, and ticagrelor for individuals carrying a CYP2C19 LoF allele ('genotype strategy'), and (3) use of ticagrelor for all individuals ('ticagrelor strategy').
Results. The genotype strategy is more effective than the clopidogrel strategy, and has an incremental cost-effectiveness ratio (ICER) below $20,000 per quality-adjusted life-year (QALY). However, the ticagrelor strategy was found to have the greatest effectiveness and an ICER of less than $30,000 compared to the genotyping strategy. If CYP2C19 gain-of-function (GoF) alleles are also tested, and assuming the effect of a GoF allele is of similar magnitude to that of a LoF allele, then genotyping for CYP2C19 may be the most cost-effective option.
Discussion. Although the genotyping strategy was cost-effective, the ticagrelor strategy is also cost-effective and is the most effective strategy overall. In addition, the genotyping strategy is associated with increased complexity and a point-of-care testing will be required for individuals with ACS. Further research is required to clarify the relative effectiveness of ticagrelor and clopidogrel for individuals carrying a CYP2C19 GoF allele.

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Liver Transplant Donor and Recipient *CYP3A5* and *ABCB1* Genetics and Tacrolimus Pharmacokinetics

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Introduction. Successful liver transplantation requires immunosuppressant drugs. Tacrolimus, the most widely used, is metabolised by CYP3A enzymes and transported by P-glycoprotein (coded by *ABCB1*).
Aims. To investigate the impact of genetic variability of recipient and donor *CYP3A5* and *ABCB1* (P-glycoprotein) on steady state tacrolimus pharmacokinetics and clinical outcomes in liver transplant patients.
Methods. Thirty-two liver transplant recipients were recruited (52 yr; 79% male), and matched donor blood samples were obtained from the Australian Red Cross Blood Service. Both recipients and donors were genotyped for *CYP3A5*3* (carriers are non-expressors) and *ABCB1* haplotype. Dose-corrected steady state tacrolimus concentrations and the incidences of nephrotoxicity and biopsy proven rejection were compared between genotype groups. Mann-Whitney or Kruskal-Wallis tests, and chi square tests were used for numerical and categorical data, respectively.
Results. Steady state tacrolimus pharmacokinetics were dependent on recipient CYP3A5 genotype: expressors had significantly lower dose-corrected concentrations than non-expressors over all time points, medians 1.15μg/L and 1.65μg/L, respectively (P=0.042); but not on donor CYP3A5 expression. In addition, CYP3A5 non-expressor recipients who also had a non-expressor donor liver had the highest concentrations (2.7μg/L) while the expressor recipient with the expressor donor liver had the lowest concentration (0.94μg/L) at final follow up (P = 0.023). In contrast, a significant impact of recipient or donor *ABCB1* haplotypes on steady-state tacrolimus concentrations was not seen. Differences in CYP3A5 expression or *ABCB1* haplotype were not associated with nephrotoxicity or biopsy proven rejection in this small study.
Discussion. Recipient *CYP3A5* genotype, present in the gastrointestinal tract, had a significant effect on tacrolimus pharmacokinetics. Donor *CYP3A5* genotype, present in the liver, had a lesser effect. This suggests that gastrointestinal wall metabolism is a major determinant of tacrolimus pharmacokinetics. Larger studies are needed to assess the impact of genetic variability in drug transporters on liver transplantation.

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Pro-migratory actions of prostacyclin in breast cancer cells that over-express cyclooxygenase-2.

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Introduction. Metastasis is the major life-threatening characteristic of many tumours. Cyclooxygenase-2 (COX-2) is over-expressed in a range of human tumours and acts in concert with downstream prostaglandin (PG) synthases to generate a series of PG mediators (Wang D & DuBois RN, 2010). While PGE_2 has been strongly implicated in metastatic disease the potential roles of alternate PGs are unclear.
Aim. To elucidate the actions of individual PGs on the invasion potential of breast cancer cells that express COX-2.
Methods. The *in vitro* invasion capacity of MDA-MB-468 breast cancer cells that were engineered to stably express COX-2 was evaluated in 3D-matrigel droplets. All data are n = 3.
Results. Inhibition of PGE_2 synthase by CAY10526 (20 μmol/L) decreased cell invasion relative to arachidonic acid alone (20 μmol/L, 79±9 versus 110±9 migrated cells, $P<0.05$). The prostacyclin synthase inhibitor U-51605 (3 μmol/L) also decreased invasion relative to arachidonic acid treated cells (76±10 versus 110±9, $P<0.05$). Consistent with the apparent role for prostacyclin, the IP-receptor antagonist CAY10441 decreased invasion (10 μmol/L, 51±9 versus 110±9, $P<0.05$), although somewhat surprisingly EP-receptor antagonists were ineffective. Inhibitors of PG synthases active in the formation of other prostanoids, and antagonists of alternate prostanoid receptors did not influence tumour cell invasion out of matrigel droplets.
Discussion. These findings implicate prostacyclin in the metastatic activity of MDA-MB-468 breast cancer cells that over-express COX-2. PGE_2 also contributed to the invasive properties of the MDA-MB-468 cells but this may be EP-receptor-independent.

Wang D & DuBois RN (2010) Nat. Rev. 10:181-193

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A new pharmacokinetic abnormality among patients with 5-fluorouracil toxicity

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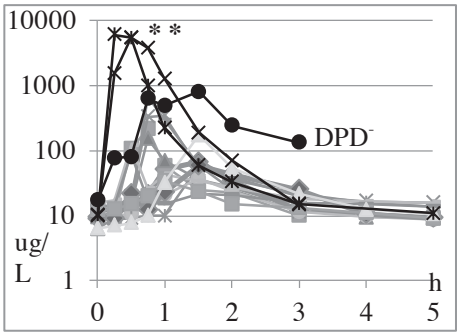
Introduction. Severe 5-fluorouracil (5FU) toxicity during cancer chemotherapy has been attributed to partial deficiency of catabolic enzyme dihydropyrimidine dehydrogenase (DPD) in about 40% of cases. The remaining 60% of toxicity is unexplained.
Aim. To develop a predictive test for 5FU toxicity.
Methods. 250 mg oral loading was trialled using thymine (5-methyluracil) as a chemical surrogate for 5FU. The loading test was combined with a sensitive and selective HPLC-MS/MS based assay for thymine and successive metabolites dihydrothymine and ureidoisobutyrate, in human plasma and urine. Thymine PK was examined first in 12 healthy male adult subjects, then retrospectively in 6 patients who had suffered severe (grade 3-4) 5FU toxicity.
Results. Fig.1 shows plasma thymine following the oral dose. Thymine was rapidly absorbed and cleared in normal subjects (grey), mean Cmax= 170 (range 34-679) ug/L; dihydrothymine Cmax was about 3-fold higher than thymine, mean= 543 (223-908) ug/L. Among the 5FU toxicity patients, 3 were clearly discernible as DPD-deficient based on delayed thymine clearance, confirmed genetically (e.g. Fig.1, filled circles)). But 3 other patients had abnormally rapid thymine uptake and up to 30-fold higher Cmax= 4190 (3000-6190) ug/L; thymine half-life was normal (e.g. Fig.1,**).



Dihydrothymine Cmax= 1277 (1070-1570) ug/L, approx 2-fold normal.
Discussion. The unexpectedly low Cmax and high CL/F= 58 L/h/kg for thymine, which exceeds human liver blood flow, was suggestive of poor systemic oral bioavailability (F), perhaps due to high first-pass clearance and involvement of a capacity-limited pyrimidine transporter. Dihydrothymine clearly showed formation-rate limited kinetics, with an apparent lower volume of distribution. The thymine load test easily distinguished DPD deficiency. We propose the unusual thymine PK phenotype models abnormal handling of 5FU that may explain the drug's toxicity in DPD-normal patients. The genetic basis for this PK behaviour remains unknown but may be a potential probe for 5FU toxicity.

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Anti-proliferative actions of sorafenib and its major metabolites in MDA-MB-231 breast cancer cells

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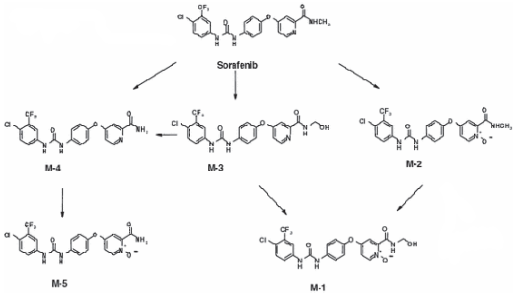
Introduction. The multi-kinase inhibitor sorafenib is approved for the clinical treatment of renal and hepatic carcinomas and is currently undergoing evaluation for the treatment of breast cancer in combination with other agents. CYP3A4 converts sorafenib to multiple metabolites (Ghassabian et al., 2012) that have been detected in human liver fractions and in patient plasma. The *N*-oxide (M2) is reported to be an active metabolite, but information on the other metabolites is not currently available.



Aims. This study evaluated the anti-proliferative actions of sorafenib and metabolites (M1-M5) in the MDA-MB-231 breast cancer cell line.
Methods. Sorafenib and its metabolites were synthesised (Ghassabian et al., 2012), and their anti-proliferative actions were evaluated by ATP assay (cell viability), cell cycle kinetics by flow cytometry and western blotting for proteins involved in cell cycle regulation, apoptosis and the MEK/ERK signalling cascade.
Results. Sorafenib and its metabolites (10 μM) decreased the viability of MDA-MB-231 cells and arrested the cell cycle in G0/G1 phase after 24-72 h of treatment. M1-M5 (10 μM) also selectively down-regulated cyclin D1 and E expression, but not cyclin A and B1. Sorafenib and M1-M5 (1 and 10 μM) all strongly down-regulated the expression of Mcl-1 and inhibited the activation of MEK and ERK.
Discussion. Sorafenib undergoes oxidation to several active metabolites (M1-M5) with the potential to impair the growth of breast cancer cells. By targeting the anti-apoptotic Mcl-1 and growth stimulatory MEK/ERK pathways these metabolites may contribute to the actions of sorafenib in breast cancer.

Ghassabian et al. (2012) Biochem Pharmacol 84, 215-223

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Differential effects of mango peel sub-fractions on lipid accumulation in 3T3-L1 adipocyte cells

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Introduction. Plant phytochemicals represent a class of bioactive molecules that may be beneficial for human health. Recent research in mangoes suggests that extracts from different cultivars can inhibit adipogenesis in the 3T3-L1 adipocyte cell line. Mango peel is reported to have greater bioactive effects on adipogenesis than does mango flesh.
Aim. In this study, peel extracts from cultivars Irwin (IW), Nam Doc Mai (NDM) and Kensington Pride (KP) were separated into four fractions and assessed for their effects on lipid accumulation in differentiating 3T3-L1 cells.
Methods. Mango peel methanol extracts were separated into four fractions using preparative HPLC. Fraction 1 contained the most hydrophilic components whilst subsequent fractions contained increasingly more hydrophobic components. 3T3-L1 pre-adipocytes were induced to differentiate with or without mango peel extract fractions (at 1, 10, 30, 100 μg/mL) for seven days. High content imaging was used to assess lipid accumulation at day seven. Mass spectrometry was used to identify unique compounds in mango peel fractions.
Results. For the three mango cultivars, the more hydrophilic peel fractions 1-3 inhibited lipid accumulation with greater potency than the more hydrophobic peel fraction 4. Fractions 1-3 displayed biphasic effects on lipid accumulation whereas fraction 4 displayed variable bioactive effects on lipid accumulation. From all cultivars, the more lipophilic fraction 4 enhanced lipid accumulation greater than fractions 1-3. Using mass spectrometry, five long chain free fatty acids were detected in fraction 4 that were not present in peel fractions 1-3. IW fraction 4 contained the highest level of free fatty acids compared to other mango cultivars, an observation consistent with its ability to promote rather than inhibit lipid accumulation at high concentrations.
Discussion. Fatty acids present within mango peel extracts may be responsible for the bioactive differences observed between different mango cultivars on lipid accumulation in 3T3-L1 cells.

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The preparation and characterisation of polypyrrole particles for tuneable drug delivery

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Introduction: Polypyrrole (PPy) is an intrinsically conducting polymer. This electroactive polymer responds to electrical stimulus and can be used in tuneable drug delivery systems. PPy particles can be prepared from soft templates including micelles and microemulsions. The model compound used in this study is risperidone, a poorly water soluble, antipsychotic drug.
Aims: To prepare and characterise the physiochemical properties of PPy particles loaded with risperidone.
Methods: Micelles and microemulsions were used as soft templates to synthesise PPy particles. Risperidone was dissolved in the template and incorporated into PPy particles during the interfacial polymerisation of pyrrole. Drug loading and entrapment efficiencies were determined by HPLC, morphology was assessed by SEM and electroactivity explored through cyclic voltammetry. Electrically triggered *in-vitro* release studies were also carried out.
Results: PPy particles loaded with risperidone were produced using soft template methods. Micellar methods yielded an entrapment efficiency of 32.5 ± 9.8% with a drug loading value of 3.5 ± 0.6%, w/w. The microemulsion system yielded an entrapment efficiency of 52.2 ± 7.5% with a drug loading of 3.2 ± 0.5%, w/w. Cyclic voltammetry demonstrated the electroactivity of the PPy particles and *in-vitro* release studies showed electrically tuneable risperidone release.
Discussion: The entrapment efficiencies of the micellar systems remained relatively constant when different risperidone concentrations were used during preparation, indicating higher drug loading could be achieved when higher drug concentrations were used. In contrast, for the particles formed from microemulsions, higher drug concentrations during preparation did not increase drug loading as the entrapment efficiency reduced proportionately. The demonstrated electroactivity of the particles makes them promising candidates for tuneable drug delivery systems.

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Characterisation of emulsions as potential saliva substitutes in xerostomia

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Introduction: Xerostomia is a condition involving reduction or absence of saliva, causing difficulties swallowing, speaking and eating, and an increased incidence of dental caries and periodontal disease (Jensen et al, 2010). Current treatments fail to provide adequate long-term relief, particularly in patients with minimal salivary gland function.
Aim: To investigate the physicochemical properties of emulsions for use as saliva substitutes.
Methods: Ricebran oil (RBO), lecithin:propylene glycol (1:1w/w, SM) and water were used to prepare compositions in a pseudoternary phase diagram. These were assessed by polarising light microscopy (MoticBA300, Hong Kong), flow and oscillatory rheology (TA Instruments, England). The rheometer was fitted with a 40mm parallel-plate and solvent trap. Shear rate was 0–50s^{-1} for flow rheology, oscillation was 0.1–10Hz for oscillatory rheology and temperature was 37ºC. Storage (G') and loss (G") moduli were used to calculate tanδ (G"/G'). Selected compositions were analysed for droplet changes with frequency using a small-angle light scattering (SALS) accessory.
Results: A small microemulsion and large liquid crystalline region were observed. Compositions with greater than 30% SM exhibited pseudoplastic flow, with apparent viscosity increasing with SM concentration. At 20-30% SM, tanδ was greater than one at frequencies below 5Hz and less than one at higher frequencies. A peak in tanδ was observed in formulations containing over 30% SM. SALS demonstrated the frequency of this peak (range: 2.4–8.7Hz) coincided with an increase in light intensity, suggesting a reversible increase in droplet size.
Discussion: Properties depended upon composition, shear rate and oscillatory frequency, which are important considerations in the oral environment. Frequency-dependent structure may be useful if liquid-like properties (tanδ>1) dominating at low frequencies assist lubrication of the oral cavity at rest, whereas solid-like properties (tanδ<1) assist in retention during high-frequency tasks such as chewing and swallowing.

Jensen S et al (2010) Support Care Cancer 18:1039-1060

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Conformational stability of various proteins in solid lipid matrices prepared by melting and cooling

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Introduction. Protein drugs are an important class of therapeutics (Antonio et al, 2007). Lipid can be used to prepare controlled release dosage forms without organic solvents (Walduck et al, 1998). However, protein may degrade by heat used to melt the lipid (Riethmier et al, 2001).
Aim. To understand conformational stability of proteins in lipid matrices prepared by melting and cooling.
Methods. Bovine serum albumin (BSA), lysozyme (LZ), horse radish peroxidase (HRP) and catalase (CT) were used. Each was heated alone at 80^0 and 140^0C and with Precirol AT05 (glycerol palmitostearate, melting point 58^0C) at 80^0C up to 96hrs. Aliquots were cooled to room temperature and analysed using ATR. Proteins were dissolved in water and enzyme activity and SEC determined.
Results. Heating solid protein to 80ºC did not alter the physical appearance whereas heating to 140ºC resulted in a colour change and reduced aqueous solubility. Enzyme activity of LZ, HRP and CT exposed to 80ºC was 88%, 70% and 33% of control respectively, suggesting a rank order of thermal stability. When heated to 140^0C, protein activity of HRP and CT was lost while LZ was reduced to 35%. SEC showed 63% BSA and 82% LZ remained following heating of protein solid at 80ºC. ATR spectra of the amide I band of heated protein (80ºC and 140ºC) were not significantly different to the control non heat-exposed protein. In the presence of lipid CT showed the greatest change in secondary structure.
Discussion. ATR spectroscopy could not predict loss of activity of enzymes exposed to heat in the solid state. In lipid, change in protein secondary structure was observed for CT which represented the most heat labile model protein.

Antonio et al (2007) Adv. Drug Del. Rev. 59:478-490.
Walduck et al (1998) J Control. Rel. 51:269-280.
Riethmier et al (2001) Int. J. Pharm. 218:133-143.

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PEGylated surfactants inhibit the digestion of co-formulated triglycerides in a PEG chain length dependent manner.

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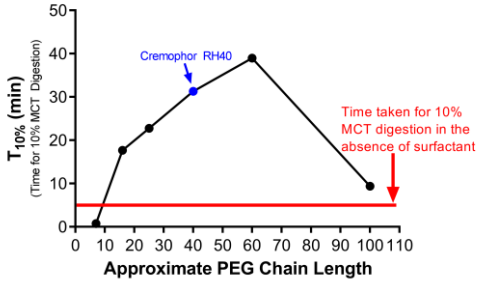
Introduction. Lipid-based formulations (LBFs) can improve the oral absorption of poorly water-soluble drugs by delivering the drug to the gastro-intestinal tract in a pre-dissolved, molecularly dispersed form. However, on entering the intestine, LBFs are rapidly digested by intestinal lipases, causing a digestion rate dependent loss in solubilisation capacity, in turn creating the risk of drug precipitation and reduced bioavailability. Surfactants containing polyethylene glycol (PEG) hydrophilic headgroups have previously been suggested to inhibit the digestion of medium-chain triglycerides (MCT).



Aims. To evaluate the effect of six classes of PEGylated surfactant on the in vitro digestion of medium-chain triglycerides

Methods. An in-vitro digestion model was used to examine the influence of PEGylated surfactants on the digestion of MCT. The time required for 10% of the MCT to be digested ($T_{10\%}$) was estimated by linear interpolation and plotted as a function of the chain length of the PEG surfactant component.

Results. The presence of PEGylated surfactants altered the digestion of co-formulated MCT. Surfactants containing short and long PEG chains were poor inhibitors of digestion. However for intermediate PEG-chain length surfactants, digestion inhibition, presumably by formation of a PEG mantle around the lipidic micellar core, was effective in increasing the time for 10% of MCT to digest.

Discussion. PEGylated surfactants are effective in modulating MCT digestion rate. The potential to delay digestion mediated changes in solubilisation capacity may provide an improved platform for oral lipid based drug delivery.

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The lymphatic system is critical in maintaining the prolonged circulation of monoclonal antibodies and in promoting absorption from the subcutaneous injection site

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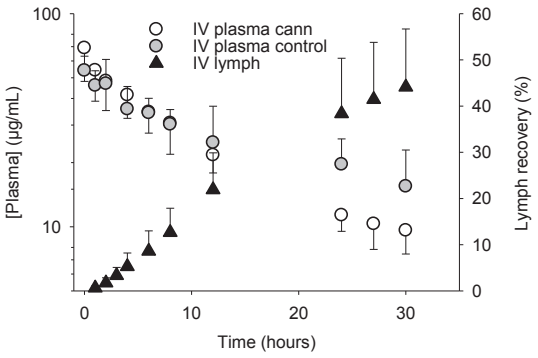
Introduction. Therapeutic monoclonal antibodies are currently delivered IV, although SC administration is a therapeutic goal. Thus far, however, little is known about the mechanisms by which antibodies are absorbed from subcutaneous injection sites, and the role of the lymphatic system in their absorption and IV PK.

Aims. To characterise the role of the lymphatic system in the absorption of a model monoclonal antibody (trastuzumab) from SC injection sites and in its IV PK.

Methods. The PK and lymphatic uptake of trastuzumab were examined in rats after IV and SC dosing via ELISA. A population PK model was developed and fitted to the data.



Results. The bioavailability of trastuzumab in rats was approximately 80% after SC administration and the antibody displayed a prolonged circulatory half life of 2 weeks following IV and SC administration in non-lymph cannulated, control rats. Plasma concentrations in lymph cannulated animals, however, were significantly lower than in control animals and approximately 44% and 27% of the administered dose was recovered in thoracic lymph over 30 h after IV and SC administration respectively (see figure). A population PK model was developed based on the data to predict the long term PK behaviour of trastuzumab in rats.

Discussion. This study highlights for the first time the significant role of the lymphatic system in maintaining the long circulatory behaviour of trastuzumab. The results of this study also show that the lymphatic system is a major pathway by which monoclonal antibodies are absorbed from SC injection sites.

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Stabilisation of amorphous indomethacin in aqueous suspensions: Effect of polymer addition method

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Introduction. Amorphous suspensions are potential formulation options for preclinical toxicology studies involving poorly water soluble drugs. However, prevention of recrystallisation during the course of study remains challenging. Polymers, through solid dispersion (SD) with drug or predissolved in solution, can be used to stabilise the amorphous form. To rationally develop approaches to stabilise the amorphous form with polymers, impact of polymer addition method should first be understood.
Aim. To investigate the effect of different polymer addition methods on crystallisation behaviour and the solution concentration-time profile of amorphous indomethacin (IND).
Methods. Amorphous IND and SD of IND with Soluplus® at 1:1 drug:Soluplus® weight ratio was prepared by melt quenching. Amorphous suspensions were prepared by either: i) adding the SD into water (20mg/mL), or ii) adding pure amorphous IND into water containing predissolved Soluplus® at total drug and polymer concentrations equivalent to those in the SD formulation. FTIR-spectroscopy (with principal component analysis) was used to understand crystallisation at different times up to 24h. Concentration of dissolved drug was assessed by UV-spectroscopy. Undissolved solid after 24h was dried completely and analysed for polymer weight by mass balance.
Results. SD did not show any crystallisation of the drug for up to 24h, whereas crystallisation occurred after 7h when the Soluplus® was added to the aqueous phase. This indicates the SD was better at inhibiting crystallisation due to drug polymer interaction. In contrast, polymer solution generated very much higher IND concentrations than SD, irrespective of the effect on the onset of crystallisation. Mass balance analysis of the solids revealed that the SD suspension contained less dissolved polymer than the predissolved solution and hence resulted in lower IND concentrations in solution.
Conclusion. Overall, the ability of Soluplus® to inhibit crystallisation of amorphous IND and maintain supersaturation in solution depends on its method of addition.

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Understanding the role of device design on the aerosolisation of a carrier-based dry powder inhaler

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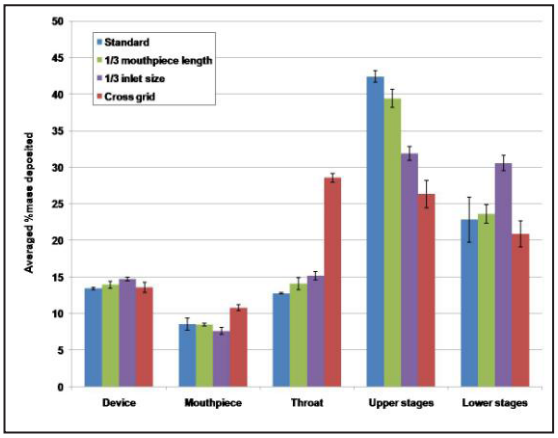
Introduction. The design of inhaler devices plays a critical role on the aerosolisation of the DPI formulations. The device design affects the drug detachment from carriers for the carrier-based formulations. Computational fluid dynamics (CFD) analysis is useful to simulate the flowfield generated in the device and thus to elucidate the mechanism of the effect of device design on the de-agglomeration.
Aims. To investigate experimentally the effect of device design on the aerosol performance of carrier-based DPI systems coupled with CFD analysis.
Methods. A commercial Aerolizer® device was modified with different geometries – air inlet, mouthpiece and grid. The *in vitro* aerosol performance was evaluated by a multi-stage liquid impinger. CFD analysis was performed to investigate the air flow pattern inside the inhaler devices.



Results. The aerosol fine particle fraction (FPF) below 5 μm was significantly improved with the reduced inlet size, attributed to the increased air velocity from CFD analysis. No significant differences were shown in the FPF with the modified mouthpiece and grid, except more drugs deposited in mouthpiece and throat as the grid voidage was increased.
Discussion. Air inlet and grid designs are critical to the aerosolisation of carrier-based DPIs. CFD analysis is useful in understanding the effect of device design.

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Impact of Alzheimer's disease on drug transport across the blood-brain barrier

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Introduction: There are various blood-brain barrier (BBB) related pathological changes reported in Alzheimer's disease (AD) but less is known about what impact such alterations have on the ability of therapeutic agents to enter the brain.
Aim: The aim of this study was to systematically assess the BBB transport of probe compounds in a relevant animal model of AD.
Methods: ^{3}H- and ^{14}C-labelled compounds were perfused into the brain of 3×Tg (TG) and wild type (WT) AD mice by transcardiac perfusion. After a 4 min perfusion, cortex, hippocampus and perfusate concentrations were determined by liquid scintillation counting, and cortex/hippocampus-to-perfusate ratios (C:P/H:P) calculated. To understand the observed *in vivo* transport alterations, molecular characterization of the BBB was performed, with collagen-IV and P-glycoprotein (P-gp) expression assessed by immunohistochemistry and western blot, respectively.
Results: C:P and H:P ratio of the paracellular marker ^{14}C-sucrose was not significantly different between WT and TG mice. BBB transport of the passive diffusion markers ^{3}H-diazepam and ^{3}H-propranolol were significantly ($p<0.05$) decreased by 54-60% in TG mice relative to WT mice, whereas the BBB transport of P-gp substrates (^{3}H-digoxin, ^{3}H-loperamide and ^{3}H-verapamil) was not different between genotypes. There was significant thickening of the basement membrane as observed by a 33% increase in collagen-IV staining in brain slices of TG mice, and a 20% reduction in P-gp expression in the isolated microvessels of TG mice.
Discussion: Consistent with that observed clinically, the BBB paracellular route is maintained in TG mice. The BBB transport of passively-diffusing compounds is reduced in TG mice likely as a result of increased cerebrovascular membrane thickness. In contrast, the BBB transport of P-gp substrates appears unaffected in TG mice, as the reduced expression of P-gp is likely compensated by a thickened basement membrane. These studies suggest that AD significantly alters disposition of therapeutics into the brain.

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Contributions of rCtr1 to the uptake and toxicity of copper and platinum anticancer drugs in sensory neurons

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Introduction. Dorsal root ganglion (DRG) neurons are affected by platinum anticancer drug-induced neurotoxicity and neurodegenerative processes associated with disturbed copper homeostasis and transport.
Aims. To understand the role and functional activity of rat copper transporter 1 (rCtr1, Slc31a1) in the uptake and toxicity of copper and platinum drugs in cultured rat DRG neurons.
Methods. Recombinant rCtr1-overexpressing cell lines and cultured rat DRG neurons were studied by methods using ICP-MS, MTT assay, immunocytochemistry and RT-PCR.
Results. Heterologous expression of rCtr1 in HEK293 cells (HEK/rCtr1 cells) increased the uptake and cytotoxicity of copper, oxaliplatin, cisplatin and carboplatin, in comparison to isogenic vector-transfected control cells. Cultured rat DRG neurons endogenously expressed rCtr1 protein on their neuronal cell body plasma membranes and cytoplasm, and displayed substantial capacity for taking up copper, but were resistant to copper toxicity. The uptake of copper by both cultured rat DRG neurons and HEK/rCtr1 cells was saturable and inhibited by cold temperature, silver and zinc, consistent with it being mediated by rCtr1. Cultured rat DRG neurons accumulated platinum during their exposure to oxaliplatin and were sensitive to oxaliplatin cytotoxicity. The accumulation of platinum by both cultured rat DRG neurons and HEK/rCtr1 cells, during oxaliplatin exposure, was saturable and temperature dependent, but was inhibited by copper only in HEK/rCtr1 cells.
Discussion. rCtr1 can transport copper and platinum drugs, and sensitizes cells to their cytotoxicities. DRG neurons display substantial capacity for taking up copper via a transport process mediated by rCtr1, but appear able to resist copper toxicity and use alternative mechanisms to take up oxaliplatin. Supported by grants from Cancer Society of New Zealand, Faculty Research Development Fund and NM McBeath Child Cancer Fund.

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PEGylation improves the lymphatic disposition of interferon 2α after subcutaneous and intravenous administration in rats and consequently improves the treatment of lymph-metastatic breast cancer

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Introduction. The lack of appropriate lymph-targeted medications has thus far hindered the successful treatment of lymph-resident diseases. For instance, protein-based immunomodulators such as interferon and interleukin have potential in the treatment of lymphatic cancers, yet they display limited lymphatic access and efficacy.
Aims. This work aimed to examine whether PEGylation improves the lymphatic disposition of interferon 2α and whether this translates into improved chemotherapeutic efficacy against lymph-metastatic breast cancer.
Methods. The lymphatic pharmacokinetics of native interferon 2α (Intron A®, 19 kDa) and PEGylated interferon (PEG-Intron®, 31 kDa; PEGASYS®, 60 kDa) were examined in rats. The chemotherapeutic efficacy of SC Intron A and PEG-Intron were compared in mice bearing axillary lymph node MDA MB-231 metastases.
Results. Intron A was poorly absorbed from the SC injection site (F_{abs} 36%) and showed little uptake into lymph after SC or IV administration (≤1%). In contrast, PEG-Intron displayed better absorption from the SC injection site (F_{abs} 82%) and lymphatic access after SC (20%) and IV (8%) dosing. PEGASYS, however, was incompletely absorbed from the SC injection site (F_{abs} 23%) and showed similar lymphatic access after SC administration to PEG-Intron (21%). The lymphatic disposition of PEGASYS after IV administration, however, was significantly greater (29%) when compared to IV PEG-Intron. SC administration of Intron A below the 3rd mammary fat pad in mice bearing axillary breast cancer metastases, or SC administration of PEG-Intron on the opposite side to axillary tumour growth had no chemotherapeutic effect. SC administration of PEG-Intron on the same side as the axillary metastasis, however, inhibited tumour growth.
Discussion. PEGylation has the potential to improve the lymphatic disposition and in vivo efficacy of small therapeutic proteins with indications in lymphatic diseases. PEG molecular weight and loading, however, need to be optimised for each protein to maximise absorption from the injection site, lymphatic access and receptor binding affinity – all critical determinants of therapeutic success.

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Association between intra-renal P-gp expression and cyclosporine concentrations in renal transplantation.

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Introduction. Although immunosuppression with calcineurin inhibitors (cyclosporine, tacrolimus) has significantly improved graft survival in renal transplantation, their long-term use is limited by nephrotoxicity. Cyclosporine and tacrolimus are substrates for the membrane efflux transporter P-glycoprotein (P-gp), and P-gp expression has been identified as a significant determinant of chronic tubulointerstitial damage in transplanted kidneys (Naesens et al, 2009). Graft P-gp expression is likely to play a role in limiting intra-renal accumulation (hence toxicity) of calcineurin inhibitors. However the relationship between P-gp expression and graft calcineurin inhibitor concentrations has not been investigated.
Aims. To determine the association between renal proximal tubular P-gp expression and graft cyclosporine concentrations in renal transplant recipients.
Methods. This was a retrospective study in 35 transplant recipients for whom routine biopsies had been performed with 1 month post-transplantation. For each subject, P-gp expression was assessed by immunohistochemistry in two paraffin-embedded biopsy samples, one taken pre-perfusion and the other within 1 month post-perfusion. Subjects were categorised by whether there was decreased, unchanged or increased P-gp expression in the post- versus pre-perfusion sample. Graft cyclosporine concentrations were measured in matching post-perfusion frozen biopsy samples by LC-MS/MS (Noll et al, 2011). Corresponding whole blood cyclosporine concentrations (2 hr post dose) were collected from therapeutic drug monitoring records.
Results. There was no correlation between cyclosporine concentrations in blood and renal tissue (r_p=0.31, P=0.07). There was no significant effect of P-gp expression on blood cyclosporine concentrations (one-way ANOVA P=0.12). However, there was a significant association between intra-renal P-gp expression and renal cyclosporine concentrations (one-way ANOVA), with mean±SD concentrations of 8.8±4.2 and 3.8±3.9 ng/mg tissue (P=0.04) in grafts with decreased (n=10) or increased (n=6) P-gp expression, respectively.
Discussion. These observations suggest that, despite therapeutic drug monitoring to minimise inter-individual variability in systemic cyclosporine concentrations, graft P-gp expression significantly determines local cyclosporine exposure.

Naesens M et al (2009) J Am Soc Nephrol 20:2468-2480
Noll B et al (2011) Ther Drug Monit 33:688-693

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Population pharmacokinetic modelling of colistin methanesulphonate and formed colistin in patients on continuous ambulatory peritoneal dialysis

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Introduction. Colistin, administered intravenously as its inactive prodrug colistin methanesulphonate (CMS), is increasingly used as last-line therapy to combat multidrug-resistant gram-negative bacteria. CMS dosing needs to be adjusted for renal function. The impact of continuous ambulatory peritoneal dialysis (CAPD) on the pharmacokinetics of both CMS and colistin formed from it in the body has not been studied.
Aims. To quantitatively describe and predict the time course of CMS and formed colistin simultaneously in plasma and dialysate of CAPD patients.
Methods. Eight CAPD patients received a single iv CMS dose (150 mg colistin base activity) over 30 min. Serial blood and dialysate samples were collected over 24 h, and cumulative urine where applicable. Concentrations were determined by HPLC. Population modelling was performed in S-ADAPT.
Results. A model with two disposition compartments for CMS, one for colistin, and first-order formation of colistin from CMS well described all data simultaneously. Total body clearance of CMS (excluding CAPD clearance) was 1.77 L/h (44%) [population mean (between subject variability)], while CAPD clearance was 0.088 L/h (64%). The population mean terminal half-life of CMS was 8.4 h. For colistin, total clearance/fm (excluding CAPD clearance; fm, fraction of CMS metabolised to colistin) was 2.74 L/h (50%), CAPD clearance was 0.101 L/h (34%), and mean terminal half-life 13.2 h. Including conversion of CMS to colistin in dialysate in the model allowed adequate description of the time courses of CMS and colistin in dialysate.
Discussion. Clearances by CAPD were low for both CMS and formed colistin. This suggests that doses should not be increased during CAPD. This model can be used to predict colistin concentrations and target attainment in CAPD patients for other than the studied dosage regimen and to optimise CMS dosage regimens in CAPD patients.

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Development of a population model of early rheumatoid arthritis disease progression treated with methotrexate, sulfasalazine and hydroxychloroquine.

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Introduction. Identification of markers that predict the rate and extent of rheumatoid arthritis progression could lead to improved guidelines for disease management and individualised treatment strategies.
Aim. To develop a disease progression model for early rheumatoid arthritis, identify factors affecting DMARD response, and apply Bayesian estimation methods to forecast an individual's response.
Methods. A population disease progression model for early rheumatoid arthritis was developed using NONMEM® and DAS28 obtained from 263 patients who attended the Royal Adelaide Hospital from initiation of therapy (consisting of methotrexate, sulfasalazine and hydroxychloroquine) until 60-weeks. Various base models and covariates were analysed to describe disease progression for the average individual in the population, and subpopulations, respectively. Bayesian estimation was performed in NONMEM® using the model and known DAS28 samples.
Results. An exponential model, additive to baseline DAS28, with covariance between parameters, and combined residual error model was developed. The population estimates from the final model were baseline DAS28 (5.62), extent (-1.28 DAS28 units) and rate of disease progression (-0.109 DAS28 units/week). Older individuals exhibited more severe baseline DAS28, those with more severe baseline disease activity received corticosteroids, and current/past smokers achieved 79% of the extent of non-smokers' response. Data rich sample schedules were needed to accurately estimate an individual's future time-course of disease progression.
Discussion. This is the first report of a disease progression model for early rheumatoid arthritis. Bayesian estimation demonstrated potential to evaluate the number, and the interval of DAS28 required to accurately forecast response.

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Evaluation of current warfarin pharmacokinetic-pharmacodynamic models and dosing algorithms.

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Introduction. Warfarin therapy is complicated by wide inter-individual variation in response, due to polymorphisms impacting pharmacokinetics (CYP2C9) and pharmacodynamics (VKORC1). Genetically-guided (PGx) dosing algorithms have been developed based in part upon population PK-PD models, to reduce the need for INR monitoring. These typically over-emphasise the performance for the 'average' patient, and have not reported the range of outcomes expected in the population.
Aims. To investigate the application of PGx dosing algorithms without INR supervision on INR outcomes and compare INR profiles with current clinical practice.
Methods. 1000 patients representative of a Caucasian population (age, weight and height) were obtained using P3M software. Using the NONMEM and R software, Monte-Carlo simulations of a PGx algorithm (Avery et al., 2011) and current clinical practice (Roberts et al., 2003) were performed using a published PK/PD model (Hamberg et al., 2010) to describe INR profiles.
Results. With the unsupervised PGx algorithm, the least sensitive genotype (CYP2C9*1/*1, VKORC1 G/G) reaches an average INR of 2. The most sensitive (CYP2C9*3/*3, VKORC1 A/A) genotype reaches an average INR of 2, with a maximum INR value of 7.5. Adjusting doses according to age and INR markedly decreases inter-individual variability (IIV).
Discussion. PGx dosing provides good estimates of doses, but without monitoring results in some patients attaining dangerous INRs. Adjusting doses in response to INR reduces variability, but provides inappropriate dosing decisions for warfarin sensitive patients. These results highlight the need for ongoing INR monitoring and incorporating PGx guided dose adjustment protocols.

Avery et al (2011) Clin Pharmacol Ther, 90(5), 701-706.
Hamberg et al (2010) Clin Pharmacol Ther 87(6), 727-734.
Roberts et al (2003) Ann Pharmacother, 37, 799-803.

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Development and application of a mechanism-based model for the multistage life-cycle of murine malaria - the effect of single and multiple dose dihydroartemisinin

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Introduction. Murine models are used to study erythrocytic stages of malaria infection, because parasite morphology and development are comparable to human malaria infections. However, mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) models for antimalarials are scarce, despite their potential to optimize antimalarial combination therapy.
Aims. To develop a mechanism-based growth model (MBGM) for *P. berghei*, and then characterize the parasiticidal effect of dihydroartemisinin (DHA) in murine malaria (MBGM-PK-PD).
Methods. Stage-specific (ring, early trophozoite, late trophozoite and schizont) parasite density data from Swiss mice inoculated with *Plasmodium berghei* were used for model development in S-ADAPT. A single intraperitoneal dose of DHA (10-100 mg/kg) or vehicle was administered at 56 h after inoculation. In an independent study, a multiple-dose regimen of DHA (10 mg/kg per dose) was administered at 0, 12, 24, 36 and 48 h post-inoculation.
Results. The MBGM explicitly reflected all four erythrocytic stages of the *P. berghei* life-cycle. Merozoite invasion of erythrocytes was described by a first-order process that declined with increasing parasitaemia. A 1-compartment model with zero order absorption described the PK of DHA, with an estimated clearance and distribution volume of 1.95 L h^{-1} and 0.851 L, respectively. Parasite killing was described by a turnover model, with DHA inhibiting the production of physiological intermediates (IC_{50} 1.46 ng/mL). The same structural model adequately described the parasiticidal effect of DHA after multiple dosing over 5 days.
Discussion. Overall, the MBGM-PK-PD described the rise in parasitaemia, the nadir following DHA dosing, and subsequent parasite resurgence. This novel model is a promising tool to study malaria infections, identify the stage-specificity of antimalarials and provide insight into antimalarial treatment strategies.

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Renal function estimation in drug development: should East Asian ethnicity be considered?

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Introduction. The growing globalisation of clinical drug development warrants consideration of the influence of ethnicity on drug pharmacokinetics, efficacy and safety. Ethnic-specific GFR-estimating equations have been developed for East Asian populations to assess renal function, a factor which can influence drug pharmacokinetics. However, the implications of using, or ignoring, East Asian-specific GFR-estimating equations in drug development programs have not yet been reported.
Aims. To evaluate the consequences of using East Asian-specific equations to assess renal function in subjects of East Asian ancestry relative to using conventional equations.
Methods. Baseline demographic data (age, sex, ethnicity, weight, serum creatinine concentration) were obtained from ethnically diverse cohorts of subjects enrolled in 17 clinical studies (including healthy subjects, subjects with renal impairment, type 2 diabetes, benign prostatic hyperplasia or renal cell carcinoma). Creatinine clearance (CrCL) was estimated in each subject using the Cockcroft-Gault (CG) equation and GFR was estimated using the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations and the ethnic-specific Chinese (C-MDRD), Japanese (J-MDRD) or Korean (K-MDRD) MDRD equations or the Japanese CKD-EPI (J-CKD-EPI) equation.
Results. Subject demographics and renal function estimates (mean±SEM) calculated using the conventional and East Asian-specific equations are given in the table.

Ethnicity	N	Age (years) [range]	Sex (% male)	CrCL (mL/min)	Estimated GFR (mL/min/1.73m^2)					
				CG	MDRD	CKD-EPI	C-MDRD	J-MDRD	J-CKD-EPI	K-MDRD
Caucasian	1049	60±0.3 [17-90]	61	91±1.2	84±0.9	79±0.7	–	–	–	–
Chinese	612	55±0.5 [19-80]	50	91±1.2	98±1.2	92±0.8	121±1.4	–	–	–
Japanese	211	55±1.2 [21-83]	70	95±2.2	113±1.7	102±1.2	–	83±1.3	83±1.0	–
Korean	56	63±1.1 [41-83]	91	65±2.7	71±2.2	69±2.2	–	–	–	88±2.4

Discussion. In East Asian subjects, renal function estimates differ when determined by ethnic-specific and conventional GFR-estimating equations. Further evaluation of East Asian-specific GFR-estimating equations is warranted to determine the implications of the differences in estimated renal function for drug development and clinical practice.

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The population pharmacokinetics of allopurinol and oxypurinol.

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Introduction. Allopurinol is used to treat gout and works by reducing serum urate concentrations. Dose requirements are highly variable between patients and there is currently no satisfactory means of predicting the maintenance dose required to achieve the serum urate target of <0.36mmol/L. A better understanding of the factors which determine the variability in allopurinol and oxypurinol pharmacokinetics between patients is required.
Aims. To develop a population pharmacokinetic model for allopurinol and oxypurinol, and, to explore the influence of patient characteristics on allopurinol and oxypurinol pharmacokinetics.
Methods: A population analysis was carried out using NONMEM. A total of 680 allopurinol and 694 oxypurinol plasma concentrations (n=104) were available for analysis. The effects of renal function, body composition, drug interactions, and genetic variability in renal urate transporters were evaluated.
Results. A parent-metabolite model with a two compartment model for allopurinol and a one compartment model for oxypurinol was fitted to the data. Renal function, fat-free mass (FFM) and diuretic use were found to predict differences in the pharmacokinetics of oxypurinol. The population estimates for allopurinol clearance, central and peripheral volume and inter-compartmental clearance were 50 L/h/70 kg FFM, 11.4 L/70 kg FFM, 91 L/70 kg FFM and 142 L/h/70 kg FFM respectively with a between subject variability of 33% coefficient of variation (CV) for allopurinol clearance. Oxypurinol clearance and volume of distribution were estimated to be 0.78 L/h/70 kg FFM for a patient with a creatinine clearance of 100 mL/min and 41 L/70 kg FFM with a between subject variability of 28% and 15% (CV) respectively.
Discussion. This research represents a step towards a pharmacokinetic-pharmacodynamic and disease progression model for the relationship between allopurinol dose, serum urate concentration and clinical outcome. Future work will explore the probability of successfully achieving serum urate targets in patients with impaired renal function.

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The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin

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Introduction. Inter-individual variation in response to metformin, first-line therapy for type 2 diabetes, is substantial. Previously, transporters have been shown to contribute to the inter-individual variability in metformin pharmacokinetics and pharmacodynamics.

Aims. We examined the effects of promoter variants in both, MATE1 (g.-66T>C, rs2252281) and MATE2 (g.-130G>A, rs12943590) on variation in metformin disposition and response in ethnically diverse healthy subjects and type 2 diabetic patients.

Methods. The pharmacokinetics and glucose-lowering effects of metformin were assessed in predominantly African-American healthy subjects (n=57) receiving metformin (1850 mg). Using electronic health records, the relative change in HbA1c within 3-9 months following initiation of metformin monotherapy was examined in predominantly Caucasian type2I diabetic patients (n=249). Statistical analysis was conducted using Student's t-test and regression analysis.

Results. In healthy subjects, the renal and secretory clearances of metformin were higher (22% and 26%, respectively) in carriers of variant MATE2 (-130G>A) who were also MATE1 (-66T>C) reference (n=32, $P<0.05$). Furthermore, both MATE genotypes were associated with altered post-metformin glucose tolerance, with variant carriers of MATE1 (-66T>C) and MATE2 (-130G>A) having an enhanced ($P<0.01$) and reduced ($P<0.05$) response, respectively. Consistent with these results, diabetic patients carrying the MATE1 (-66T>C) variant and OCT1 reference allele showed greater relative reduction in HbA1c (n=145, mean [95%CI], -0.12 [-0.14-0.09]) compared to those carrying the MATE1 reference (-0.16 [-0.20-0.12], P=0.01), after adjustment for dose and ethnicity.

Discussion. These findings suggest that promoter variants of MATE1 and MATE2 are important determinants of metformin disposition and response in healthy subjects and type 2 diabetic patients. Further, the study provides evidence that MATE1, MATE2 and OCTs work in concert and should be considered together when ascertaining the genetic determinants of renal elimination and response to metformin. Finally, the results of our study suggest an important role of MATE2 in the pharmacokinetics and pharmacodynamics of metformin.

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Gentamicin directed therapy: Which program to use?

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Introduction. Australian Therapeutic Guidelines recommends initiating directed therapy of gentamicin if administration exceeds 48 hours. The directed dose of gentamicin is based on a 24-hour AUC using gentamicin plasma concentrations and is calculated by a dosing prediction program. TCIWorks or Aladdin have been suggested for this purpose but the outputs have not been assessed for concordance.

Aims. To determine if the directed doses predicted by TCIWorks, Aladdin and Excel (adapted from Begg et al., 1995) agreed with those predicted using Abbottbase.

Methods. Retrospective peak and trough plasma concentrations after the first and second administered doses of gentamicin were available for three groups (n=20-23) of varying creatinine CLs (<40 mL/min, 40-80 mL/min and >80 mL/min). The directed dose needed to produce 24-hour AUC values of 80 mg.h/L was calculated using each program. The qualification was that the peak concentrations must be >10 mg/L and trough concentrations <0.5 mg/L. If the qualification criteria were not satisfied, the dosage interval was extended to 36 or 48 hours and the AUC target was adjusted proportionally to 120 or 160 mg.h/L, respectively. Agreement of dose predictions was examined by Bland-Altman analysis.

Results. The ratio (95% agreement limits=1.96*SD) of the directed doses determined following the first administered dose of gentamicin by TCIWorks, Aladdin and Excel compared to Abbottbase were 106% (96% to 116%), 102% (87% to 118%) and 108% (91% to 125%), respectively. Similar mean ratios were seen following the second dose of gentamicin. For each of the three renal function groups, the programs yielded similar directed doses compared to Abbottbase.

Discussion. The four programs used in the calculation of directed doses of gentamicin yielded similar results. Any would be suitable for use in clinical practice. Prospective comparison of all four packages is still required.

Begg EJ et al (1995). Br J Clin Pharmacol 39:605-609.

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Clozapine-induced myocarditis: characterisation using case-control design

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Introduction. Myocarditis is a hypersensitivity reaction, typically occurring in the third week after commencing clozapine, the most effective treatment available for schizophrenia.
Aims. A case-control design is commonly used to investigate risk factors, we show that it can be used to characterise an adverse reaction more broadly.
Methods. Cases and controls were documented from patients' medical records. Controls were matched by unit at which clozapine was commenced and approximate start date.
Results. 105 cases and 296 controls met entry criteria. Time to onset for cases was 10-33 days, with 82% developing 14-21 days after commencing clozapine. Almost 90% of cases and controls had tachycardia. Eosinophilia developed in 64% of cases and 30% of controls, but onset among cases was delayed 0-8 days after the peak in troponin. However, 87% of cases had C-reactive protein (CRP) > 50mg/L and CRP could be raised up to 5 days before the rise in troponin.
Multivariate regression analysis indicated that the risk of myocarditis increased with increasing age (31% per decade; 95% CI 7-60%), increasing rate of clozapine dose titration (26% per 250mg during days 1-9; 95% CI 2-55%) and concomitant sodium valproate (odds ratio 2.59; 95% CI 1.51-4.42).
Discussion. Comparison of cases and controls permitted identification of the features of myocarditis, and avoided confounding by features associated with introduction of clozapine. Monitoring for myocarditis should use troponin and CRP but not eosinophil counts. Clozapine should be introduced by slow dose titration and sodium valproate is best avoided, if clinically feasible.

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Relationship between high risk prescribing and adverse outcomes in people with and without Alzheimer's disease

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Introduction. There is a lack of empirical data in relation to possible negative outcomes associated with use of anticholinergic and sedative medicines in older adults with Alzheimer's disease (AD). Aims. The objective of this study was to investigate the relationship between exposure to medicines with anticholinergic and sedative properties with hospitalisation and mortality in people with and without AD in Finland. Methods. Community-dwelling individuals (n = 16,897) with AD in 2005 were identified by the Social Insurance Institution. For each person with AD, a comparison person individually matched in terms of age (±1 year), sex, and region of residence was identifed. Records of reimbursed medicines purchased from 1st September–31st December 2005 were extracted from the Finnish National Prescription Register. High risk prescribing was defined using the Drug Burden Index (DBI), a dose-normalised measure of exposure to anticholinergic and sedative medicines. Mortality and hospitalisation data were extracted from National Registers. Cox and logistic regression analyses were used to investigate the relationship of high risk prescribing with mortality and hospitalisation over a one-year follow-up period. Results. The age of the participants with and without AD ranged from 65-101 (mean 79.2) years, with women comprising 67% of participants. For every unit increase in DBI, the adjusted hazard ratio (HR) for mortality was 1.22 (95% confidence intervals [CI]: 1.11-1.35) among AD participants, and 1.43 (95%CI: 1.27-1.62) for non-AD participants. In relation to hospitalisation data, for every unit increase in DBI, the odds ratio (OR) for being hospitalised over one year in AD participants was 1.38 (95%CI: 1.31-1.46) compared with an adjusted OR of 1.81 (95%CI: 1.70-1.93) amongst non-AD participants. Discussion. These data imply a dose-response relationship of higher DBI exposure with hospitalisation and mortality in both people with and without AD, with a greater relative risk among individuals without AD.

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A collaboration to measure adverse drug events in three district health boards

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Introduction. The three DHBs worked to implement the Adverse Drug Event Trigger Tool (ADETT), a modified IHI trigger tool for medications. The collaboration was maintained with frequent teleconference. Definitions were agreed on and triggers refined.
Methods. A random sample of charts (from March 2010 to February 2011) was obtained excluding patients admitted for <48 hours, children <18 and psychiatric admissions. In each DHB, trained reviewers scanned these in a structured way to identify any of the 19 triggers. If triggers were identified, a more detailed, time-limited review of the chart, was done to determine whether an ADE had occurred. The severity of patient harm was categorised using the National Coordinating Council for Medication Error Reporting and Prevention Index. No attempt was made to determine preventability. ADEs from acts of omission were excluded.
Results. The ADE TT was applied to 1210 charts and 353 ADE were identified, with an average rate of 28.9/100 admissions and 38/1,000 bed days. 94.5% were in the lower severity scales with temporary harm, however in 5 patients the ADE contributed to their death, and 9 required intervention to sustain life. The most commonly implicated drugs were opioids and anticoagulants. Patients who suffered ADE were more likely to be older females.
Conclusions. The rate of medication-related harm identified by the ADE TT is higher than identified through voluntary reporting. The ADE TT provides a standardised measure of harm over time that can be used to determine trends and the effect of medication safety improvement programmes.

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Risk management plans in the Australian regulatory environment

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Introduction: A Risk Management Plan (RMP) is a set of pharmacovigilance activities and interventions designed to identify, characterise and minimise risks relating to a medicine. RMPs are commonly required as part of applications submitted to the Therapeutic Goods Administration (TGA).
Aims: To characterise additional pharmacovigilance (APhV) and additional risk minimisation (ARiM) activities and study timeframe compliance as part of RMPs for applications submitted to the TGA.
Methods: A retrospective analysis was conducted for RMPs associated with applications submitted to the TGA between January 2009 to August 2012 for new chemical entities (NCEs), extension of indications (EOIs), major variations (MVs; e.g. dose form or route of administration) and other (e.g. new combinations of medicines). APhV activities included planned or ongoing studies at the time of application. ARiM included activities such as Healthcare Professional education. Relevant information was extracted and compiled using the TGA's electronic records management systems.
Results: One hundred and thirty-four (134) applications with RMPs were submitted with approved products, 35% NCEs, 33% EOIs, 17% MVs and 15% other. Approximately 76% of these RMPs proposed at least one APhV activity and 23% ARiM activities. Preliminary results indicate 443 APhV activities were included with RMPs, with 33% planned and 67% ongoing studies. Seventy percent (70%) of planned studies were ongoing or completed at the date of approval. Approximately 70% of studies were 'on-target' and within timeframes specified.
Discussion: A large number of APhV and ARiM activities are proposed with applications to the TGA for NCEs, EOIs, MVs and other. Compliance with study timelines within RMPs appears to be good. However, preliminary results indicate most planned studies are already completed or initiated at the date of product approval, suggesting RMPs submitted to the TGA are outdated because they are not reflective of post-marketing pharmacovigilance commitments proposed at the time of submission.

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β-alanine, A GABAc partial agonist becomes antagonist when one residue at loop C of binding pocket mutated

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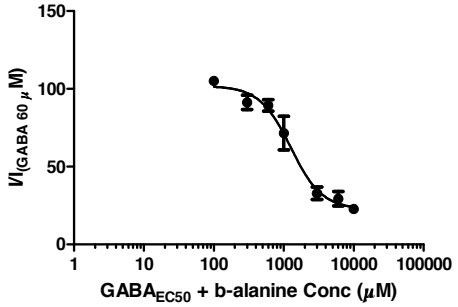
Introduction: The GABAc Receptors are member of the LGIC superfamily. They are structurally related to $GABA_A$ receptors but have a distinct pharmacology (1). Actually, Extensive research has been done on GABAc receptors in order to identify amino acids involved in GABA- mediated activation. Recently, the functionality of threonine 244 residues has been studies with various ligands (2). In this study we continue our investigation on T244 with the partial agonist β-alanine.

Aims: Structural and functional studies of residues located at the extracellular domain of GABAc receptor.

Methods: 1. Schrödinger suite 2012, Glide 5.8; was used to study β-alanine docking intothe receptor homology model (3) 2.Site-directed mutagenesis; used to prepare mutant receptors.3. Two Electrode voltage clamp electrophysiology used to measure the response of receptors to β-alanine (4).

Results: β-alanine activity has been tested on GABAc WT and T244S mutant. On WT, β–alanine acts as a partial agonist by activating only 50% of the GABA^{max} response (10 mM). On the other hand, when applied to the T244S mutant, a drastic decrease in sensitivity was noticed, and instead it becomes an antagonist (Figure 2).



Discussion: our docking studies predict that β–alanine forms a direct hydrogen bond with threonine 244 in loop C of the binding pocket (Figure 1). The conversion of β–alanine to an antagonist on the T244S mutant indicates that this residue is essential for channel activation. This suggests that the T244 is involved in the early processes of coupling agonist binding to channel gating.

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Analgesic efficacy and mode of action of a small molecule angiotensin type 2 receptor antagonist in a rat model of prostate cancer induced bone pain.

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Introduction. Our laboratory has previously shown that single bolus doses of small molecule angiotensin type 2 receptor (AT_2R) antagonists, produced dose-dependent analgesia in nerve-injured rats by a mechanism that involves blockade of p38 and p42/p44 mitogen-activated protein kinases (MAPK) activation in lumbar dorsal root ganglia (DRGs) (Smith et al., 2012). Hence, the present study was designed to investigate a role for angiotensin II/AT_2R signaling in the pathobiology of prostate cancer induced bone pain (PCIBP).

Aims. To investigate the analgesic efficacy and model of action of a selective AT_2R antagonist, PD123,319, in an AT3B prostate cancer cell (APCCs)-induced rat model of PCIBP.

Methods. Rats received a unilateral intra-tibial injection of $4x10^4$ APCCs or heat-killed cells (sham) (Muralidharan et al., 2012). Dose-response curves were generated for single i.v. bolus doses of PD123,319 and the ~ED_{50} dose was estimated using nonlinear regression (GraphPad Prism v5.03). The expression levels of phospho-p38 (pp38) and phospho-p42/44 (pp42/pp44) MAPK were determined in lumbar DRGs using immunohistochemistry (IHC) and western blot analysis at the time of peak analgesia of i.v. PD123,319 (3mg/kg).

Results. The mean ED_{50} doses for PD123,319 to alleviate mechanical allodynia and thermal hyperalgesia in the ipsilateral hindpaws were 0.9 (95% CI: 0.5 to 1.5) and 3.7 (95% CI: 1.7 to 8.0) mg/kg i.v., respectively. Administration of PD123,319 but not vehicle, reduced mean DRG expression levels of pp38 and pp42/pp44 MAPK to match the corresponding levels in sham-controls.

Conclusion. PD123,319 produced dose-dependent analgesia in a rat model of PCIBP by a mechanism suggesting that small molecule AT_2R antagonists hold promise as novel analgesics for the relief of PCIBP.

Muralidharan A et al (2012) Abstract PF291, 14th World Congress on Pain, 25-31 August 2012 Milan, Italy. IASP Press.
Smith MT et al (2012) Abstract PW285, 14th World Congress on Pain, 25-31 August 2012 Milan, Italy. IASP Press.

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Attenuation of Toll-like receptor 4 reduces reward-like behaviours in mice

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Introduction. Alcohol abuse is a significant social and economic problem. Recent evidence suggests alcohol-induced pro-inflammatory central immune signalling may also be involved in the actions of alcohol[1]. Specifically Toll-like receptor 4 (TLR4), a pattern recognition receptor, has found to be essential for many of alcohol actions[1].
Aims. To determine whether TLR4 is involved in the rewarding properties of alcohol in mice
Methods. The TLR4 signaling pathway was analysed using (+)-naltrexone - a TLR4 antagonist, and genetic TLR4 knockout mice. Reward-like behaviour was assessed using two-bottle choice and conditioned place preference. Control experiments to eliminate confounds of taste were also conducted. Finally, to determine the cell type and which TLR4 signalling pathways were activated in response to alcohol, immunohistochemistry was performed.
Results. TLR4 K.O. mice and (+)-naltrexone treated mice demonstrated a reduced preference for consuming alcohol compared to wild-type and saline treated mice respectively (main effect of genotype $p<0.0001$, and (+)-naltrexone $p<0.0001$). However, mice did not differ in saccharin or quinine intake ($p>0.05$). Pre-treatment with (+)-Naltrexone reduced the time spent in alcohol conditioned chamber compared to saline treated mice in conditioned place preference (two-way ANOVA $p=0.0005$).
Discussion. Genetic and pharmacological TLR4 blockade reduced alcohol preference when assessed by two-bottle choice and conditioned place preference, which is indicative of a reduced reward. This difference was not due to altered taste. Immunohistochemical analysis suggests glia are critical for this response. Collectively, the results suggest that TLR4 contributes to the generation of alcohol-induced reward, suggesting that blocking this signalling may prove beneficial in treating alcohol-abuse disorders.

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Potent anti-inflammatory effects of andrographolide and its major metabolite, andrographolide sulfonate

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Introduction. Chronic inflammation is a contributing factor for many ageing-related diseases including Alzheimer's disease (AD). In order to provide effective, yet safe anti-inflammatory treatments, there is a renewed interest in the search of plant based novel secondary metabolites. Andrographolide, an ent-labdane diterpene from an ayurvedic herb Andrographis paniculata has been traditionally used for the treatment of chronic inflammatory diseases. However, andrographolide exhibits poor bioavailability (< 3%), and is known to rapidly metabolize to a sulfonate with unknown potency, which was investigated in this study.
Methods. Anti-inflammatory activity was determined by nitric oxide production in LPS + IFN activated RAW264.7 macrophages (n=3, in triplicate). Cell viability was measured using the MTT reduction assay (n=3, in triplicate),
Results. Andrographolide and its major metabolite, andrographolide sulfonate both demonstrated strong anti-inflammatory activity with IC_{50} values of 12.4 ± 0.6 μM and 14.2 ± 0.3 μM, respectively. Both compounds were much more potent than the NSAIDs aspirin and ibuprofen or paracetamol (IC_{50} values > 1 mM). The LC_{50} concentrations for andrographolide and andrographolide sulfonate were determined to be 272 ± 20 μM and 489 ± 11 μM, respectively.
Discussion. The nearly equipotent anti-inflammatory activity of andrographolide sulfonate (which exhibits > 20 times higher plasma levels than andrographolide), together with its extended half-life, might account for its purported clinical efficacy.

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Anaesthesia increases markers of inflammation and pro-inflammatory cytokines in serum and hippocampus: relationship to post-operative cognitive dysfunction.

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Introduction. Post-operative cognitive dysfunction (POCD) occurs in ~10% of all surgical patients but the pathogenesis is unknown. Studies in young and aged rats suggest anaesthesia and/or inflammation induced by surgical trauma may contribute to POCD. We have previously found differences between anaesthetic agents and age-dependent effects on memory in rats (Callaway *et al*, 2012, *Anesthesiology*).

Aim. Here we investigated whether anaesthesia alone has any effect on markers of inflammation in serum and hippocampus in rats.

Methods. Young adult (3 mo) male Sprague Dawley rats were exposed to equivalent concentrations (1 MAC, 4h) of the volatile anaesthetics sevoflurane or desflurane or no-anaesthesia condition. At 6, 24, 48h or 7d after exposure (n=3-6 per time point) rats were deeply anaesthetised (Isoflurane 5%) and blood samples taken via cardiac puncture prior to transcardial perfusion with PBS. Brains were removed and hippocampus dissected and processed for cytokine analysis. C-reactive protein was analysed in serum and cytokines were analysed in serum and hippocampus lysates (Bioplex Assay).

Results. C-reactive protein levels were increased 2-fold in serum of rats exposed to volatile anaesthetics compared with no-anaesthesia controls and increases were still evident at 7d. Multiple pro-inflammatory cytokines including IL-1β (4 fold increases, $P<0.001$) and IL-6 ($P<0.01$) were significantly increased in serum 6h after exposure. Significant increases in IL-1β and IL-6 were also found in the hippocampus 6h after desflurane exposure compared with controls ($P<0.02$).

Discussion. Volatile anaesthethics alone increased markers of inflammation and pro-inflammatory cytokines in the periphery and the CNS. Increased inflammation has been proposed as a mediator of POCD.

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Hypoxia-inducible factor - 1 (HIF-1) prolyl hydroxylase inhibitors have neuroprotective actions in a neonatal rat model of hypoxic-ischemic brain injury.

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Introduction. Hypoxia-inducible factor-1 (HIF-1) is the key transcription factor regulating the expression of many hypoxia-responsive genes, including erythropoietin and vascular endothelial growth factor. Under normoxic conditions HIF-1α protein is constantly degraded due to HIF-1 prolyl hydroxylase enzymes (PHDs) which hydroxylate proline residues on HIF-1α causing ubiquitination and proteosomal degradation and consequently, constitutive levels of HIF-1α protein are almost undetectable. Hypoxia and drugs that can inhibit PHD activity can cause accumulation of HIF-1 and increase target gene expression. Previously, we have shown that preconditioning with hypoxia and PHD Inhibitors (cobalt chloride (CoCl2) and desferrioxamine (DFX)) can protect the brain against hypoxic-ischemic (HI) brain injury and this protective effect is largely due to expression of HIF-1 and its target genes.

Aims. Here we have examined the neuroprotective effects of PHD Inhibitors administered after brain injury.

Methods. Sprague-Dawley rat pups (postnatal day 7) were anaesthetised with isofluorane (1-5%, via inhalation in oxygen) and underwent a unilateral common carotid artery ligation and were then exposed to 3 hours of 8% oxygen. A single, subcutaneous injection of drug treatments: DFX (200mg/kg), CoCl2 (60 mg/kg), ethyl-3,4-dihydrobenzoate (EDHB; 200mg/kg) or saline vehicle was performed immediately after the HI procedure. One week post-injury, brains were removed for histological analysis, using cresyl violet and immunohistochemistry for the neuronal marker - neuronal nuclear antigen.

Results. This combined HI procedure results in a significant loss of brain tissue in the ipsilateral hemisphere. Treatment with DFX (n=12), CoCl2 (n=12) and EDHB (n=10) significantly reduced the degree of damage in the ipsilateral hemisphere by 38%, 42% and 37%, respectively, when compared with vehicle treated littermate HI only controls (n=18; $p<0.05$, ANOVA, Dunnett's post-hoc test).

Conclusion. Our findings indicate that modulation of HIF-1 and its target gene expression after HI brain injury is an effective neuroprotective strategy.

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Oxycodone-induced activation of Toll-Like Receptor 4 contributes to drug reward.

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Introduction. Traditional opioid action to create reward was thought to result solely via the initial agonism of neuronal opioid receptors. Previous studies have implicated proinflammatory immune signaling within the brain in potentiating the rewarding properties of opioids. However, the mechanism for the initiation of this proinflammatory central immune signal was unclear.
Aims. Identify if proinflammatory central immune signaling is a novel contributor to opioid reward: Toll-like receptor 4 (TLR4), and its MyD88-dependent signaling.
Methods. Conditioned place preference and hotplate latencies for oxycodone was examined in wild-type Balb/c, *TLR4* -/- and *MyD88* -/- mice.
Results. Oxycodone (20 mg/kg) induced a 127 +/- 31 sec change in preference for the oxycodone-paired environment, compared to 32 +/- 32 sec for *TLR4* -/- and -25 +/- 32 for *MyD88* -/- mice. Two-way ANOVA revealed significant effects of oxycodone ($p = 0.049$), strain ($p = 0.046$) and interaction ($p = 0.01$). Hotplate latency revealed a 5-fold leftward shift in the *TLR4* -/- oxycodone dose response curve (ED50 wild-type 1.36 mg/kg versus *TLR4* -/- 0.26 mg/kg; $P < 0.0001$) compared to wild-type mice.
Discussion. Collectively, these data indicate that the actions of opioids at classical opioid receptors, together with actions at TLR4/MD2, possibly affects the mesolimbic dopamine system and may explain altered opioid reward behaviors. Thus, the discovery of TLR4/MD2 recognition of opioids as foreign xenobiotic substances adds to the existing hypothesized opioid reward mechanisms, identifies a new drug target in TLR4/MD2 for the treatment of addiction, and provides further evidence supporting a role for central inflammation in drug reward.

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Exploring cannabinoid receptor activity of synthetic compounds identified in "Spice"-related products using novel assays for CB1 and CB2 receptor activation.

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Introduction. Over the counter/over the internet products that mimic the effects of cannabis are a potentially dangerous alternative to smoking cannabis. These cannabimimetics are structurally unrelated to the components of cannabis and their pharmacological activity is largely undefined.
Aims. To define the cannabinoid receptor-1 (CB1) and -2 (CB2) activity of synthetic components of cannabimimetic products using a fluorescence-based assay of membrane potential.
Methods. Mouse AtT-20 cells stably expressing rat CB1 (AtT20-CB1) or human CB2 (AtT20-CB2) receptors were grown in 96 well plates. CB receptor signalling was measured using a proprietary membrane potential dye (Molecular Devices) in a Flexstation 3 microplate reader. The synthesis of *N*-(adamtan-1-yl)-1-pentyl-1*H*-indole-3-carboxamide (SB-001), *N*-(adamtan-1-yl)-methyl-1-pentyl-1*H*-indole-3-carboxamide (SB-002), adamantan-1-yl(1-pentyl-1*H*-indol-3-yl)methanone (AB-001), 2-(adamantan-1-yl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone (AB-002) will be reported elsewhere.
Results. WIN-55212, a non-selective CB agonist, hyperpolarized AtT20-CB1 cells (*p*EC50 7.5±0.1, max change in fluorescence 45±3%) and AtT20-CB2 cells (*p*EC50 7.1±0.1, max 32±2%). Data for other drugs were normalized to WIN-55212 (1μM) for ready comparison. Δ9-Tetrahydrocannabinol (THC), the major psychoactive component of cannabis, hyperpolarized AtT20-CB1 cells (*p*EC50 7.2±0.1, max 78±5% of WIN-55212) but showed limited activity in AtT20-CB2 cells (5% at 10 μM). SB-001 showed similar activity at CB1 (*p*EC50 7.5±0.1, max 98±6%) and CB2 (*p*EC50 7.5±0.1, max 90±5%) but SB-002 was more efficacious at CB1 (*p*EC50 7.4±0.1, max 84±6%) than CB2 (7.2±0.25, max 23±4%). AB-001 had similar activity at CB1 (*p*EC50 7.2±0.2, max 80±7%) and CB2 (*p*EC50 7.5±0.2, max 84±10%), but AB-002 was a poor agonist (CB1 max 17±3% at 10 μM; CB2 max 38±3% at 10μM).
Discussion. AtT-20 cells expressing CB1 or CB2 receptors are a platform for rapid screening of ligands in a relatively naturalistic environment. This novel screen identified that some of the components found in synthetic cannabimimetic blends can be potent and selective CB agonists.

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The role of the secretory pathway calcium ATPases (SPCAs) in breast cancer

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Introduction. The secretory pathway Ca^{2+} ATPase (SPCA) is a Ca^{2+} pump localized to the Golgi. The Golgi is a major site of protein processing and trafficking, which are pathways that are altered in some cancers. Microarray studies show that SPCA1 is overexpressed in some basal-like breast cancers, a molecular subtype of breast cancer that infers a poor prognosis.
Aims. To identify proteins that have altered expression levels due to SPCA1 silencing in MDA-MB-231 basal breast cancer cells.
Methods. MDA-MB-231 cells were seeded into 6-well plates (75 000 cells/well) and treated with Dharmacon siRNA targeted to SPCA1 or non-targeting control siRNA 24 h after plating. Protein was isolated 72 h post siRNA treatment and SPCA1 knockdown was confirmed using real time RT-PCR and immunoblotting. 2D-DIGE and electrospray ionization tandem mass spectrometry was used as a high throughput method to identify proteins sensitive to SPCA1 silencing in MDA-MB-231 breast cancer cells. Results were confirmed using independent immunoblotting with appropriate antibodies.
Results. A total of 215 protein spots were identified to be significantly different between non-targeting and SPCA1 siRNA treatments. Tandem mass spectrometry was used to identify 20 of these spots, and one of these was determined to be heat shock protein 60 (HSP60). Immunoblotting confirmation from 3 independent experiments demonstrated that HSP60 expression was reduced by 81±2% (n=3, $P<0.05$) upon SPCA1 silencing in MDA-MB-231 cells.
Discussion. These studies show that 2D-DIGE is a suitable method to identify proteins sensitive to SPCA1 inhibition in MDA-MB-231 breast cancer cells. This approach may be an effective method for studying changes in protein expression in other cancer cell types or with silencing of other Ca^{2+} transporters. Further studies are needed to fully characterize the functional consequences of SPCA1-silencing mediated HSP60 downregulation in breast cancer cells.

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Structure-function analysis of allosteric and bitopic ligand binding at adenosine A_1 receptors.

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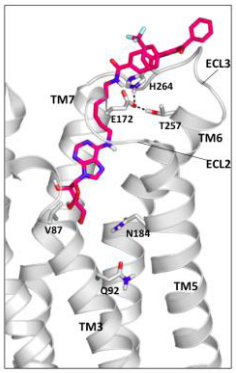

Introduction. The adenosine A_1 receptor (A_1AR) represents a potential therapeutic target for a variety of disorders. A_1AR ligands can interact with the orthosteric site, a topographically distinct allosteric site, or concomitantly bridge both sites via a "bitopic" mechanism (Valant *et al*, 2012). A_1AR therapeutic applications would benefit immensely from the rational design of more selective and efficacious A_1AR ligands, however this approach requires greater structural knowledge of the A_1AR binding sites.
Aim. To probe the key residues involved in conferring A_1AR allosteric and bitopic ligand affinity, efficacy and allosteric cooperativity.
Methods. Homology modelling of the A_1AR predicted key residues involved in allosteric and bitopic ligand binding. A_1ARs containing alanine substitutions were stably expressed in FlpINCHO cells. Radioligand binding and ERK1/2 phosphorylation assays were used to investigate the influence of receptor mutations on orthosteric (NECA), allosteric (PD81723), and bitopic (VCP746) ligand affinity, efficacy and cooperativity.
Results. The extracellular mutations, T257A, H264A and E172A, significantly enhanced the affinity of VCP746 but decreased or had no effect on NECA affinity (n=4; $p<0.05$). Transmembrane mutations, V87A, Q92A, N184A, significantly decreased the positive cooperativity between NECA and PD81723 (n=4; $p<0.05$).
Discussion. T257, H264 and E172 likely form a hydrogen bond network between extracellular loops 2 and 3. Breaking this network may open up an extracellular cavity to facilitate VCP746 binding (see figure). Residues involved in conferring allosteric cooperativity cluster around a region proximal to the orthosteric site. Structural knowledge gained from these studies will inform ongoing structure-activity studies and rational drug design efforts at this therapeutically relevant receptor family.

Valant C et al (2012) Annu. Rev. Pharmacol. Toxicol. 52:153-178

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Glucocorticoids inhibit breast tumour cell migration but increase metastasis to the lung in a mouse model of breast cancer

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Introduction: Chemotherapy-induced nausea and emesis is commonly treated by the administration of glucocorticoids. Glucocorticoids are also known to influence tumour cell behaviour. We have previously identified a class effect of glucocorticoids in inhibiting serum-induced migration in the human breast tumour cell line, MDA-MB-231, in a 2-dimensional scrape wound healing assay and a 3-dimensional modified Boyden chamber assay. This glucocorticoid effect appears to be dependent on transactivation rather than transrepression. Interestingly, the murine breast tumour cell line, 4T1.2, showed dex-induced inhibition of migration in the 3-dimensional but not 2-dimensional migration assays.
Aims: To investigate the effect of glucocorticoids in a mouse model of breast cancer.
Methods: mCherry-expressing 4T1.2 murine breast tumour cells (500,000) were injected into the 4th mammary fat pad of Balb-c mice. Dexamethasone (dex) was administered sc at 0.1mg/kg/day, commencing 2 days after the tumour was first palpable. Primary tumour and organs were harvested after a further 23 days. DNA was extracted from lung, spine and femur using phenol-chloroform and levels of mCherry were measured using qPCR along with vimentin as a control. mCherry content was assessed as a measure of metastasis.
Results: Dex treatment reduced final body weight (Vehicle: 19±0.3g, Dex: 18±0.3g, $P<0.05$) but there was no affect on primary tumour weight. There was a significant increase in mCherry content (metastasis) in the lungs of dex-treated mice (Vehicle: 1.0±0.3, Dex: 2.0±0.6, $P<0.05$).
Discussion: Dexamethasone had no effect on primary tumour growth but increased metastasis to the lung. This effect was opposite to expectations based on previous *in vitro* studies. Our findings suggest Dex may promote tumour spread. Confirmation of these findings in xenograft models of human breast tumours in mice would lead us to advocate for the use of other non-steroidal anti-emetics in treating breast cancer.

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Oleoyl-L-Glycine and N-Arachidonyl-Glycine Inhibit the Glycine Transporter GlyT2

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Introduction: Concentrations of extracellular glycine in the central nervous system are regulated by Na+/Cl--dependent glycine transporters, GlyT1 and GlyT2. Selective inhibitors of GlyT1 have been developed for the treatment of schizophrenia, whilst selective inhibitors of GlyT2 are analgesic in animal models of pain. We have investigated the inhibitory actions of a series of endogenous lipids on GlyT2.
Methods: Human GlyT2 was expressed in Xenopus laevis oocytes and the inhibitory actions of a series of acylcarnitines and arachidonyl-amino acids on glycine transport was measured using electrophysiological analysis.
Results: Oleoyl-L-carnitine was the most potent inhibitor of GlyT2 identified with an IC50 of 340 nM, which is 15-fold more potent than N-arachidonyl-glycine. Both Oleoyl-L-carnitine and N-arachidonyl-glycine are non-competitive inhibitors of GlyT2 and show slow onsets of inhibition and slow washouts. The rate of washout can be greatly increased by the inclusion of beta-cyclodextrin in the wash solution, which suggests that these lipid inhibitors may be acting on the GlyT2 via a lipid exposed site on the transporter. Using a series of chimeric GlyT1/2 transporters and point mutant transporters we have identified a leucine residue in extracellular loop 4 of GlyT2 that confers differences in oleoyl-L-carnitine and N-arachidonyl-glycine sensitivity between GlyT2 and GlyT1.
Discussion: Oleoyl-L-carnitine and N-arachidonyl-glycine represent a novel class of lipid-based inhibitors of glycine transport by GlyT2, which have the potential for further development as analgesics.

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A bitopic (orthosteric/allosteric) ligand can differentiate monomeric from dimeric forms of a Family A G protein-coupled receptor

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Introduction: The dopamine D_2 receptor (D_2R), a prototypical Family A G protein-coupled receptor (GPCR) is an important therapeutic target for the treatment of central nervous system disorders including schizophrenia. SB269652 was identified as the first drug-like allosteric modulator of this receptor.
Aims: To understand the mechanism of action of SB269652 at the D_2R
Methods: Progressively truncated derivatives of SB269652 were synthesized and characterized at the human D_2R using both functional assays and radioligand binding studies. We used a novel functional D_2R complementation system to control of the identity of the individual protomers comprising a dimeric D_2R signalling unit.
Results: We identified a purely orthosteric pharmacophore and a purely allosteric pharmacophore indicating that the parent molecule represents a hitherto-unappreciated bitopic (dual orthosteric/allosteric) ligand. Using the complementation system we show that SB269652 exerts its cooperative effect by binding in a bitopic mode within one protomer but allosterically modulating the other. Mutational impairment of SB269652 binding to one protomer converts the interaction between the bitopic molecule and dopamine into simple competition, thus indicating that the molecule has the ability to differentiate monomers from dimers via switching between allosteric and competitive pharmacology. We used SB269652 to demonstrate the presence of D_2R oligomers in rat striatum.
Discussion: By utilizing a combination of biochemical, cellular and functional complementation assays, medicinal chemistry, analytical pharmacology and molecular modelling, we have identified and validated a chemical probe with a unique mechanism of action, characterized by a "switch" in pharmacology from allosteric to competitive, depending on whether the interaction is occurring at a functional monomeric versus dimeric (or higher order) Family A GPCR.

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Delineating determinants of cooperativity, affinity and bias for mGlu5 allosteric modulators

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Introduction. Metabotropic glutamate receptor 5 ($mGlu_5$) has emerged as an exciting new therapeutic target; $mGlu_5$ enhancers are desired for treatment of schizophrenia and cognitive disorders, whilst inhibitors are being sought for autism and depression. Traditionally, efforts have sought to competitively mimic or block glutamate activity. An alternative approach is to target distinct allosteric sites; these compounds are termed allosteric modulators. Negative allosteric modulators (NAMs) inhibit, while positive allosteric modulators (PAMs) enhance, the activity of glutamate. $mGlu_5$ allosteric modulator structure-activity relationships are notorious for being either "steep" or "flat" and also for their propensity to show "molecular switches" whereby a PAM is derived from a NAM scaffold or vice versa. In addition, there is evidence for multiple allosteric sites and biased modulation.
Aims. Identify the molecular determinants that govern $mGlu_5$ allosteric modulator affinity and cooperativity.
Methods. Mutations were introduced into $mGlu_5$, guided by a comparative model of $mGlu_5$ based on the β_2-adrenergic receptor crystal structure. All mutations were assessed for perturbation of allosteric modulation of glutamate induction of intracellular calcium mobilization. Where practical, effects on ligand affinity were quantified utilizing the radiolabelled allosteric ligand [^{3}H]methoxyPEPy.
Results. We identified novel mutations within the transmembrane domains that influence allosteric modulator binding and cooperativity, including residues that differentially affect PAMs compared to NAMs. Interestingly, point mutations in TMs 6 and 7 were discovered that engendered "molecular switches" in modulator cooperativity, such that PAMs became NAMs or neutral modulators or NAMs behaved as PAMs.
Discussion. Use of our homology model combined with the systematic mutagenesis performed will provide tools to drive our understanding of how allosteric modulators exert their effects. Ultimately, these studies will aid drug design efforts, to rationally predict pharmacological profiles and minimize undesirable activities.

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Nordihydroguaiaretic acid activates human TRPA1

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Introduction. Nordihydroguaiaretic acid (NDGA), is an anti-oxidant and broad spectrum lipoxygenase inhibitor that has been used in topical medications for skin conditions and trialled as an anti-caner drug. While assessing the role of arachidonic acid metabolites in the activation of the human transient receptor potential ankyrin repeat 1 (hTRPA1) channel, we sought to inhibit lipoxygenase activity with NDGA.
Aims. To define the effects of NDGA at hTRPA1.
Methods. HEK 293 cells stably transfected with hTRPA1 or a mutant where cysteine residues Cys619, Cys639 and Cys663 had been changed to alanine (3xCys mutant) were grown in 96 well plates, loaded with a proprietary calcium sensitive dye (Molecular Devices) and intracellular calcium ($[Ca]_i$) levels measured in a Flexstation 3 microplate reader.
Results. At 37°C, NDGA increased $[Ca]_i$ in hTRPA1 expressing cells with a pEC_{50} of 5.4 ± 0.1, and a maximum increase in fluorescence of 402 ± 30% (n=5). Cinnamaldehyde, a prototypic TRPA1 agonist, elevated $[Ca]_i$ with a pEC_{50} of 4.95 ± 0.05, with a maximum increase of 480 ± 20% (n=5). The effects of NDGA were blocked by the TRPA1 inhibitor HC 030031, and strongly reduced in the 3xCys mutant (100 ± 16% increase in $[Ca]_i$ at 100 μM NDGA). The effects of cinnamaldehyde were virtually abolished in the 3xCys mutant (42 ± 7% increase at 300 μM cinnamaldehyde). The maximum effect of both NDGA (550 ± 80%) and cinnamaldehyde (690 ± 40%) were increased when the experiments were performed at 24°C.
Discussion. We have shown that NDGA, a compound widely used to inhibit lipoxygenase activity in studies of animal nociception and previously used as a drug in humans, is an agonist at TRPA1, an ion channel associated with nociception and inflammation. The contribution of actions at TRPA1 to the biological effects of NDGA remain to be established.

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Pharmacoregulation of distinct molecular phenotypes of the human CaSR caused by naturally occurring mutations

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Introduction. More than 200 naturally occurring mutations have been identified in the human CaSR, which are linked to dysregulation of extracellular Ca^{2+} homeostasis. They have classically been termed "loss-" or "gain-of-function" mutations, which is an oversimplification given that amino acid changes can alter numerous molecular properties of a receptor.
Aims. To characterise 21 naturally occurring CaSR mutations and determine their effects on CaSR pharmacoregulation.
Methods. Cell surface expression levels were measured using flow cytometry and measurements of intracellular Ca^{2+} mobilization and ERK1/2 phosphorylation were used to monitor receptor signalling.
Results. We identified distinct molecular phenotypes caused by naturally occurring amino acid substitutions, which include combinations of loss- and gain-of-expression and intrinsic signalling capacity. Importantly, we have identified some mutations that result in receptor conformations that differentially alter receptor coupling preferences across two pathways. Cinacalcet, a positive allosteric CaSR modulator predominantly used to treat secondary hyperparathyroidism, rescued functional impairments in all loss of function/expression mutant CaSRs via a restoration of receptor expression and/or signalling. Similarly, the negative modulator, NPS-2143, corrected the signalling of gain-of-function mutants, indicating that these drugs may be effective in treating a substantial number of patients. NPS2143 also effectively rescued mutants whose cell surface expression was substantially impaired, suggesting that both positive and negative allosteric modulators can stabilise a receptor conformation that is trafficked more effectively to the cell surface.
Discussion. These findings have important implications for understanding the causes of diseases linked to the CaSR and for the treatment of these diseases using allosteric CaSR modulators. A full understanding of the molecular effects of these amino acid changes and their effects on the pharmacoregulation of the CaSR, will enable the development of therapeutics that specifically target the molecular determinant of impairment in the receptor.

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Newer drugs in older people: better the devil you know when it comes to medication safety?
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Australian consumers have been recently advised that they should wait at least seven years from the date of release on the market to take any new drug, unless it is a rare 'breakthrough' drug.[1] In part, this was based on analyses of new drug entities approved by the US Food and Drug Administration and subsequent post-marketing labelling changes or product withdrawal after the drugs had been used in large numbers of patients.[2,3] Similar analyses have been performed on drugs approved in Canada.[4]
The rationale for this advice will be discussed and analysed, with particular reference to the marketing and clinical use of newer antithrombotic drugs.

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Complex patients, complex models: PBPK made easy?
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The application of physiologically-based pharmacokinetic (PBPK) modelling is coming of age in drug development and regulation, reflecting significant advances over the past 10 years in the predictability of key pharmacokinetic parameters from human *in vitro* data and in the availability of dedicated software platforms and associated data bases. With respect to understanding co-variates and variability, the quantitative impact of drug-drug interactions, age, genetics, racial differences, food effects and pharmaceutical formulation have been assessed. In principle, it is also possible to incorporate pathological features in PBPK models to predict PK in specific disease states defined by aetiology and/or severity. These extensions of PBPK modelling, along with the incorporation of the PK of biologicals and moves towards linking PBPK to pharmacodynamic outcome, are clearly of benefit in understanding extremes of risk in different patient populations as part of the process of drug development. Apart from this application, PBPK also has potential use in the health care arena as an educational tool and for the provision of computerised, 'point of care' advice on personalised drug dosage. Multi-drug treatment of the complex patient (*e.g.* an elderly, obese lady with cardiac failure, rheumatoid arthritis, renal impairment, Alzheimer's disease, and a 'poor metaboliser' to boot) is a considerable clinical challenge. One day, when sufficient information is available on the patient, clinicians may be able to link that person to his or her virtual twin within a PBPK-PD model on an iPad to provide safe, effective, individualised dosage, and to avoid undesired drug-drug interactions. If the physician considers this too complex, the friendly clinical pharmacist will be looking over his/her shoulder to provide further guidance. Although there are no simple solutions to this complexity, we should neither fear nor ignore it.