100

And then there were three – lessons from a convoluted drug development project

Martin C. Michel, Dept. Pharmacology, Johannes Gutenberg University, Mainz, Germany

The existence of β_3-adrenoceptors (B3AR) only became accepted after its cloning in 1989. Initial attempts to develop B3AR-targeted drugs for the treatment of obesity and type 2 diabetes failed, because species differences in ligand recognition profiles, tissue distribution and functional role had been underappreciated. Around the year 2000 some of these programs were repurposed to develop B3AR agonists as treatment of the overactive bladder syndrome. The translational pharmacology programs accompanying the clinical development of B3AR agonists faced several challenges. First, concepts of the pathophysiology underlying urinary bladder dysfunction were only poorly developed and have considerably emerged in the past 15 years. Specifically, the smooth muscle-centric view of bladder function changed into a multi-player concept including urothelium, afferent nerves and blood vessels. This questioned the original rationale for B3AR agonists, which had been based on muscle strip experiments. Second, the overall role of B3AR in humans was largely unknown and no validated tools existed for their detection at the protein level, complicating prediction of possible side effects in patients. Third, the selectivity of pharmacological tools for functional studies was limited, often leading to false conclusions. Fourth, B3AR polymorphisms had been described but their impact on target function was controversial. Fifth, generating meaningful selectivity in agonists is much more difficult than in antagonists as cells expressing another receptor subtype but high receptor reserve may show responses even if the agonist occupies only a minor fraction of these receptors. The concept of biased agonism also evolved only while clinical development was already ongoing. Finally, any new treatment concept requires supporting mechanistic data to obtain acceptance and support of the concept among scientists and physicians. Despite these challengers, the first B3AR agonist has meanwhile been launched successfully in major markets. A translational pharmacology program was a relevant success factor by combining in-house data of the originating company, collaboration with leading academic investigators and contract research organizations, complemented by data generated independent of the originating company.

101

Pharmacogenetics including personalisation of treatment in cardiovascular medicine through next generation sequencing

Ana Alfirevic. The Wolfson Centre for Personalised Medicine, Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, L69 3GL, UK

Pharmacogenomics has remarkable potential to maximise benefit and minimise harm associated with drug therapy. Our rapidly increasing knowledge of all genes in the human genome together with the development of multiparallel, next generation sequencing technology, facilitates the rapid discovery of genetic variants that lead to variable dose requirements, reduced efficacy and increased risk of toxicity of cardiovascular drugs. However, implementation of genetic testing into clinical practice has been rather slow. Our research within the Wolfson Centre for Personalised Medicine in Liverpool aims to facilitate implementation of personalised medicine into clinical practice by incorporating clinical and basic research, training of clinicians and scientists, regulatory authorities' involvement and patient and public engagement. We will discuss four phases in translation of a biomarker into clinical practice from discovery, clinical validity and utility to implementation and the effect of testing on public health. We will use cardiovascular drugs as paradigms for pharmacogenetic study design which includes randomised controlled trials and implementation studies using point-of care devices related to genotype-guided dosing of coumarin derivatives. We will also discuss next generation sequencing approaches to identify rare variants that may lead to severe and potentially life threatening adverse reactions such as statin-induced myotoxicity and angiotensin-converting-enzyme (ACE) inhibitor-induced angioedema.

Several barriers to implementation of novel biomarkers into clinic include strength of evidence, training and education and patient and public- engagement. Our multi-disciplinary team and global collaborations as well as access to electronic healthcare records facilitate assessment of patient outcomes in real-world clinical settings with the aim to personalize patient treatment.

Alfirevic et al. (2013) Pharmacogenomics 14(8):861-7.
Alfirevic et al. (2014) Clin Pharmacol Ther 96(4):470-6

102

Pharmaceutical drug misuse: Implications for post-market surveillance

Nick A Buckley[1,2]. Pharmacology, University of Sydney[1], Sydney, NSW; RPA Hospital/NSW Poisons Information Centre[2], Sydney, NSW.

In recent years, pharmaceutical drug misuse has apparently become more prevalent than illicit drug misuse. This is seen in hospital presentations and coronial data. This is generally perceived to be a rapidly expanding problem to be stamped out; but the effects of rescheduling to reduce abuse and reformulation for abuse deterrent formulations have been mixed and not always resulted in a change in misuse or improved health outcomes. The better information on misuse of pharmaceutical products (cf illicit substances) opens up new opportunities for exploring the spectrum and trajectory of misuse in the community.

Roughly 6% of premature loss of life in Australia is attributed directly to adverse drug effects or poisoning (ABS 2009) and a similar or greater proportion of admissions to hospital. It is generally accepted that there are many medication safety issues that will only become apparent after marketing approval and misuse is one of them. In most OECD countries there is a program of post-marketing surveillance which are summarised in Risk Management Plans (RMPs) for each drug that attempt to identify and mitigate these risks. However, few of these contain an active or specific plan to monitor for misuse, even for products extremely likely to be subject to misuse.

Passive 'routine post marketing surveillance' is generally the core component of these RMPs. This is currently under-resourced, heavily reliant on spontaneous reports and haphazard. There is extreme underreporting of the details on serious or fatal adverse drug events, and in particular those due to misuse or overdose. Consequently, interpretation of any 'signals' generated or lack thereof is problematic. However, patterns of drug dispensing that are highly likely to indicate misuse can be seen in routinely collected data that contains person level data. A final problem is that the translation of identified risks into stronger drug regulation has to overcome a legislative framework that favours individual and company 'rights' over broader public health considerations.

103

Opioid substitution therapy among prisoners: longitudinal findings from a data linkage study

N Gisev[1]. National Drug and Alcohol Research Centre, University of New South Wales, Sydney, NSW

Introduction. Prisoners experience high rates of opioid and other drug dependencies, health problems and premature mortality. While in prison, opioid dependent people face increased mortality risks upon entry into prison and after their release, yet the effectiveness of opioid substitution therapy (OST) in reducing mortality has not been examined.
Aims. To evaluate the impact of OST in reducing mortality among opioid dependent people in prison, and after prison release, as well as examine the cost-effectiveness of post-release OST in reducing mortality.
Methods. Population-based, retrospective data linkage study using records of OST entrants in New South Wales, with data on charges and court appearances, prison episodes and death notifications. Multivariable Cox regressions were undertaken to examine the association between OST exposure and mortality.
Results. A total of 16 715 opioid-dependent people entered prison between 2000 and 2012. Individuals were in prison for 30 998 person-years (PY), during which time there were 51 deaths. The all-cause crude mortality rate (CMR) in prison was 1.6/1000 PY (95% CI 1.2 to 2.2/1000 PY). Compared to time out of OST, the hazard of all-cause death was 74% lower while in OST (Adjusted Hazards Ratio (Adj. HR) 0.26; 95% CI 0.13-0.50). A total of 16,453 people were released from prison at least once and 1,050 deaths occurred. Individuals were receiving OST in 51% of releases. Lowest post-release mortality was among those continuously retained in OST post-release (CMR 4-weeks post-release: 6.4 per 1,000PY; 95% CI 5.2-7.8). OST exposure in the four weeks post-release reduced the hazard of death by 75% (Adj. HR 0.25; 95%CI 0.15, 0.52); OST receipt in prison had a short-term protective effect that decayed quickly across time. In the cost-effectiveness analysis, the final average costs were lower for the group that received OST post-release ($7206 versus $14 356). The incremental cost-effectiveness ratio showed that OST post-release was dominant, incurring lower costs and saving more lives. The probability that OST post-release is cost-effective per life-year saved is 96.7% at a willingness to pay of $500.
Discussion. OST treatment in prison and immediately post-release, is highly protective against mortality both while in prison and after release. This study provides strong evidence to support the value and cost-effectiveness of OST programs within the criminal justice system, highlighting the need for OST programs in prison to be scaled up and that the continuation of OST post-release be maximised.

104

Misuse of herbal medicines: An emerging issue

Ian F Musgrave[1], Claire L Hoban[1], Rachael L Farringtion[1], Roger W Byard[2,3]. Discipline of Pharmacology, University of Adelaide[1], Adelaide, SA; Dept of Pathology, Forensic Sciences SA[2], Adelaide, SA; Dept of Pathology, University of Adelaide[3], Adelaide, SA.

Herbal medicines have become increasing popular in developed nations, including Australia. While there are significant concerns over the efficacy and safety of herbal medicines when used as directed, less well recognised is their increasing potential for misuse. Misuse is a broad classification, but for the purposes of herbal medicines we can concentrate on the following: use for non-clinically indicated conditions (for example the anti-depressant St. John's Wort for "sadness"), use with contraindicated conventional medications or herbal medicines, poisonings and suicides. Drug-Herb interactions are of the most concern as they have significant potential for serious or fatal outcomes. Adverse drug reactions and poisoning notifications for herbal medicines have steadily increased over recent years, with children under 5 being most affected group in terms of poisonings. Herbal weight loss products are over represented in poisonings and admission to emergency wards. A significant contribution to these adverse events is the popular belief that herbal products are natural and therefore safe. This is exacerbated by inadequate safety and usage information provided for herbal medicines. The use of herbal medicines for suicide is surprising, given their reputation for safety. The herbals most used in suicide attempts appear to be St. John's Wort and Ephedra containing herbals. With increasing use of herbals, especially with purchase via the internet, their misuse will continue to grow. Changes to herbal medicine labelling, health care provider and consumer education programs are needed to limit this growth

Gryzlak BM, et al., (2007). Pharmacoepidemiology and drug safety. 16: 947-957.
Geller AI, et al., (2015). N Engl J Med. 373: 1531-40s

105

Trends in pharmaceutical drug-related harms

Belinda Lloyd[1]. Turning Point, Eastern Health, and Eastern Health Clinical School, Monash University[1], Melbourne, VIC

Introduction. There has been growing concern in Australia and internationally regarding harms associated with the misuse of pharmaceutical drugs. Increases in prescribing, availability and diversion have also been identified as cause for concern, with calls for strategies to address harms associated with prescribed drugs.

Methods: Through examination of multiple population level data sources, this presentation explores trends and characteristics of pharmaceutical drug-related harms, including demographic, geographic and temporal factors, as well as patterns of drug involvement and the nature of harms experienced.

Conclusions: Through identification of populations most at risk of harm, and patterns of prescribing and prescription drug use that contribute to drug-related harms, appropriate and evidence-based prevention and intervention strategies can be developed that support prescribers, patients and the community.

106

Development of novel anti-fibrotic treatment for chronic kidney and heart disease

Darren J Kelly, Yuan Zhang Department of Medicine, St Vincent's Hospital, The University of Melbourne, VIC.

Introduction. Fibrosis is a final common pathway in the development of chronic kidney and heart disease. Locally active profibrotic cytokines have been implicated in the pathogenesis of fibrosis. The use of drug interventions for intensive control of blood glucose and blood pressure, and in particular, blockade of the renin-angiotensin system merely delays fibrosis progression in both diabetic and non-diabetic chronic kidney and heart disease patients. We have developed a library of novel compounds with enhanced anti-fibrotic actions than that of tranilast, a known anti-fibrotic agent that is approved for the treatment of asthma, allergic rhinitis and atopic dermatitis in humans.

Aims. We sought to evaluate the effects of a series of novel, small molecule anti-fibrotic drugs (FT compounds, Fibrotech Therapeutics, Australia), in experimental rat models of chronic kidney and heart disease in the absence and presence of diabetes.

Methods. *In vitro*, we studied effects of FT compounds on cell proliferation and collagen synthesis in cultured mesangial cells and cardiac fibroblasts in response to profibrotic cytokines stimulation. *In vivo*, we evaluated anti-fibrotic therapeutic efficacy in clinical predicative rat models of chronic kidney disease (sub-total nephrectomised rat), diabetic nephropathy (STZ-diabetic HTZ Ren-2 rat), chronic heart failure (myocardial infarction rat) and diabetic cardiomyopathy (STZ-diabetic HMZ Ren-2 rat).

Results. In the *in vitro* setting, FT compounds inhibited cell proliferation and collagen synthesis in response to profibrotic cytokines stimulation in cultured mesangial cells and cardiac fibroblasts. Consistent with these *in vitro* actions, treatment of sub-total nephrectomized or STZ-diabetic HTZ Ren-2 rat with FT compounds was associated with a reduction in proteinuria, glocmerulosclerosis and tubulointerstitial fibrosis. Similarly, FT compounds preserved both systolic and diastolic function along with a reduction in myocyte hypertrophy and interstitial fibrosis in either myocardial infarction or STZ-diabetic HMZ Ren-2 rat.

Discussion. Together these studies suggest that inhibiting fibrosis has the potential to protect the kidney and heart from progressive injury in both the diabetic and non-diabetic setting. FT compounds, currently in clinical development, is a first-in-class drug therapy to treat the underlying pathological fibrosis associated with chronic kidney and heart disease.

107

Role of oxidative stress in the development of pulmonary fibrosis

Ross Vlahos. School of Health Sciences & Health Innovations Research Institute, RMIT University, Bundoora, VIC, Australia.

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease characterized by progressive fibrosis of the alveolar interstitium. IPF affects more than 5 million people worldwide and the median survival rate for IPF patients is less than 3 years. Unfortunately, there are currently no effective therapies that have been shown to influence survival. The aetiology of IPF is still unknown but the pathogenesis is thought to involve abnormal re-epithelialization and dysregulated remodelling of the extracellular matrix (ECM) after alveolar injury. It is now evident from human and animal studies that oxidative stress is a key mediator in the pathogenesis of IPF. Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS) in excess of the capacity of cells/tissues to detoxify or scavenge them. Such a state of oxidative stress may alter the structure/function of cellular macromolecules that eventually leads to tissue/organ dysfunction. Lung tissues from IPF patients demonstrate "signatures" of chronic oxidative damage. Bronchoalveolar lavage fluid (BALF) isolated from IPF patients has elevated levels of oxidative damaged proteins (e.g. complement C3, transferrin, immunoglobulin light chains, immunoglobulin A), increased eosinophilic mediators and myeloperoxidase concentrations, and enhanced levels of lipid peroxidation products (e.g. 8-isoprostane), than controls. Neutrophils, macrophages and eosinophils from BALF of IPF patients overproduce ROS causing epithelial injury. Increased activation and abundance of these inflammatory cells in IPF may explain the high levels of hydroxyl radical and superoxide anion concentrations in IPF. In addition, extracellular matrix degradation products produced by ROS may promote fibrogenesis by influencing epithelial, mesenchymal and inflammatory cell activity. Recent studies have demonstrated a critical role for the ROS-generating enzyme NADPH oxidase-4 (Nox4) in pulmonary fibrosis. Lung tissues from human subjects with IPF had high expression of Nox4 in fibroblastic foci. Furthermore, *in vivo* knockdown of Nox4 and pharmacologic targeting of Nox4 during the persistent phase of lung fibrosis in aged mice restored the capacity for fibrosis resolution. In conclusion, targeting oxidative stress may be a valuable strategy to help treat IPF.

108

Relaxin: A pleiotropic hormone with potent anti-fibrotic actions

Chrishan S. Samuel[1]. Dept of Pharmacology, Monash University[1], Clayton, VIC (introduced by K. Pfleger, University of Western Australia, Perth, WA).

Originally described for its ability to remodel the birth canal during pregnancy, the hormone relaxin has emerged as both an endogenous inhibitor of collagen turnover and a potential therapy for the progressive fibrosis that results from aberrant wound healing, and which eventually contributes to end-stage organ disease. Based on the latter and the added ability of relaxin to promote systemic vasodilation and increase global arterial compliance, serelaxin (recombinant human relaxin-2) was developed and is currently being assessed in clinical trials as a treatment for acute heart failure. Furthermore, serelaxin has now been shown to rapidly inhibit (and in many cases reverse) fibrosis in diverse experimental models of dermal, cardiovascular, renal, pulmonary/airway and hepatic disease, regardless of etiology. Importantly, serelaxin only inhibits pro-fibrotic cytokine (angiotensin II, TGF-β1, interleukin-1β)-stimulated collagen and fibronectin deposition in primary fibroblast culture models *in vitro* and animal models of injury/disease *in vivo* without affecting basal matrix turnover; suggesting that it is a safe anti-fibrotic. By interfering with TGF-β1 signal transduction, at the level of Smad2 phosphorylation, serelaxin primarily acts to prevent TGF-β1-induced myofibroblast differentiation and aberrant collagen production. An added feature of the drug is its ability to degrade existing collagen via the promotion of various matrix metalloproteinases and inhibition of tissue inhibitors of metalloproteinase activity. Recent findings have also demonstrated a superior anti-fibrotic efficacy of serelaxin compared to ACE inhibition[1] and angiotensin receptor blockade in experimental models of heart and kidney disease. These combined actions along with its angiogenic and wound healing properties highlight its potential as an anti-fibrotic therapy.

[1]Samuel CS et al (2014) Hypertension 64:315-322

109

Insulin regulated aminopeptidase: A novel target to treat cardiac fibrosis

Tracey A Gaspari[1], Huey Wen Lee[1], Siew Yeen Chai[2], Robert E Widdop[1]. Dept of Pharmacology[1], Dept of Physiology[2], Monash University, Clayton, VIC. (introduced by K. Pfleger, University of Western Australia, Perth, WA).

Cardiovascular diseases (CVDs) remain the world's leading cause of morbidity and mortality with risk factors such as ageing, excessive acute (e.g. myocardial infarct) or chronic (e.g. hypertension) cardiac injury, leading to increased cardiac fibrosis, chronic heart failure and/or end organ damage. Despite clinical advances there are still relatively few effective treatments directed against fibrosis, with inhibition of the renin angiotensin system (RAS) by either angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor subtype 1 blockers (ARBs) being the current standard of care in fibrotic indications. However, these drugs cause only a modest regression of fibrosis. Therefore development of cardio-protective agents alone or in combination with standard care is urgently warranted given our ageing population. Here I will present the work of our group examining the therapeutic potential of targeting the enzyme, insulin regulated aminopeptidase (IRAP), for the development of such cardioprotective agents based on 3 striking observations: (1) IRAP expression is upregulated in cardiac tissue in pathophysiological states; (2) age-induced cardiac fibrosis is absent in 2-year-old IRAP-deficient mice, with hearts from these aged mice resembling the organs of young mice, and (3) first-in-class specific IRAP inhibitors completely reverse age-induced cardiac fibrosis. These exciting observations build on our early data of vaso- and athero-protective effects mediated by IRAP inhibitors. Collectively, these findings suggest that IRAP plays a key role in the pathogenesis of cardiovascular disease and highlight the potential of pharmacological inhibition of IRAP as a novel therapeutic strategy, particularly for difficult-to-treat end-organ damage that occurs with ageing and/or hypertension.

110

The Pharmacokinetic intersection of academia, hospital and pharmaceutical industry

Vidya Perera[1,2], Brenda Cirincione[1,2], Department of Clinical Pharmacology and Pharmacometrics, Bristol-Myers Squibb[1], Princeton NJ, USA; School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo[2], Buffalo NY, USA.

Introduction. Pharmacokinetics is commonly understood as *'what the body does to the drug'*. Expansion of traditional pharmacokinetics to population pharmacokinetics, pharmacokinetic/pharmacodynamic modelling and optimal sampling have enabled greater applications to clinical settings. Assessment of a drug's clinical or therapeutic utility index, obtained through the integration of pharmacokinetic and pharmacodynamic (e.g. clinical endpoints) data, in addition to real world effects seen in clinical trials such as placebo response and dropout patterns, provides a mechanism to translate information from multiple sources into information that is meaningful to treatment providers. These modelling approaches have been shown useful in the pharmaceutical industry to assess key claims of the benefit:risk ratio of drugs when comparing treatment options.

Aims: This presentation will highlight examples of leveraging existing pharmacokinetic data and the role this plays in the development of clinical utility scores to compare the benefit:risk profile of drugs. The presentation will discuss key elements of pharmacokinetic, pharmacodynamic, placebo response and dropout response models in order to capture the necessary information to compare and contrast medicines in a fair and objective manner. The role that academia, hospitals and the pharmaceutical industry can play in obtaining data through industry research and through investigator initiated trials, the development of these models and the challenges in their application will also be discussed. Several therapeutic areas including psychiatry and cardiovascular disease will be highlighted.

Discussion: The integration of knowledge from multiple sources may enable a more 'hypothesis-driven' approach to generating objectives and may also provide additional support when seeking research funding. The information required for the development of clinical or therapeutic utility scores require input from academicians, industry scientists and clinicians to assess the most appropriate pharmacokinetic data, modelling methodologies and clinical endpoints. This tool serves as an ideal example for a potential framework to serve as a basis for translational research across environments which centre on benefiting the patient.

111

Dose individualisation of sunitinib and tamoxifen

Howard Gurney, Westmead Hospital, Sydney, NSW

Most anti-cancer drugs have a narrow therapeutic window. Despite this, individualisation of dose has not advanced appreciably since the introduction of body surface area in the late 1950s. Over the last 20 years we have undertaken a number of studies examining the use of various measures of drug elimination for dose individualisation, including: phenotype markers, pharmacogenomics, toxicity-adjusted dosing and therapeutic drug monitoring (TDM). Phenotype and genotype markers for CYP3A and ABCB1 were not found to be of practical use for epirubicin, vinorelbine, imatinib or sunitinib. TDM may be of potential use for monitoring anti-cancer drugs that are given orally, as well as intermittent agents with long half-life such as monoclonal antibodies. We have evidence that TDM is superior to *CYP2D6* genotype for prediction of endoxifen level in breast cancer patients treated with tamoxifen, and that TDM may also be useful for sunitinib in renal cell cancer. Toxicity-adjusted dose (TAD) requires a toxicity-anti-cancer effect relationship and this has now been shown for multiple agents and preliminary evidence of its practical application will be presented for sunitinib. On the other hand, we found no relationship between tamoxifen-induced toxicity (hot flushes) and endoxifen levels. Conclusion: Dose individualisation of anti-cancer drugs is critical, especially for those with a narrow therapeutic window. It is important that the oncology community recognises the importance of this area and incorporate markers that account for variation in drug elimination in early phase trials of new agents, as well as for established drugs.

112

Across the continuum: DMPK experiences from preclinical to market

Bill J Smith[1], Yazdi K Pithavala[2], Michael Zientek[3], Theunis Goosen[4], Jean-Claude Marshall[4]. Drug Metabolism, Gilead Sciences[1], Foster City, CA; Clinical Pharmacology[2], Pharmacokinetics, Dynamics and Metabolism, Pfizer, San Diego, CA[3], and Groton[4], CT, USA.

Introduction. This presentation will describe in vitro and in vivo studies to understand the metabolism and disposition of axitinib, a medication for advanced renal cell carcinoma.

Aims. The studies in the presentation were conducted during the continuum from discovery, development and post-market to fully describe the disposition, metabolism, pharmacokinetics (PK) and pharmacogenomics of axitinib.

Methods. In vitro metabolism, phenotyping, radiolabeled mass balance, metabolite identification, PK and genotyping studies were conducted using well established methodologies. As development continued, new advances in the field were considered and applied, even during the post-marketing phase.

Results. Axitinib is a low dose medication (5 mg, twice daily) that is metabolized mainly by CYP3A4 with lesser contributions from CYP3A5, CYP2C19, CYP1A2, and UGT1A1. UGT1A3 and UGT1A9 are minor contributors relative to UGT1A1. Sulfoxidation and N-glucuronidation of axitinib yield inactive metabolites that circulate in plasma followed by further metabolism to secondary metabolites identified in excreta. Two well controlled, retrospective genotyping studies have been performed to examine the involvement of polymorphic enzymes in PK variability. These studies were unable to associate PK variability with CYP3A4, CYP3A5, UGT1A1, or POR genotypes in addition to genotypes of other drug metabolizing enzymes and transporters.

Discussion. During the discovery and early development of axitinib it was clear that CYP3A forms were the main enzymes involved in axitinib clearance.   During late development and post-market, application of contemporary in vitro metabolism approaches provided stronger quantitative confirmation that CYP3A5 and UGT1A1 were minor enzymes contributing to axitinib clearance. This was further supported by retrospective genotyping studies, including newly discovered genotypes. During the sometimes lengthy period of drug development, advances in the field may become available and the sponsor may need to pursue new studies to support the understanding of the medication's PK/metabolism.

Smith et al. (2014) DMD 42:918-931. Zientek et al. (2015) DMD in review. Marshall et al. (2015) AHSG Conference Poster 688W, Baltimore, MD.

113

Medicines regulation and pharmacokinetics: An academic perspective

Andrew J McLachlan. Faculty of Pharmacy and Centre for Education and Research on Ageing, The University of Sydney and Concord Hospital, Sydney, NSW.

Understanding the fundamental aspects of drug and metabolite systemic exposure and the factors that influence this are essential for establishing the quality, safety and efficacy of a medicine. High quality and relevant pharmacokinetic information plays a critical role in the regulation of medicines and can guide their use once approved from marketing. The type of pharmacokinetic studies provided in regulatory submissions is typically informed by internationally harmonised regulatory guidelines. It is often emphasised that these documents provide "guidance" and not formal rules. This is particularly the case when evaluating novel therapeutics such as biological medicine products. Pharmacokinetic information provides the backbone of Australian Approved Product Information which informs the quality use of newer medicines. There is an increasing role of pharmacometric analyses in supporting regulatory submissions, informing recommended dosing regimens, dose adjustments in special patient populations, drug-drug interactions and investigating exposure-repose relationships. Innovations in pharmacokinetic study design and analysis by sponsors and academics needs to be matched by the expertise within the regulator and its expert committees. The move towards greater international collaboration between regulators creates some important opportunities for sharing the skills and expertise needed for a comprehensive regulatory framework, reflecting the global nature of the therapeutics industry. Finally, while biopharmaceutical and pharmacokinetic information plays an important role in medicines regulatory decisions, all of the evidence (related to safety and efficacy) needs to considered by the delegate when recommendations are made regarding market authorisation.

114

Sensory innervation of the gut and bladder - role of purines

Simon JH Brookes[1], Vladimir Zagorodnyuk[1,]. Human Physiology, FMST, School of Medicine, Flinders University, Adelaide, SA, Australia.

Introduction. Primary afferent (sensory neurons) are excited by mechanical, chemical, thermal and other forms of stimuli and trigger both sensations and motor responses. Classes of sensory neurons are sensitive to specific combinations of stimuli; this determines their role in behaviour. Distinguishing the classes of sensory neurons is important to allow pharmacological intervention to target specific sensations such as satiety, pain and urgency.

Aims. We have developed techniques to distinguish different classes of extrinsic sensory neurons to the gut and bladder by the morphology of their peripheral endings and relate this to their physiology and pharmacology.

Methods. Extracellular recordings are made from extrinsic nerve trunks close to isolated preparations of gut and bladder. Mechanical, thermal and pharmacological stimuli are applied to characterise responses. At the end of the recording, biotinamide is applied to the recorded nerve trunk and the filled sensory axon is identified by its location.

Results. Throughout the gut five major classes of extrinsic sensory neurons have been distinguished. In the upper gut, distinctive low threshold mechanoreceptors have distinctive intraganglionic laminar endings in enteric ganglia. They express P2x receptor immunoreactivity, are strongly excited by P2x agonists; an effect blocked by the P2x antagonist, PPADS. However, PPADS has no effect on their stretch-induced firing. Similar low threshold mechanoreceptors in the rectum are not excited by ATP. Another class of highly sensitive mechanoreceptors with endings beneath the mucosal epithelium are strongly activated by 5-HT but not by ATP. The most abundant extrinsic sensory neurons to the gut are polymodal mechano-nociceptors with transduction sites on intramural and extramural blood vessels. They are highly responsive to ATP, in addition to other inflammatory and damage mediators including bradykinin, glutamate, mast cell mediators (including histamine and proteases), nerve growth factor, prostaglandins, 5‑hydroxytryptamine and cytokines including IL‑1β, IL‑6 and TNF-alpha.

Discussion. The extrinsic sensory innervation of viscera comprises a number of discrete classes. Each expresses distinctive combinations of receptors for mediators that tailor their sensitivity to their roles in health and disease.

115

Effect of infection and inflammation on purinergic signalling in the urinary bladder: A trigger for Detrusor Overactivity?

Kylie J Mansfield[1]. Graduate School of Medicine, University of Wollongong[1], Wollongong, NSW

Detrusor overactivity is a debilitating disorder characterized by symptoms of urgency, frequency and nocturia usually with urge urinary incontinence. While this is a relatively common condition, affecting approximately 17% of people over the age of 40 years the etiology of detrusor overactivity remains poorly understood. Over the last 5 years a number of studies have exposed a role for infection and inflammation in the pathophysiology of many bladder pathologies including detrusor overactivity with infection demonstrated in up to 50% of patients with refractory detrusor overactivity. The symptoms associated with bladder inflammation, including urgency, frequency and pain have been proposed to be mediated by ATP binding to purinergic receptors on suburothelial afferent nerves. Enhanced release of urothelial ATP has been demonstrated in several models of chronic bladder inflammation including human diseases such as interstitial cystitis/ painful bladder syndrome and animal models of inflammation such as cyclophosphamide induced cystitis. However enhanced urothelial ATP release is not a universal finding with some studies of acute inflammation demonstrating a decrease in urothelial ATP release. In cell culture studies, decreased urothelial cell ATP release has been described in the presence of inflammatory mediators including histamine and serotonin. Similarly, incubation of urothelial cells with bacterial virulence factors including lipopolysaccharide and pyocyanin decrease ATP release. This decrease in ATP release seems to be counterintuitive given the accepted role of ATP in afferent signaling. This presentation will explore the factors associated with bladder infection and inflammation that could serve as triggers for the development of detrusor overactivity and could be responsible for the alterations in purinergic signaling seen in these patients.

116

P2X1-purinoceptor antagonists as potential male contraceptives

Sabatino Ventura. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC.

Introduction. According to the World Health Organization, there are 75 million unwanted pregnancies worldwide each year. Aside from the issue of global overpopulation, this is also a problem in affluent societies such as the U.S.A. and Australia. The NIH reported that in 2002, 49% or 2.65 million pregnancies (including abortions) in the U.S.A. were reported as unintended. Similarly, the Australian Bureau of Statistics recorded a teenage birthrate of ~15 per 1000 women in Australia in 2012, higher than most other developed countries. While present contraceptive methods are effective, there is clearly a need to develop additional methods of contraception for males, a market which is clearly lacking. Therapeutic targets for male contraception are associated with numerous problems due to their focus on disrupting spermatogenesis or hormonal mechanisms to produce dysfunctional sperm. This project describes the dual genetic deletion of α_{1A}-adrenoceptors and P2X1-purinoceptors in male mice thereby blocking sympathetically mediated sperm transport through the vas deferens during the emission phase of ejaculation.

Results. This modification produced 100% infertility without effects on sexual behaviour or function. Sperm taken from the cauda epididymides of double knockout mice were microscopically normal and motile. Furthermore, double knockout sperm were capable of producing normal offspring following intracytoplasmic sperm injection into wild type ova and implantation of the fertilized eggs into foster mothers. Blood pressure and baroreflex function was reduced in double knockout mice but no more than single knockout of α_{1A}-adrenoceptors alone.

Discussion. These results suggest that this autonomic method of male contraception appears free from major physiological and behavioural side effects. In addition, they provide conclusive proof of concept that pharmacological antagonism of the P2X1-purinoceptor and α_{1A}-adrenoceptor provides a safe and effective therapeutic target for a non-hormonal, readily reversible male contraceptive. A two pronged medicinal chemistry approach is currently under way to discover a suitable P2X1-purinoceptor antagonist to use in combination with tamsulosin for this purpose.

117

Postjunctional GPCR regulation of lower urinary tract purinergic responses

Russ Chess-Williams. Centre for Urology Research, Faculty of Health Sciences & Medicine, Bond University, Gold Coast, QLD

Neurotransmission in the autonomic nervous system usually involves multiple transmitter systems. In the bladder electrical field stimulation (EFS) results in the release of acetylcholine (Ach) and ATP as co-transmitters. Ach induces contraction via muscarinic M3 receptors which are G-protein coupled receptors coupled to IP3 and DAG, whilst ATP induces contraction via P2X1 receptors which operate as ion channels allowing sodium and calcium influx. Muscarinic M3 receptors and other GPCRs produce contractions not only via an increase in intracellular calcium, but also by inducing calcium sensitisation via the rho kinase pathway. Such a sensitisation may operate to enhance responses to ATP.

In porcine detrusor muscle, carbachol and NKA activate the rho kinase pathway via M3 and NK2 receptors respectively, and in doing so potentiate contractile responses to ATP. Thus detrusor responses to ATP (1mM) are potentiated by carbachol (1μM, 924±211%) and NKA (100nM, 226±56%), whilst these agonists do not potentiate each other. In the presence of the rho kinase inhibitor Y27632 (10μM) the potentiation of responses to ATP by carbachol was greatly reduced (70%), whilst the potentiation produced by NKA was abolished. This interaction may have consequences for neurotransmission where Ach and ATP co-release occurs. Thus electrical field stimulation of detrusor strips induced contractions that were reduced by 74±3% in the presence of atropine (10μM). However in the presence of NKA, the inhibitory effect of atropine was significantly reduced and atropine depressed responses by only 38±5% (P<0.05).

Conclusions and implications: Potentiation of detrusor responses to ATP occurs following stimulation of G-protein coupled receptors including M3 receptors. Since Ach and ATP are released as co-transmitters, full responses to ATP may be dependent on simultaneous M3 receptor stimulation and calcium sensitization. The potentiation of purinergic responses by GPCRs may influence the degree of atropine resistance of neurogenic detrusor responses, raising the possibility that detrusor responses to ATP may be altered by disease- or drug-induced changes in other receptor systems.

118

Standardised over-the-counter medicine labels – can we do it?

Vivien Tong[1], David K Raynor[2], Parisa Aslani[1]. Faculty of Pharmacy, Univ of Sydney[1], Sydney, NSW; School of Healthcare, Univ of Leeds[2], Leeds, UK.

Introduction. In 2012 and 2014, the Therapeutic Goods Administration initiated consultations proposing the implementation of a standardised over-the-counter (OTC) medicine label format in Australia. Previous research has indicated mixed consumer opinions regarding the proposed label format presented in the 2012 consultation paper (Tong et al, 2015). Furthermore, there is limited evidence demonstrating the usability of the proposed formats.

Aims. (i) To develop and examine the performance of alternative OTC medicine label formats for standardisation and; (ii) Explore consumer perspectives on the alternative label formats and required label improvements

Methods. Findings from an initial qualitative consumer needs analysis were reviewed by an international expert panel and used to guide the development of alternative label formats. A total of four alternative OTC label formats were developed for the exemplar medicine diclofenac. Individual face-to-face interviews with demographically matched cohorts of 10 consumers (total n=50) are currently being conducted to user test each alternative label format, as well as a current label for an existing diclofenac product. Each interview consists of: (i) administration of a user testing questionnaire to quantitatively measure consumers' ability to find and understand key points of information pertaining to diclofenac when using one of the labels and; (ii) a semi-structured interview exploring consumer perspectives.

Results. To date, 21 interviews have been conducted. Overall, the label formats have performed well in supporting consumers' ability to both find and understand key points of information for diclofenac such as the indication, dosage, maximum daily dose, contraindications, treatment duration and further information sources. When shown all labels, consumer perspectives on the label formats varied. Factors such as perceived usability, use of colour, design, content, and/or content ordering impacted consumer preferences and subsequent rankings of the labels.

Discussion. Despite the overall satisfactory performance demonstrated by the label formats, consumer perspectives are diverse and should be considered when working towards an OTC medicine label format for standardisation.

Tong V et al (2015) Health Expect. DOI: 10.1111/hex.12389.

119

How do the stakeholders perceive the changing situation in Australian community pharmacy?

Andi Hermansyah[1,2], Erica Sainsbury[1], Ines Krass[1]. Faculty of Pharmacy, University of Sydney[1], Sydney, NSW; Faculty of Pharmacy, Airlangga University[2], Surabaya, INDONESIA.

Introduction. Community Pharmacy (CP) in Australia has evolved in response to a variety of societal changes and other developments in health care. However, the government agenda to contain healthcare expenditure has influenced its viability. As a result, CP must adapt with the changing situation to survive. Understanding the context of contemporary CP is important to investigate the extent to which such changes affect CP practice.

Aims. To explore stakeholders' perspectives of contemporary CP, including facilitators and constraints in the development of professional practice.

Methods. In-depth, semi structured interviews were conducted from December 2014 to August 2015 with a range of CP stakeholders regarding contemporary CP practice in Australia. Interviews were recorded, transcribed verbatim and analysed for emerging themes. Ethics approval was obtained from the University of Sydney.

Results. A total of 27 key informants participated. Three main themes emerged from the data: the societal value of pharmacy, the economic context, and the policy context. Participants unanimously recognised the value to the community delivered by CP and the professional roles enacted by the pharmacist. Despite this, the role continues to focus predominantly on medicines provision and counselling. As a result, professional service oriented health care delivery is underdeveloped. In addition, over-reliance on a dispensing model, combined with price disclosure and the expansion of discount chemists, has influenced financial viability. Further, participants have differing views about the extent to which the Community Pharmacy Agreements (CPAs) have enabled or constrained developments in contemporary CP practice.

Discussion. CP is once again at the crossroads. The business model, centred on the dispensing function is under threat with reductions to its profitability. In this changing context, CP needs to adapt and innovate to address contemporary challenges. This may include optimising opportunities afforded both by CPA funded services and by emerging health care needs of the population to move towards a sustainable professional service model.

Pharmaceutical Society of Australia (2014)
Cross, P., & Annabel, B. (2015). AJP 96: 12-16

120

Unconscious implicit attitudes as a predictor of medicines adherence and self-medication

James A. Green[1], Mudassir Anwar[1], Pauline Norris[1], Oulmann Zerhouni[2], Aurélie Gauchet[2]. School of Pharmacy, University of Otago[1], Dunedin, NZ; Laboratoire Interuniversitaire de Psychologie, Université Grenoble Alps[2], Grenoble, FRANCE.

Introduction. Unintentional non-adherence most often means forgetting, whereas intentional non-adherence involves a conscious choose to stop taking a medicine. However, dual process models of cognition suggest that automatic unconscious processes can underpin conscious, apparently intentional, choices. That is, our prior experiences with medicines can produce attitudes and associations that we are not necessarily consciously aware of, and that these may be key predictors of intentional choice.

Aims. To determine whether implicit attitudes contribute unique prediction to both medicines adherence and to self-medication.

Methods. Study 1 was a prospective cohort study, with a random sample of 152 people aged 18-65 recruited from the Dunedin telephone directory. Each participant completed a series of questionnaires at Time 1, including an implicit measure of attitudes towards medicines (Green et al, in press). They were then followed up each day for 30 days and asked if they had experienced symptoms, and if so, what actions they took in response. Study 2 was a cross-sectional study, with a convenience sample of 74 participants. Participants completed implicit and explicit measures of attitudes towards medicines as well as measures of adherence.

Results. In Study 1, having a negative attitude towards conventional medicines predicted great use of self-medication, O.R. 0.51, $P < .05$. In Study 2, positive attitudes towards conventional medicines were associated with higher levels of adherence, $r (74) = .45$, $P < .01$.

Discussion. The implicit measure of attitudes to medicines was a strong predictor of both self-medication and medicines adherence, even when controlling for explicit attitude. Therefore, interventions to improve adherence and promote rational self-medication need to consider the role of automatic processes alongside conscious choice.

Green J et al (in press) J Health Psychol

121

What do consumers want to know about drugs (medication and lifestyle) in their pregnancy journey?

Eva L Pijpers[3,4], Treasure M McGuire[,1,2,5] Sanne Kreijkamp-Kaspers[3], Laura Deckx[3], Wendy Brodribb[3], Mieke L van Driel[3]

School of Pharmacy, The University of Queensland[1], Brisbane QLD; Mater Pharmacy Services, Mater Health Services[2], Brisbane, QLD; Discipline of General Practice, School of Medicine, The University of Queensland[3], Herston, QLD; Faculty of Medicine, Rijksuniversiteit Groningen[4], Groningen, the Netherlands; Faculty of Health Sciences & Medicine, Bond University[5], Gold Coast, QLD.

Introduction. Drug use during pregnancy is common; yet data to support safety are mainly case reports and retrospective studies. There is also growing evidence for harmful fetal effects when treatment of certain maternal conditions is inadequate. Seeking information is a coping strategy and can assist with decision-making.

Aim. This study aimed to identify the medicines information needs of women across pregnancy stages and explore whether their questions could be justified by actual pregnancy risk.

Methods. We conducted a retrospective, mixed method study of pregnancy-related calls to an Australian consumer medicines call centre, NPS Medicines Line (September 2002-June 2010). Call characteristics were compared for pregnancy (n = 4,573) and non-pregnancy (n = 118,547) questions. Drugs of interest, motivations to call and question themes were analysed across pregnancy stages, with medicines assessed for risk, using the Australian categorisation system for prescribing medicines in pregnancy. Call narratives were explored for stage-specific themes.

Results. Pregnancy-related enquiries were prompted more often by conflicting or inadequate information and request for second opinion. Most questions concerned safety. Psychotropic medication and fertility were strong drivers to seek information in preconception. Everyday illnesses and self-medication with Over-The-Counter products were of increasing concern as pregnancy progressed, with medicines classified as 'safe' accounting for 34% of questions.

Conclusion. Analysis of real world, pregnancy-related questions demonstrates that women are concerned about safe drug use in pregnancy and are likely to overestimate risk. Health care professionals should proactively address drug information gaps of women's pregnancy stage-specific information needs during routine consultations.

122

Over-the-counter supply of combination analgesics containing codeine in community pharmacy: A simulated patient study

Georgia A. Byrne[1], Pene J. Wood[1], M. Joy Spark[1]. School of Pharmacy and Applied Science, LaTrobe University[1], Bendigo, VIC.

Introduction. Over-the-counter combination analgesics containing codeine (OTC-CACC) are Pharmacist Only Medicines used for short term treatment of pain; misuse of OTC-CACC has become a significant health issue in Australia. Pharmacists are required to establish a therapeutic need for every sale. However current quantitative evidence on the practice behaviour of community pharmacists during OTC-CACC product requests is limited.

Aims. To investigate the current management of OTC-CACC requests in Australian community pharmacies.

Methods. A covert simulated patient (SP) methodology was used to observe the responses of pharmacy staff during an OTC-CACC request. SPs were trained for two scenarios involving a direct product request for Nurofen Plus® (OTC-CACCI, 200mg ibuprofen, 12.8mg codeine). Each SP provided identical information relating to reason for use, symptoms, and medical history; Scenario One (Sc1) SPs had no previous history of OTC-CACC use and Scenario Two (Sc2) SP had used OTC-CACCI regularly for the past month. Data were recorded immediately post-visit on a data collection form.

Results. SPs visited 38 metropolitan and 37 non-metropolitan pharmacies (73 Sc1 and 72 Sc2). An OTC-CACCI was purchased in 71% (75%, n=55 Sc1; 67%, n=48 Sc2) of pharmacy visits; an alternative treatment was recommended five times. Adequate counselling was received for 25% of product purchases for both scenarios. The SP for Sc2 was referred to a health professional in 19% (n=14) of visits. In 18% (n=19) of product purchases the SP was not provided with medication counselling. A positive outcome (Sc1-product with counselling, Sc2-referral, product, counselling) was reported in 19% of Sc1 visits and 5.6% of Sc2 visits. There was a significant association between scenario and outcome (χ^2(2, n=145) = 47.5, $p < 0.005$, Cramer's V = 0.57) with a large effect size.

Discussion. Outcomes varied depending on whether the requester was initiating or continuing treatment; a negative outcome for the requester was more likely for repeat purchase primarily because the need to refer was not identified. Overall minimal product advice was received and needs to be increased to ensure appropriate and safe use of these products.

123

Consumer perceptions of service quality in Australian community pharmacies

Ardalan Mirzaei[1], Stephen R. Carter[1], Carl R. Schneider[1]. Faculty of Pharmacy, The University of Sydney[1], Sydney, NSW.

Introduction. The landscape of Australian pharmacy has rapidly evolved in the past decade. As such, different marketing strategies have developed, yet little is known about how these strategies have influenced how consumers choose pharmacies. Understanding more about how consumers conceptualise service quality could assist pharmacies to improve market share. A theoretically grounded hierarchical model of service quality was developed within medical practice clinics (Dagger et al, 2007). The applicability of the model which includes four primary dimensions of service quality, has not been tested in the community pharmacy sector.

Aims. To validate a model of service quality within a pharmacy using a price-focused marketing strategy (PFMS).

Methods. A cross-sectional study was conducted using an electronic survey, completed by consumers while waiting for their prescriptions in an Australian metropolitan pharmacy. Inclusion criteria were adults who visited the pharmacy regularly for a prescription or non-prescription medicine. The 12 items pertaining to the four primary dimensions of service quality were subject to Confirmatory Factor Analysis (CFA) to confirm the hypothesised factor structure and to determine the psychometric properties of the sub-scales.

Results. Data from 145 respondents were analysed. Results indicate that among competing models, a three factor CFA model using 11 items provided an optimal fit for the data (TLI=0.945, CFI=0.975, RMSEA=0.073) while providing acceptable convergent & discriminant validity of the three derived scales: Interpersonal & Technical Quality; Environmental Quality; and Administrative Quality.

Discussion. It appears that the conceptual framework that consumers use to assess service quality within a PFMS pharmacy had three primary dimensions, counter to the four described by Dagger and colleagues. Within the present setting, consumers' assessments of interpersonal and technical qualities overlap considerably. Further survey research is planned with other pharmacies that use different marketing strategies. It is intended that the conceptual framework may be useful in the optimisation of marketing strategies for community pharmacies.

Dagger TS et al. (2007) Journal of Service Research. 10(2):123-142.

124

Examining hospital pharmacists' goals for medication counselling within the communication accommodation theoretical framework

Bernadette AM Chevalier [1], Bernadette M Watson [2], Michael A Barras [1, 3], W Neil Cottrell [1]; School of Pharmacy [1] and School of Psychology [2], University of Qld, Brisbane, QLD; Pharmacy Dept, Royal Brisbane and Women's Hospital [3], Brisbane, QLD

Introduction. Medication counselling opportunities are key times for pharmacists to speak to patients about their medications and any changes made during their hospital stay. Communication Accommodation Theory (CAT) posits that an individual's goals drive their communication behavior. The way in which pharmacists communicate with patients may be determined by the goals they set for these medication counselling sessions.

Aim. To examine hospital pharmacists' goals in patient medication counselling within the CAT framework.

Methods. Hospital pharmacist focus groups were held in two public hospitals. Eligible pharmacists had provided medication counselling within the previous six months (inpatient and/or outpatient settings). Interested pharmacists completed a demographic questionnaire, provided consent and attended a focus group designed to elicit their goals in patient medication counselling. Six one hour focus groups (24 pharmacists) were recorded and transcribed verbatim. NVivo® software was used to assist in coding and organisation. The codes were reviewed for reliability by pharmacists independent of the focus groups. An inductive thematic analysis was applied to the data.

Results. Saturation of information was achieved after four focus groups. Greater than 80% consensus was achieved for reliability of the identified codes. Patient-centred themes constructed from these codes were to build rapport, to empower patients and to improve patients' experience, health and safety. Exemplars provided by pharmacists for the goals of building rapport and empowering patients aligned well with five CAT communication behaviours (approximation, interpretability, discourse management, emotional expression and interpersonal control).

Conclusion. Patient-centred goals described by hospital pharmacists for medication counselling aligned well with CAT behaviors necessary for effective communication. These findings will be used to develop strategies for effective communication and inform data collection tools for the next phase of this project. This further research will determine to what extent these goals identified by pharmacists occur in actual medication counselling sessions.

125

Exploring the role of health beliefs in medication adherence in individuals with asthma

Holly Foot [1], Adam La Caze [1], Gina Gujral [1], Peter Baker [2] and Neil Cottrell [1]. School of Pharmacy, University of Queensland [1], Brisbane, QLD; School of Population Health, University of Queensland [2], Brisbane, QLD.

Introduction. Many individuals with asthma are not using their preventer medication regularly and this contributes to increased exacerbations, healthcare utilisation and mortality. Health beliefs appear to play an important role in medication adherence; however, the relationship is still poorly understood. A better understanding of the role of health beliefs in medication-taking in asthma is important to inform strategies on ways to support adherence.

Aim. To investigate the relationship between beliefs about medicines, illness perceptions and locus of control beliefs with medication adherence in individuals with asthma.

Methods. Adults prescribed a preventative asthma inhaler were recruited from community pharmacies and online through Asthma Australia. Participants completed a survey of validated questionnaires to elicit: medication adherence (Medication Adherence Report Scale [MARS®]), with a higher score indicating better adherence and: health beliefs (Beliefs about Medicines Questionnaire [BMQ], Brief Illness Perception Questionnaire [B-IPQ] and Multi-Health Locus of Control Scale [MHLCS]). Multiple linear regression with interaction effects was used to identify significant independent predictors of medication adherence and interactions between health beliefs.

Results. A total of 198 participants completed the survey, 161 (81.7%) were female and the mean (±SD) age was 39.8 (±12.7) years. The mean (±SD) MARS score was 19.2 (±4.5) with a range from 7 to 25. Doctor locus of control beliefs and concern beliefs independently predicted MARS score. Chance locus of control beliefs (MHLCS) moderated the relationship between concern beliefs (BMQ) and medication adherence. From the B-IPQ, understanding of illness moderated the relationship between treatment control beliefs and medication adherence. This model produced an adjusted R^2 =0.39, F (7,124) = 12.81, p=<0.001.

Discussion. Analysis of health beliefs identified two groups of individuals based on the relationship between their health beliefs and medication adherence. In those who have strong beliefs in chance to control their asthma, their concern beliefs were a significant predictor of adherence. In those with a poor understanding of their asthma, strong beliefs in the benefit of treatment were also predictive of adherence. Personalising adherence interventions targeted to changing these beliefs have potential to better support adherence in these groups.

126

Prioritising interventions to address polypharmacy in Australian aged care facilities

Kate N Wang[1], Natali Jokanovic[1,2], Samanta Lalic[1,3], Justin P Turner[1], Michael J Dooley[1,2], Carl M Kirkpatrick[1], J Simon Bell[1]. Centre for Medicine Use and Safety, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University[1], Parkville, VIC; Pharmacy Department, Alfred Health[2], Melbourne, VIC; Pharmacy Department, Austin Health[3], Melbourne, VIC.

Introduction. Polypharmacy is highly prevalent in aged care facilities. Although not necessarily inappropriate, polypharmacy has been associated with hospital admissions, lower quality of life, and increased mortality. Few studies have investigated strategies to address polypharmacy.

Aims. To identify and prioritise a range of potential local, regional or state level interventions to address polypharmacy in aged care facilities.

Methods. Two nominal group technique (NGT) sessions were convened in August 2015. The first session involved a purposive sample of clinicians, researchers and managers working in the aged care sector. The second session involved representatives of consumer, professional and health policy organisations. Participants were asked "What interventions or innovations are needed to address the prevalence and appropriateness of medicines use in aged care facilities?" Nineteen participants identified and prioritised the five most important interventions.

Results. A total of 16 distinct interventions or innovations were identified. Preliminary findings indicate the top five interventions, ranked from most important to fifth most important, are 'implementation of a pharmacist-led medication reconciliation service for new residents', 'conduct facility level audit and feedback on prevalent or high risk medicines', 'develop deprescribing scripts to assist general practitioners (GPs) and other clinicians proactively discuss medicine discontinuation', 'develop or revise prescribing guidelines specific to older people with multimorbidity in aged care facilities, and 'implement electronic medication charts and records accessible to GPs, pharmacists, nurses and aged care workers'.

Discussion. This study produced a prioritised list of interventions and innovations potentially well suited for implementation and evaluation at the local, regional or state level. This list should assist clinicians and policy makers develop a comprehensive strategy to address the increasing prevalence of polypharmacy in aged care facilities.

127

What's most important to residents and health care professionals when deprescribing in residential aged care facilities?

Justin P. Turner[1,4], Susan Edwards[2], Melinda Stanners[3], Sepehr Shakib[4], J. Simon Bell[1]. Centre for Medicine Use and Safety, Monash University[1], Parkville, Vic. Drug and Therapeutics Information Service, Repatriation General Hospital[2], SA. Torrens University[3], Adelaide, SA. Dept. of Clinical Pharmacology, Royal Adelaide Hospital[4], SA.

Introduction. Polypharmacy and multimorbidity are common in residential aged care facilities. Reducing polypharmacy may reduce adverse events and maintain quality of life. Deprescribing refers to reducing medications after consideration of therapeutic goals, benefits and risks, and medical ethics.

Aims. To use Nominal Group Technique (NGT) to rank factors that general medical practitioners (GPs), nurses, pharmacists and residents perceive are most important when deciding whether or not to deprescribe medications.

Methods. Discipline-specific groups of GPs, nurses, pharmacists and residents/representatives were convened across South Australia. Using NGT each group ranked factors they perceived to be most important when deciding whether or not to deprescribe. Factors generated by individual groups were then prioritized by a metropolitan and regional multidisciplinary group which included resident representatives.

Results. A maximum variation sample of participants were recruited across six separate groups including, GPs (n=19), nurses (n=12), pharmacists (n=14) and residents/representatives (n=11). No two groups had the same priorities. GPs ranked "evidence for deprescribing" and "communication with family/resident" as most important factors. Nurses ranked "GP receptivity to deprescribing" and "nurses ability to advocate for residents" as most important. Pharmacists ranked "clinical appropriateness of therapy" and "identifying residents' goals of care" as most important. Residents ranked "wellbeing of the resident" and "continuity of nursing staff" as most important. The multidisciplinary groups ranked "adequacy of medical and medication history" and "identifying residents' goals of care" as most important.

Discussion. Each group ranked different factors as most important when deciding whether or not to deprescribe. Future deprescribing interventions need to recognize and address the range of factors prioritized by both residents and health professionals.

128

Older adults' and carers' beliefs and attitudes towards deprescribing

Emily Reeve[1], Lee-Fay Low[2], Sarah N Hilmer[,1,3]. Cognitive Decline Partnership Centre, Kolling Inst, Sydney Med Sch, Univ of Sydney[1], St Leonards, NSW; Fac Health Sciences, Univ of Sydney[2], Sydney, NSW; Dept of Aged Care and Clin Pharmacol, Royal North Shore Hosp[3], St Leonards, NSW.

Introduction. Use of harmful and/or unnecessary medications in older adults with and without dementia is prevalent and knowledge of older adults and carer attitudes will contribute to medication optimization in practice.

Aims. The aims of this study were to develop and validate a tool to investigate and score older adults and carers beliefs and attitudes towards medication use and barriers towards deprescribing.

Methods. The previously validated Patients' Attitudes Towards Deprescribing questionnaire (which does not identify barriers or score participants) was expanded based on literature review, expert opinion and focus groups and a carer specific version was developed. Following piloting, the self-administered questionnaires and a trust in physician validated tool were distributed to older adults and carers. Psychometric validity and reliability were examined. Responses to questions identified to group into a factor were combined to produce a score.

Results. A total of 383 older adult (mean age=74, 47% taking 6 or more medications) and 200 carer (mean age of care recipient=81, 54% taking 6 or more medications) questionnaires were returned. Exploratory factor analysis revealed four common factors in both older adult and carer versions of the questionnaire (with 4-5 questions in each factor). The factors were burden of medications, appropriateness of medication use (harms and benefits), concerns about stopping and involvement/knowledge of their medications. The vast majority of older adults and carers are willing to stop a medication if their doctor said it was possible (88.2% / 84.6%). The concern factor score was correlated with reduced willingness to stop in older adults (P=0.004) but not in carers (P=0.245). Higher burden (P=0.002), higher involvement (P=0.002) and lower appropriateness (P<0.001) factor scores were associated with increased willingness to have a medication stopped in carers. Additionally, higher trust in physician was correlated with lower burden and higher belief in appropriateness in older adults and carers (P<0.002 for all).

Discussion. Most older adults and carers are willing to have a medication deprescribed although this is influenced by different factors in each group. This newly validated tool may provide insight into individuals' willingness and barriers to deprescribing which may be utilized in research and in practice to optimise shared-decision making.

129

Are polypharmacy and medication regimen complexity associated with all-cause mortality in older people? A population-based cohort study

Barbara C Wimmer[1], J Simon Bell[1,2], Johan Fastbom[3], Michael D Wiese[2], Kristina Johnell[3]. Centre for Medicine Use and Safety, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University[1], Melbourne, VIC; Sansom Institute, School of Pharmacy and Medical Sciences, University of South Australia[2], Adelaide, SA; Aging Research Center, Karolinska Institutet and Stockholm University[3], Stockholm, SWEDEN.

Introduction. Older people have a high prevalence of polypharmacy and increasingly complex medication regimens.

Aims. To investigate whether polypharmacy and medication regimen complexity are associated with all-cause mortality in older people.

Methods. Data were collected as part of the Swedish National Study of Aging and Care Kungsholmen (SNAC-K) study. Polypharmacy was analysed as a continuous variable (number of medications). Medication regimen complexity was assessed using the 65-item Medication Regimen Complexity Index (MRCI) in 10-unit steps. Mortality data were obtained from the Swedish National Cause of Death Register. Cox proportional hazard models were used to compute unadjusted and adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between polypharmacy and regimen complexity with all-cause mortality over a three-year period. Sub-analyses were performed stratifying by age (≤80 and >80 years), sex and cognition (Mini-Mental State Examination [MMSE] <26 and ≥26).

Results. Overall, 3348 people aged ≥60 years participated. During follow-up, 14.0% of the participants (n=470) died. After adjusting for age, sex, comorbidity, educational level, activities of daily living, MMSE and living place, polypharmacy was not associated with mortality (adjusted HR=1.03 95% CI 0.99-1.06). In adjusted analyses, higher MRCI was associated with mortality (HR=1.12 95% CI 1.01-1.25). When stratifying by sex, both polypharmacy and MRCI were associated with mortality in men but not in women. MRCI was associated with mortality in participants ≤80 years and participants with MMSE ≥26 but not in participants aged >80 years or with MMSE <26.

Discussion. Regimen complexity was a better overall predictor of mortality than polypharmacy. However, regimen complexity was not predictive of mortality in women, in participants aged >80 years, or those with MMSE <26. These different associations with mortality deserve further investigation.

130

Statin discontinuation in older people with dementia: A pharmacoepidemiological study

Sujita W Narayan[1], Sarah N Hilmer[2], Prasad S Nishtala[1]. [1]School of Pharmacy, University of Otago, Dunedin, OTAGO and [2]Kolling Institute of Medical Research and Sydney Medical School, University of Sydney, NSW

Introduction. In people with dementia, the effects of continuing and discontinuing statins on cognition are unclear.

Aims. Investigate statin discontinuation in the last year of life by older adults with a diagnosis of dementia compared to those without dementia, stratified by indication for primary versus secondary prevention of cardiovascular diseases.

Methods. Retrospective analysis using national level data of 4290 New Zealanders aged ≥75 years who had at least one dispensing record of a statin in the year prior to death for the study period 2007 – 2011. Propensity score matching was used to select cases with diagnosis of dementia (dementia group) and matched controls (no-dementia group) based on sex, age, ethnicity and Charlson Comorbidity Index scores. Cox regression and Kaplan–Meier survival analysis tested the relationship between statin discontinuation and a diagnosis of dementia and indication for statin.

Results. The prevalence of statin discontinuation was 60.98% in the dementia group (n=2145), in comparison to 57.39% in the no-dementia group (n=2145; p <0.05). In dementia patients discontinuation was greater in the primary prevention category, hazard ratio (HR) 1.17, 95% CI 1.07 – 1.29 compared to secondary prevention category HR 0.97, 95% CI 0.90 – 1.06, in the last 12 months of life.

Discussion. Statins were discontinued in more than half of New Zealanders in the last year of life and was more likely in those with a diagnosis of dementia and in those using statins for primary prevention. Further research is required to understand the clinical effects of discontinuing statins in individuals with dementia.
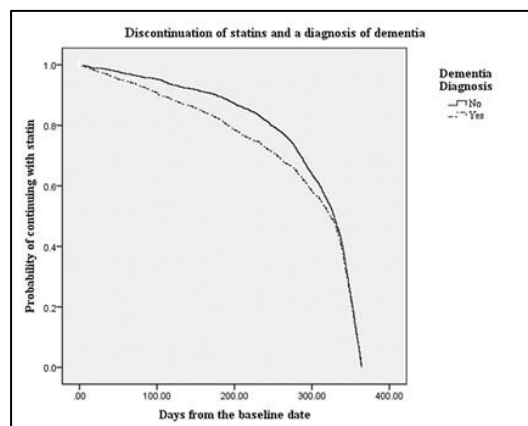
131

Patterns in use and costs of disease-modifying anti-rheumatic drugs (DMARDs) and biologic DMARDs (bDMARDs) in Australia

Ellen Donges[1], Christine E Staatz[1], Samantha A Hollingworth[1]. School of Pharmacy, Uni of QLD[1], Brisbane, QLD.

Introduction. Pharmacological intervention with disease-modifying anti-rheumatic drugs (DMARDs) and biologic DMARDs (bDMARDs) significantly enhances patient outcomes in rheumatoid arthritis (RA). Public subsidy ensures these medicines are accessible to all Australians.

Aims. To characterise utilization patterns and associated costs of DMARDs and bDMARDs in Australian RA patients from 2004-2014 as an update and expansion of previous research (Chan et al, 2006).

Methods. We extracted data on dispensing and government expenditure for these medicines on the Pharmaceutical Benefits Scheme (PBS) from Medicare Australia and analysed for temporal trends. We used the World Health Organization's defined daily dose (DDD) per 1000 population per day measure to standardize medicine use and compare between individual drugs.

Results. Total Australian dispensed use of DMARDs and bDMARDs increased 65% from 4.68 (2004) to 8.01 DDD/1000 population/day in 2014. Although bDMARD use was a small proportion of total use, dispensed use increased by a huge 1,164% over the study period. Methotrexate was the most frequently used DMARD and adalimumab the most used bDMARD. There was a 10% decrease in expenditure on DMARDs from 2004-2014, with a huge 1,369% increase in the expenditure on bDMARDs ($26.0 million in 2004 to $381.4 million in 2014).

Discussion. Use of both DMARDs and bDMARDs to treat RA has increased over the past decade concurrently with the subsidy of new bDMARDs and changes to therapeutic guidelines that recommend intensive DMARD treatment in early disease states. The subsidy of bDMARDs is a substantial cost to government and current use and expenditure trends necessitate further research on health outcomes to ensure value for money.

Chan V and Tett SE (2006) Pharmacoepidemiol Drug Saf 15:462-468
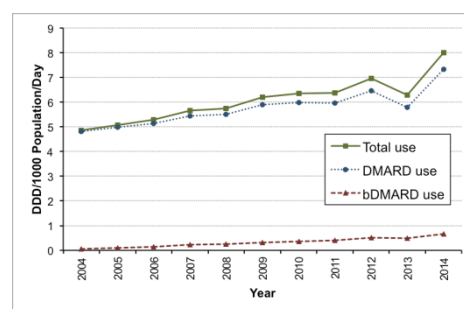
132

A model for predicting high bleeding risk among potential warfarin users

Saranya Ramesh, Lahn D Straney, Judy A Lowthian, Basia O Diug
School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC.

Introduction. Warfarin can effectively minimise stroke. However, a major side effect is haemorrhage. Several classification schemes have been developed to identify high-risk patients. These schemes only consider non-specific biological risk factors.

Aims. To develop a bleeding risk scheme including psychosocial and biological risk factors, for use prior to prescribing warfarin.

Methods. Secondary data was analysed from a case-control study comprising patients previously stabilised patients on warfarin. Predictor variables were identified through a literature survey of bleeding risk factors and univariate analysis. The outcome variable was defined as an individual with an International Normalised Ratio ≥ 6. A prediction model was developed using multivariable logistic regression. Backward step-wise elimination using the Akaike Information Criterion was used to determine the final model. The out-of-sample predictive performance was assessed in 200 random splits using the c-statistic and goodness of fit tests. Sensitivity and the positive predictive value were used to assess the prognostic utility at different probability cut points.

Results. The final predictive model comprised nine risk factors: mild cognitive impairment, depression, prior stroke/haemorrhage, myocardial infarction, valve replacement, chronic obstructive pulmonary disorder, asthma, diabetes mellitus and poor visual acuity. The model had adequate discrimination (c-statistic: 0.72) and a non-significant goodness of fit test. There was high sensitivity at cut points less than 0.4 (85% at 0.2) and specificity was still moderate (47%) at a cut point of 0.2.

Discussion. This model provides a bleeding risk scheme that considers both biological and psychosocial factors, and was tested on a real world population. It is sensitive for identifying high-risk patients and could also be used to inform clinical practice to improve safety of warfarin prescription and management in an ageing population.
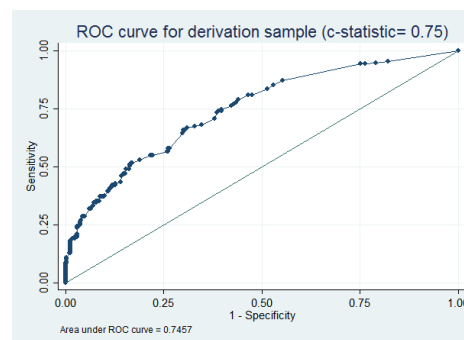
133

Improving the assessment of drug safety: an analysis of the clopidogrel-proton pump inhibitor interaction

Adam La Caze. School of Pharmacy, The University of Queensland, Brisbane, QLD

Introduction. There is a significant amount of work in philosophy of medicine and philosophy of science that is relevant to addressing the challenge of assessing drug safety.

Aim. Motivate a re-evaluation of how drug safety evidence is amalgamated using the clopidogrel-proton pump inhibitor interaction as a case study.

Method. Two approaches to amalgamating drug safety evidence are contrasted. Approach One is the standard approach used in systematic review methodology. In Approach Two, drug safety evidence is amalgamated according to explicit causal *arguments*. Clinicians and scientists provide such arguments when contributing to or discussing the primary literature.

Results. The two approaches to evidence amalgamation provide different summaries of the evidence in the clopidogrel-proton pump inhibitor case. The summary from Approach One is that high-quality evidence does not support a clinical effect from the interaction. This summary has informed the clinical guidance provided on the interaction. Approach Two provides different advice: the clinical studies to date do not provide a reliable test of the specific interaction supported by the pharmacological evidence (i.e. the interaction between omeprazole and clopidogrel).

Discussion. Appropriate evidence amalgamation in drug safety is an unmet challenge. Approach One was designed to be a quick and reliable method of evidence amalgamation in assessing drug efficacy and has some under-recognised limitations when used to assess drug safety. The clopidogrel-proton pump inhibitor case highlights the need for an explicitly causal approach with a greater focus on the specific inferences warranted by the observed data as opposed to methods used to generate the data.

134

NOX2 Oxidase promotes prostate tumour development

Ian P Harrison[1], Antony Vinh[1], Grant R. Drummond[1], Christopher G. Sobey[1], Ian RD Johnson[2], Doug A Brooks[2], Ajay Shah[3], Elizabeth D Williams[4] and Stavros Selemidis.[1] [1] Dept of Pharmacol, Monash Univ, VIC, Aus, 3800. [2] Sansom Institute for Health Research, Univ of SA, Adelaide, SA 5001. [3] King's College London, UK, [4] Australian Prostate Cancer Research Centre, Queensland Univ of Tech, Brisbane, Aus.

Introduction. Reactive oxygen species (ROS) promote vascular endothelial growth factor (VEGF) signaling and have potent angiogenic properties. However, in the context of tumour angiogenesis, the precise enzymatic source of ROS generation *in vivo*, and the subcellular localization of ROS production have yet to be defined.

Aims. To determine the role of the ROS generating enzymes NOX2 and NOX4 oxidases in VEGF signalling and prostate tumourigenesis.

Methods. We analysed mRNA microarray databases and used fresh-frozen tissue samples to quantify gene expression for NOX2 and NOX4 in primary and metastatic prostate cancers. We used a syngeneic, orthotopic mouse model of prostate cancer and assessed tumours for size, weight, immune cell populations and angiogenesis. Using confocal fluorescence microscopy, we determined both the co-localisation of the VEGFR2 with EEA1 and NOX2 and endosomal ROS production in endothelial cells in the presence of VEGF-A (30ng/ml).

Results. We show increased gene expression for NOX2 in human primary prostate cancer and NOX4 in human metastatic but not primary prostate cancer, yet mice overexpressing endothelial NOX4 developed similar-sized primary prostate tumours compared to wild-type mice. NOX2 deletion resulted in significantly ($P<0.05$) reduced angiogenesis, significantly ($P<0.05$) more tumour-associated F4/80$^+$ macrophages and almost total failure in tumour development. In endothelial cells treated with VEGF-A, the VEGFR2 co-located with both the early endosomal marker EEA1 and NOX2, which correlated with significant ROS production within acidified endosomal compartments. Furthermore, the endothelial cell proliferation to VEGF-A was dependent on the endocytosis of VEGFR2, endosomal acidification and NOX2 oxidase-dependent ROS production in endosomes.

Discussion. NOX2 oxidase is expressed in endosomes, which is important for prostate cancer growth and positive regulation of the VEGF pathway. This mechanism could provide rationale for a novel treatment strategy for prostate cancer patients that involves endosome-targeted NOX2 oxidase inhibitors.

135

Des-acylated ghrelin limits brain injury and blood-brain barrier disruption after stroke

Jacqueline Ku[1], Ali Taher[1], Connie Wong[2], Sarah J. Spencer[3], Zane B. Andrews[4] & Alyson A. Miller[1]. School of Medical Sciences and Health Innovations Research Institute, RMIT University[1], Melbourne, VIC; Department of Medicine, Monash University[2], VIC; School of Health Sciences, RMIT University[3], Melbourne, VIC; Department of Physiology, Monash University[4], Melbourne, VIC

Introduction. The ghrelin-related peptides, acylated ghrelin (AG) and des-acylated ghrelin (DAG), are produced primarily by the stomach and are best known for their neuroendocrine actions. Recent evidence, however, suggests these peptides may have other important biological functions. For example, we have recently reported that DAG exerts important protection actions on the cerebral endothelium by stimulating vasoprotective nitric oxide and limiting oxidative stress. Moreover, both peptides are reported to have neuroprotective actions. Thus, these peptides may protect the brain and its vasculature against ischaemic stroke.

Aims. Examine whether AG and/or DAG can limit brain injury, oedema, and blood-brain barrier (BBB) disruption after cerebral ischaemia and reperfusion (I/R) in mice.

Methods. Transient ischaemic stroke was induced in mice by middle cerebral artery occlusion (MCAo) for 0.5 h followed by reperfusion (23.5 h). Brain injury and oedema were assessed by measuring infarct and oedema volumes using thionin, and by quantifying apoptosis. Evans blue (EB) extravasation was used to assess BBB disruption.

Results. Post-stroke treatment with DAG (1 mg/kg) reduced infarct and oedema volumes by ~50% (Infarct: DAG, 19±6 vs. Veh, 37±5, mm^3, n=14), whereas AG (1 mg/kg) had no effect. DAG also reduced the number of apoptotic cells in peri-infarct regions (DAG, 165±37 vs. Veh, 51±9, mm^3, n=6). DAG markedly reduced Evan's blue leakage into ischaemic hemispheres (DAG, 1.6±0.5 vs. Veh, 7.8±1.2, mg, n=8), indicative of decreased BBB permeability and hence reduced BBB disruption. In addition, DAG attenuated the increase in paracellular permeability of brain endothelial cells (bEnd.3) caused by oxygen glucose deprivation and reoxygenation (*in vitro* model of I/R) by preventing oxidative-induced downregulation of tight junction proteins.

Discussion. Collectively, these findings indicate that DAG, but not AG, can limit brain injury and BBB disruption after I/R in mice. Thus, DAG or longer-acting analogues such as AZP-531 could be exploited therapeutically for the acute treatment of ischaemic stroke.

136

The HNO donor isopropylamine NONOate offers haemodynamic advantages over the NO donor diethylamine NONOate in the diabetic rat myocardium ex vivo.
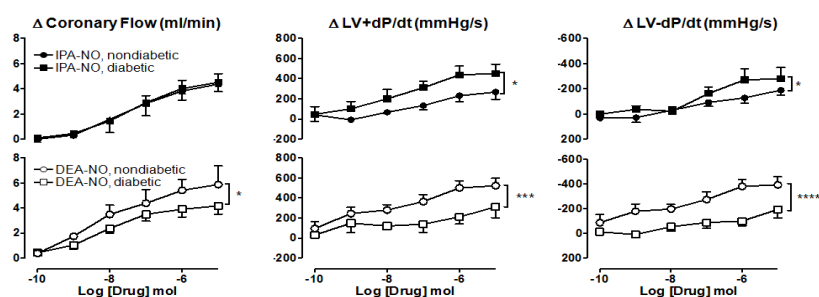
Jarryd Anthonisz[1,2]*, Chengxue H Qin[1]*, Nicola Kahlberg[4], Chen Huei Leo[4], Edwina Jap[1], Laura J Parry[4], Barbara K Kemp-Harper[3], Rebecca H Ritchie[1] [1]Baker IDI Heart & Diabetes Inst, Melb, VIC; [2]CCS, Monash Univ Melb VIC; [3]Dept. Pharmacology, Monash Univ Melb, VIC; [4]School of BioSciences, Univ of Melb, Parkville, VIC,

Introduction. The NO redox sibling nitroxyl (HNO) elicits vasodilatation and enhances left ventricular (LV) con-traction and relaxation. The efficacy of HNO has not yet been fully characterised in disease. We tested the hypothesis that the HNO donor isopropylamine NONOate (IPA-NO) offers haemodynamic advantages over the NO donor diethylamine NONOate (DEA-NO) in the diabetic myocardium.

Methods. After 8wks of streptozotocin diabetes (55mg/kg i.v., blood glucose ~30mM) or sham, hearts were isolated from adult male rats, anaesthetised (ketamine-xylazine 100-12mg/kg i.p.) and Langendorff-perfused. Following U46619 preconstriction of the coronary vasculature to 50%, dose-response curves to acute bolus doses of IPA-NO or DEA-NO, both 10pmol-10μmol were performed.

Results. HNO vasodilatation was preserved, and the positive inotropic and lusitropic effects were increased in diabetic hearts; in contrast, those of NO were attenuated (Figure). The impaired NO vasodilatation persisted in hearts from moderately hyperglycaemic rats (6-7U insulin s.c./day, blood glucose ~22mM).

*$P<0.05$, ***$P<0.001$ and ****$P<0.0001$ (n=5-8/group; 2-way ANOVA, Tukey's post-hoc)*

Discussion: Haemodynamic responses to HNO are preserved or enhanced in diabetes, whereas those of NO are attenuated.

137

Loss of Alzheimer's-associated tau (MAPT) impairs cardiovascular function.

Ashenafi H Betrie[1], Peng Lei[2]. Scott Ayton[2], Ashley I Bush[2], James A Angus[1], Christine E Wright[1]. Cardiovascular Therapeutics Unit, Dept of Pharmacol and Therapeutics, Univ of Melbourne[1], Oxidation Biology Unit, The Florey Institute for Neuroscience and Mental Health, Univ of Melbourne[2], Parkville, VIC

Introduction. Aggregation of tau protein (MAPT) causes neuronal death in Alzheimer's disease and various neurodegenerative disorders termed tauopathies. The normal function of tau is unknown, which is important because soluble levels of the protein drop in disease. This could lead to toxic loss of function and lowering tau protein is a prominent therapeutic strategy. But the potential side effects are unknown. We showed that loss of tau protein causes Parkinsonism in mice (Lei et al., 2012). We have now observed that tau protein is surprisingly abundant in cardiac tissue, so we explored its functional role in the cardiovascular system, and the consequences of its absence.

Aims. To investigate the role of tau protein in the cardiovascular system.

Methods. Blood pressure, cardiac and vascular functions were assessed in tau knock-out (KO) and wild type (WT) mice of two different age groups (14 and 22 months old). Tail-cuff and carotid catheterisation were used to measure BP, wire myography was used for isolated mesenteric arteries and organ baths for isolated atria.

Results and Discussion. The 14 mo tau KO mice were hypertensive, displayed increased maximal contractility to isoprenaline and calcium in the left atria, and lower resting rate in the right atria as compared to WT mice (P<0.05). There was also a significant increase in the sensitivity of tau KO isolated mesenteric arteries to vascular relaxation by sodium nitroprusside (SNP). Responses to a range of other pharmacological agonists such as relaxation to Ach, contraction to methoxamine or angiotensin II (AngII) were not changed. In the older (22 mo) age group, similar responses were seen in the resting right atrial rate, left atrial maximum response to isoprenaline, vascular relaxation to SNP and Ach and contraction to methoxamine as compared to the WT mice. However, there was no difference in the BP or maximum inotropic response to calcium. We reveal that the Alzheimer's tau protein, canonically a brain protein, has an important role in the maintenance of normal cardiac function. These data have implications in understanding the normal function of tau protein for neuroscience applications – including Alzheimer's disease pathogenesis. Furthermore, we introduce a novel protein involved in the maintenance of cardiovascular physiology.

Lei P et al. (2012) Nature Med. 18:291-295.

138

Phosphoinositide 3-kinase [p110α] (PI3K) gene therapy attenuates type 1 diabetic cardiomyopathy in mice

Darnel Prakoso[1,2], Miles J De Blasio[1], Helen Kiriazis[1], Hongwei Qian[1], Kate L Weeks[1], Sarah Rosli[1], Chengxue Qin[1], Laura J Parry[2], Xiao-Jun Du[1], Paul Gregorevic[1], Julie R McMullen[1], Rebecca H Ritchie[1], Baker IDI Heart & Diabetes Institute[1], Melbourne, VIC, 3004. School of Biosciences, University of Melbourne[2], Melbourne, VIC, 3010

Introduction. Diabetic cardiomyopathy is characterised by left ventricular (LV) diastolic dysfunction accompanied by LV fibrosis and cardiomyocyte hypertrophy, which currently has no specific treatment. We tested the hypothesis that PI3K gene therapy, administered after LV diastolic dysfunction onset, attenuates diabetic cardiomyopathy.

Methods. Diabetes was induced in male FVB/N mice by streptozotocin (55mg/kg/day i.p. for 5 days; nondiabetic shams received citrate vehicle). After 8wks of untreated diabetes, LV diastolic dysfunction was confirmed (echocardiography, impaired E/A). A single tail vein injection of recombinant adeno-associated virus containing PI3K (rAAV6-PI3K, 2×10^{11}vg) or null vector was administered, and mice were followed for a further 6 or 8wks (all n=7-9/group).

Results. Diabetes-induced increases in LV remodelling were significantly attenuated 8wks after rAAV6-PI3K, accompanied by improvements in both LV diastolic (E/A, isovolumic relaxation time IVRT, deceleration time DT) and systolic function (fractional shortening, FS) (Table).

Discussion. These results suggest PI3K gene therapy may be a promising approach for treating diabetic cardiomyopathy.

Table (mean±SEM)	6 weeks after AAV				8 weeks after AAV			
	Sham		Diabetic		Sham		Diabetic	
	Null	PI3K	Null	PI3K	Null	PI3K	Null	PI3K
LV collagen content (%)	2.5±0.2	2.7±0.3	4.0±0.7*	2.3±0.3#	3.4±0.4	2.6±0.3	6.0±0.2*	3.0±0.2#
Cardiomyocyte width (μm)	11.5±0.9	11.8±0.2	12.6±0.5	11.8±0.3	11.8±0.6	12.3±0.2	13.5±0.5*	11.9±0.5#
E/A (% sham null)	100±10	96±9	61±2*	83±8#	100±8	117±8	75±8*	90±6#†
DT (% sham null)	100±4	94±10	120±2*	92±2	100±7	101±7	126±11*	90±4#
IVRT (% sham null)	100±8	105±5	122±7*	111±4	100±5	95±8	118±3*	97±5#
FS (%)	42±4	42±3	32±3*	50±3#	49±3	55±3	34±7*	60±3#†

*P<0.05 vs age-matched non-diabetic-null; #P<0.05 vs age-matched diabetic-null; †P<0.05 vs 6wks diabetic+PI3K.

139

The effects of Th2 cytokines to modulate macrophage phenotype and reactive oxygen species generation

Tara E McConaghy, Caitlin V Lewis, Christopher G Sobey, Grant R Drummond & Barbara K Kemp-Harper. Biomedicine Discovery Inst. & Dept. of Pharmacol, Monash Univ, Clayton, VIC.

Introduction. Macrophages are central to atherogenesis and although the Th2 cytokines, interleukin-4 (IL-4) and interleukin-13 (IL-13) both promote a protective M2 phenotype, they have pro- and anti-atherogenic effects, respectively (Bhattacharjee *et al.*, 2013). Whilst IL-4 increases the activity of macrophage reactive oxygen species (ROS) generating enzyme (NOX2 oxidase), the impact of IL-13 is unknown. Differential effects of Th2 cytokines on macrophage ROS generation may underlie their opposing roles in atherosclerosis.

Aims. To compare the effects of IL-4 and IL-13 on macrophage phenotype and ROS generation in human primary macrophages.

Methods. Human primary monocytes (from donor buffy coat) were differentiated to macrophages with macrophage colony stimulating factor (50 ng/ml, 7d) and treated with IL-4 or IL-13 (0.005-50 ng/ml, 24h). Macrophages were characterised by (a) mRNA expression of M2 markers (CCL18, CCL22) and NOX2 subunits (qRT-PCR) and (b) ROS generation by L012-enhanced chemiluminescence.

Results. IL-4 and IL-13 caused concentration-dependent increases in mRNA expression of CCL18 (500-fold, n=5-6, P<0.01) and CCL22 (5.5-fold, n=6). p38MAPK and STAT3 inhibitors appeared to attenuate IL-4 and IL-13 (2.5 ng/ml)-induced increases in CCL18 mRNA by 50% and 90%, respectively (n=2). The Th2 cytokines (50 ng/ml) augmented expression of the NOX2 cytosolic subunits, p67phox (3-fold, n=7-8, P<0.01) and p47phox (2.5-fold, n=7-8, P<0.05) whilst decreasing NOX2 expression (5-fold, n=6-7, P<0.01). PDBu-stimulated superoxide production was unchanged by IL-13 yet increased 40% by IL-4 (n=6, P<0.01).

Discussion. IL-4 and IL-13 promote an M2 phenotype, potentially via STAT3 signalling. The greater impact of IL-4 on ROS generation, as compared to IL-13, may contribute to its pro-atherogenic properties.

Bhattacharjee A*, et al.* (2013). Free Radic Biol Med 54: 1-16.

140

Chronic β1-blockade impairs ischaemic tolerance and preconditioning in murine myocardium

Louise See Hoe[1*], Jan M Schilling[2,3], Anna R Busija[2,3], Kristofer J. Haushalter[2,3], Victoria Ozberk[1], Malik M. Keshwani[3], David M. Roth[2,3], Eugene Du Toit[1], John P. Headrick[1], Hemal H. Patel[2,3], Jason N. Peart[1]. Menzies Health Institute Queensland, Griffith University[1], Southport, QLD; VA San Diego Healthcare System[2], San Diego, CA; University of California San Diego[3], San Diego, CA.

Introduction. β-adrenoceptor (β-AR) antagonists are commonly used in ischaemic heart disease (IHD) patients, yet may impair signalling and efficacy of 'cardioprotective' interventions.

Aims. To assess the effects of chronic β_1-AR antagonism on myocardial resistance to ischaemia-reperfusion (I-R) injury and the ability of cardioprotective interventions [classic ischaemic preconditioning (IPC); *novel* sustained ligand-activated preconditioning (SLP)] to reduce I-R injury in murine hearts.

Methods. Young male C57Bl/6 mice were untreated or received atenolol (0.5 g/l in drinking water) for 4 weeks. Subsequently two cardioprotective stimuli were evaluated: vehicle vs. morphine pellets (75 mg) were subcutaneously implanted to induce SLP (controls received vehicle) 5 days prior to Langendorff heart perfusion, and IPC in perfused hearts (3 x 1.5 min ischaemia/2 min reperfusion). Langendorff-perfused hearts were exposed to 25 min global ischaemia/45 min reperfusion. Post-ischaemic cell death was determined by coronary lactate dehydrogenase (LDH) efflux.

Results. Atenolol significantly reduced *in vivo* heart rate. Untreated control hearts exhibited substantial left ventricular dysfunction (~50% pressure development recovery, ~20 mmHg diastolic pressure rise) with significant release of LDH (tissue injury indicator) after I-R. Contractile dysfunction and elevated LDH were reduced >50% with IPC and SLP. While atenolol treatment did not modify baseline contractile function, post-ischaemic function was significantly depressed compared to untreated hearts. Atenolol pre-treatment abolished beneficial effects of IPC, whereas SLP protection was preserved.

Discussion. These data indicate that chronic β_1-AR blockade can exert negative effects on functional I-R tolerance and negate conventional IPC (implicating β_1-ARs in IR injury and IPC signalling). However, novel morphine-induced SLP is resistant to inhibition by β_1-AR antagonism.

141

Deficiency of endogenous annexin-A1 exaggerates cardiomyopathy in a mouse model of type 1 diabetes

Sarah Rosli[1, 2], Chengxue Qin[1], Helen Kiriazis[1], Minh Deo[1], Yuan H Yang[3], Xiao-Jun Du[1], Rebecca H Ritchie[1, 2]. [1]Baker IDI Heart & Diabetes Institute, Melbourne, VIC; [2]Central Clinical School, Monash University, Melbourne, VIC; [3]Centre of Inflammatory Diseases, Monash University, Clayton, VIC.

Introduction: Diabetes is a chronic inflammatory disease associated with increased risk of heart failure. We have recently shown that deficiency of anti-inflammatory annexin-A1 (ANX-A1) exaggerates myocardial infarction; its impact on other cardiac pathologies has not been investigated. We tested the hypothesis that ANX-A1^{-/-} exaggerates type 1 diabetic cardiomyopathy. Methods: ANX-A1^{+/+} and ANX-A1^{-/-} male mice were followed for 16wks after streptozotocin (55mg/kg/day i.p. for 5 days)-induced diabetes or vehicle sham. Left ventricular (LV) function was assessed in anaesthetised mice (85/8.5/0.8mg/kg ketamine/xylazine/ atropine, i.p.). Results: At study end, diabetes induced LV inflammation, LV remodelling, and LV diastolic dysfunction (Table); inflammation and diastolic dysfunction were further exacerbated in ANX-A1^{-/-} diabetic mice. Conclusion: Deficiency of ANX-A1 exacerbates diabetes-induced cardiomyopathy. ANX-A1 may thus represent a therapeutic target for the treatment of diabetes.

RESULTS: mean±SEM (n)	ANX-A1^{+/+} sham (10)	ANX-A1^{+/+} diabetes (9)	ANX-A1^{-/-} sham (8)	ANX-A1^{-/-} diabetes (6)
Blood glucose (mM)	8.7±0.2	30.4±1.01*	9.4±0.4	30.8±1.5*
Final bodyweight (g)	33.4±0.6	28.7±1.0*	31.1±0.2	25.8±0.7*
LV macrophage content (AU)	61±2	87±3*	62±4	105±5*[#]
β-myosin heavy chain mRNA (fold)	1.0±0.3	5.1±1.4*	1.4±0.5	11.1±5.3*
Connective tissue growth factor mRNA (fold)	1.0±0.2	2.0±0.2*	0.9±0.2	2.0±0.6*
Cardiac collagen content (AU)	5.1±0.8	9.5±0.7*	5.3±0.5	10.6±1.2*
LV E:A ratio (AU)	1.80±0.11	1.61±0.10*	1.64±0.08	1.32±0.09*[#]
LV-dP/dt (mmHg/s)	9340±560	8070±284*	8710±514	7181±890*
LV+dP/dt (mmHg/s)	12100±652	9940±553*	9100±444	7860±1020*[#]

*$p<0.05$ genotype sham; [#]$p<0.05$ vs diabetic ANX-A1^{+/+} (2-way ANOVA, Sidak post-hoc for multiple comparison).

142

Dimer interfaces are implicated in the structural basis of allosterism at metabotropic glutamate receptor 5

Vyvyan T. Mai[1], Elizabeth D. Nguyen[2], Avinash Chavda[2], P. Jeffrey Conn[2], Arthur Christopoulos[1], Karen J Gregory[1]. Drug Discov Biol, Monash Inst Pharm Sci, Monash Univ[1], Parkville, VIC; Vanderbilt Univ Med Ctr[2], Nashville, TN.

Introduction. Dysfunctional glutamatergic neurotransmission is implicated in the pathophysiology of numerous CNS disorders. Allosteric modulation of metabotropic glutamate receptor 5 (mGlu5) represents an attractive therapeutic strategy to restore glutamatergic neurotransmission in multiple disorders including depression, schizophrenia and anxiety. Recent mGlu crystal structures have definitively localised one allosteric binding pocket, the so-called 'MPEP site'. However, three allosteric ligand chemotypes (termed 'second site modulators') are non-competitive with ligands binding to the MPEP-site. The location of additional allosteric site or sites remains unknown.

Aims. To identify the binding site/s of putative 'second site' mGlu5 positive allosteric modulators

Methods. Mutations in mGlu5 were rationally designed on the basis of homology models and crystal structures of mGlu receptor seven transmembrane (TM) spanning domains. Stable HEK293A cells lines were generated and relative expression levels compared with the wild-type receptor via western blotting. The impact of mutations were assessed on the pharmacology of the 'second site' positive allosteric modulators (PAMs), CPPHA and VU0357121, in parallel with a well-characterised MPEP-site PAM, VU29. Allosteric interactions were assayed using glutamate mediated intracellular Ca^{2+} mobilisation.

Results. Removal of the extracellular N-terminal domain that mediates receptor homodimerisation resulted in a complete loss of CPPHA and VU0357121 activity, whereas MPEP-site PAMs including VU29 retained activity. Based on these data we focussed on putative dimer interfaces and extracellular loop regions to probe the binding site of second site PAMs. Mutations in the proposed TM1-TM1 interface and second extracellular loop had no effect on potentiation of glutamate by second site PAMs or VU29. Four residues in TM4/5 were identified that reduced either CPPHA or VU0357121 potentiation of glutamate, but had no effect on VU29 potentiation.

Discussion. Our data suggest that dimerisation of mGlu5 may be crucial for the activity of second site allosteric modulators. Understanding the structural basis of allosteric interactions at mGlu5 is crucial to improve elucidation of structure-activity relationships and in the future facilitate rational drug discovery approaches.

143

Enhanced glucose uptake in brite/beige adipocytes following adrenoceptor activation

Dana S Hutchinson[1], Jon Merlin[1], Masaaki Sato[1], Bronwyn A Evans[1] Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC

Introduction. The high prevalence of obesity and diabetes has provoked substantial interest in adipocyte biology as a means of expending excess energy and utilizing glucose as a fuel. While brown adipose tissue (BAT) has an established role in thermogenesis and glucose uptake, and white adipose tissue (WAT) in lipid storage, the role of the newly described beige/brite adipocyte is less clear. Brite adipocytes are derived from the conversion of a fat-storing white adipocyte into a brown-like genetic phenotype by multiple stimuli including the PPARγ activator rosiglitazone (Petrovic et al 2010), but little is known of their role in glucose uptake or utilization.

Aims. To determine the effect of rosiglitazone on adrenoceptor (AR) function in mouse brite adipocytes, focussing on glucose uptake capacity.

Methods. Primary inguinal white preadipocytes from FVB mice were differentiated *in vitro*, and brite adipocyte conversion induced by 1μM rosiglitazone (ROSI) treatment. $β_3$-AR expression (qPCR, confocal microscopy) and function (cAMP production, [^{3}H]-2-deoxyglucose uptake) was assessed in response to noradrenaline (NE) in the absence or presence of ROSI. P was evaluated using 2-way ANOVA or Student's t-tests.

Results. ROSI treatment of white adipocytes increased $β_3$-AR expression (determined by qPCR and confocal microscopy) and NE-stimulated cAMP production (control: max 5.7±0.6% forskolin (100μM), pEC_{50} 7.2±0.4; +ROSI: max 12.9±1.3%, pEC_{50} 7.0±.3, n=5, p=0.01). This coincided with an increase in NE-stimulated [^{3}H]-2-deoxyglucose uptake (control: 124.3±11.6% basal, pEC_{50} 8.3±0.7; +ROSI: 175.9±17.5% basal, n=7, pEC_{50} 9.2±0.3, p=0.03). This enhanced glucose uptake was correlated with a significant (p<0.05) increase in glucose metabolism genes including GLUT4, Hk2, Pfkm, Gapdh and Pgk1, but not GLUT1 mRNA levels.

Discussion. ROSI-induced brite adipocytes show an increased capacity for glucose uptake compared to classical white adipocytes, taking on a brown adipocyte metabolic phenotype. This study highlights the potential of brite/beige adipocytes as a target for diabetes therapy.

Petrovic N et al (2010) JBC *285*: 7153-64

144

Identifying the orthosteric vestibule and an allosteric binding site of the α_{1A} adrenoceptor

Angela M. Finch[1], Karmen Xu[1], Woo-Ju Noh[1], Junli Chen[1], Adrian P. Campbell[1]. School of Medical Science, UNSW Australia[1], Kensington, NSW

Introduction. C9 bisquinoline (C9Q) and C9 bisacridine (C9A) are bitopic, negative allosteric modulators of the α_{1A} adrenoceptor (α_{1A} AR). Docking studies have predicted that one of C9Q's quinoline moieties interacts with $S83^{2.61}$, $F86^{2.64}$, $E87^{2.65}$ and $W102^{3.28}$ outside of the orthosteric binding site (Chen *et al.*, 2014).

Aims. To identify novel sites on the α_{1A} AR which regulate orthosteric ligand binding.

Methods. Mutant α_{1A} ARs were made by DpnI-mediated site-directed mutagenesis. The effect of these mutations on orthosteric and allosteric ligand binding was characterised using saturation, competition and kinetic radioligand binding assays using [^{3}H]prazosin.

Results. The W102A and F86A mutations resulted in reduced affinity of the orthosteric ligands [^{3}H]prazosin and norepinephrine (NE) compared to wildtype (WT) ([^{3}H]prazosin K_D (nM): WT 0.4±0.02, F86A 2.3±0.9; W102A >12: NE pK_i: WT 5.5±0.4, W102A 4.29±0.1; n=3-4, p<0.05), and increased [^{3}H]prazosin dissociation rates, (K_{off} (min^{-1})WT 0.06±0.01; F86A 0.16±0.02; W102A 0.88±0.001, n=3, p<0.05), while having no effect on association rates. Conversely, [^{3}H]prazosin association and dissociation rates decreased for S83A (K_{on} (nM.min^{-1}): WT 0.10±0.01; S83A 0.04±0.004, p<0.05; K_{off} (min^{-1}): S83A 0.01±0.01, p<0.05). Mutation of E87 did not affect orthosteric ligand binding or modulatory effects of C9Q and C9A. The ability of 100 µM C9A to negatively modulate [^{3}H]prazosin dissociation was increased by S83A and decreased by F86A (K_{obs}/K_{off} : C9A WT 24±0.5; S83A 72±8; F86A 12±1, n=3, p<0.05). Preliminary data suggests that S83A also increases the ability of C9Q to negative modulate ^{3}H]prazosin dissociation (K_{obs}/K_{off} : C9Q 100 µM, WT 5±1; S83A 51±13, n=2).

Discussion. We propose that $S83^{2.61}$, $F86^{2.64}$ and $W102^{3.28}$ are important for the orthosteric ligand's transit in and out the orthosteric binding site and that $S83^{2.61}$ and $F86^{2.64}$ form part of an allosteric site on the α_{1A}-AR. This orthosteric vestibule has a location distinct from the vestibule identified computationally in the β_2-AR and Muscarinic receptors (Dror *et al.,* 2011; Dror *et al*., 2013) and supports a two-step binding process for the biogenic amines.

Chen *et al.* 2014 Bioorg Med Chem 21: 5910; Dror *et al*., 2011 PNAS 108: 13118; Dror *et al*., 2013 Nature 503:29

145

Mechanisms of selectivity for allosteric modulators of the muscarinic acetylcholine receptors (mAChRs)

Celine Valant[1], Alice Berrizi[1], Chris Felder[2], Patrick M Sexton[1], Christopher J Langmead[1] and Arthur Christopoulos[1]. Monash Institute of Pharmaceutical Sciences, Drug Discovery Biology[1], Parkville, VIC, AUSTRALIA, Eli Lilly and Company[2], Indianapolis, Indiana, USA[2].

Introduction. Allosteric modulators of G protein–coupled receptors have the potential to engender subtype selectivity via two mechanisms, namely, divergent binding pockets between receptor subtypes and/or due to different levels of cooperativity with the orthosteric ligand.

Aims. To uncover the mechanisms of subtype selectivity of positive allosteric modulators (PAMs) of the five mAChRs.

Methods. BQZ-12, an M_1 mAChR PAM, LY-2033298 and LY-2119620, two M_2/M_4 mAChR PAMs were used in this study. We performed radioligand binding, and various functional experiments between each modulators and ACh, at all five M_1-M_5 subtypes.

Results. In [^{3}H]NMS equilibrium binding assays, all modulators bound to each of the five mAChRs, with micromolar affinities. However, using three different signalling assays, [^{35}S]GTPγS binding, [IP_1] accumulation and ERK1/2 phosphorylation, distinct differences were observed in the allosteric modulators' pharmacological profiles that varied between receptor subtypes and signalling pathways.

Discussion. PAM subtype selectivity at the mAChRs appears to be mostly driven by selective cooperativity rather than binding, suggesting that the extracellular allosteric site of mAChRs is more conserved than previously appreciated. In contrast, the interaction network between orthosteric, allosteric and intracellular coupling sites is likely dramatically different. Additionally, our functional data also suggest that mAChR modulators may exhibit allosteric bias of endogenous ligand signalling, differentially modulating ACh response in one pathway compared to another, adding another layer of complexity to the 'allostery paradigm' of mAChRs.
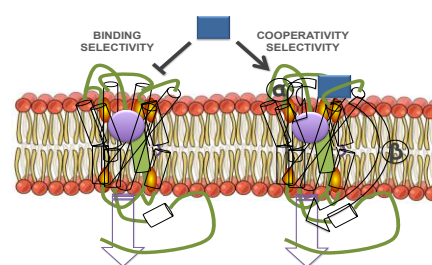
Christopoulos A (2014) Mol Pharmacol 86(5):463-78.

146

Probing the pharmacology of the α_{1A}-adrenoceptor with fluorescent prazosin

Linzi Lim[1], Cameron Nowell[1], Dana S. Hutchinson[1], Roger J. Summers[1], Bronwyn A. Evans[1], Sabatino Ventura[1], Lauren T. May[1]. Drug Disc Biol, Monash Inst of Pharmaceutical Sci[1], Monash Univ, Parkville, VIC, AUS.

Introduction. Fluorescent ligands are normally used to visualize receptor localization in the native cellular environment. BODIPY-prazosin (QAPB) is a fluorescent analogue of the high affinity non-selective α_1-AR antagonist, prazosin but validation of its pharmacology at α_{1A}-ARs which play an important role in regulating the prostate, heart, arteries and blood vessels has not been conducted.

Aims. The study aimed to characterize and assess the localization and pharmacological properties of QAPB in different cellular compartments through short and long incubation times in live CHO cells expressing the human α_{1A}-AR.

Methods. Calcium mobilization assays was first used to assess the functionality of prazosin and its fluorescent analogue QAPB. Competition binding studies were conducted using ^{3}H-prazosin and 10nM QAPB to compare pK_i values. Confocal microscopy was used to assess the association and dissociation kinetics of QAPB at the plasma membrane and cytoplasm at 25°C and 37°C following 5 min and 1 h incubation.

Results. QAPB displays 10-fold lower affinity in comparison to prazosin, although the Schild slopes were not different (p>0.05). Total fluorescence binding with 10nM QAPB produced similar pK_i values of α_1-AR antagonists (n=4-7) compared to ^{3}H-prazosin binding in intact cells. Dissociation kinetics of QAPB at the plasma membrane was faster than in the cytoplasm (n=3-6).

Discussion. QAPB is able to probe the spatial organisation of α_{1A}-ARs in the plasma membrane and cytoplasm with high specificity and is able to provide a high throughput and robust approach to probe for α_{1A}-AR pharmacology. It offers an alternate approach that allows localization of receptors to explain differences in functionality which cannot be done with radioligand binding assays.

147

Identification of the molecular determinants of adenosine A_1 receptor allosteric modulation.

Lauren T May[1], Anh TN Nguyen[1], Karen J Gregory[1], Patrick M Sexton[1] & Arthur Christopoulos[1], Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash Univ[1], Parkville, VIC.

Introduction. The adenosine A_1 receptor (A_1AR) is an important therapeutic target for a number of cardiovascular and neuronal conditions (Jacobson *et al,* 2006). A_1AR allosteric modulators interact with a topographically distinct binding site from that of adenosine and offer a number of theoretical advantages, including increased subtype selectivity and preservation of the spatial and temporal pattern of endogenous agonist signalling. To facilitate the rational design of more selective and efficacious A_1AR allosteric ligands, greater structural knowledge of the allosteric binding site is required.

Aim. To investigate the role of the second extracellular loop on allosteric ligand binding at the A_1AR.

Methods. Mutant A_1ARs containing single alanine substitutions were expressed in FlpINCHO cells. Radioligand binding interaction studies between the orthosteric agonist, NECA, and the allosteric ligand, PD81723, quantified allosteric ligand affinity and cooperativity. Docking and molecular dynamic simulations were performed using a human A_1AR 3D homology model based on an active A_{2A}AR structure (PDB ID: 3QAK).

Results. Substitution of E172 for alanine resulted in a significant reduction in PD81723 affinity. Substitution of F144, N147, N148, L149, E153, N159, G160, S161, V166, I167, K168, E170, K173, V174, S176 and E178 for alanine significantly reduced the allosteric binding cooperativity between NECA and PD81723. Docking and molecular dynamics were subsequently performed to assist with interpretation. The predicted allosteric binding site for PD81723 was located in the extracellular region within TM2/6/7 and ECL2/3. Molecular dynamics suggested E172 forms a hydrogen-bond interaction with PD81723. PD81723 is also flanked by a hydrogen bond with another glutamate residue E170, which forms hydrogen bonds with two adjacent lysine residues K168 and K173.

Discussion. A_1AR mutagenesis and molecular modelling suggest that E172 forms a hydrogen bond with PD81723. Hydrogen bonding between the extracellular residues, K168, E170, E172, K173 may explain their role on the transmission of allosteric cooperativity.

Jacobson K et al (2006) Nat Rev Drug Discov 5:247-264

148

Compartmentalised Signalling of the Calcitonin Gene-Related Peptide (CGRP) Receptor

Meritxell Canals, Rebecca E Yarwood, Michelle L Halls and Nigel W Bunnett. Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC.

Introduction. The calcitonin receptor-like receptor (CLR) and the receptor activity-modifying protein 1 (RAMP1) comprise a receptor for calcitonin gene-related peptide (CGRP). CGRP is a potent mediator of neurogenic inflammation and pain transmission. Moreover, CGRP has a causative role in migraine headaches, and is thus a mediator of human disease. We have recently demonstrated that neurokinin 1 receptor (NK$_1$R) signalling from endosomal compartments participates in pain transmission. However, whether endosomal signalling is also a feature of other neuropeptide GPCRs is still unknown.

Aims. To investigate the link between CGRP receptor trafficking and signalling in real time in live cells.

Methods. Confocal imaging and Bioluminescence Resonance Energy Transfer (BRET) were used to investigate the trafficking of CGRP receptor. To assess compartmentalised signalling, we used Förster Resonance Energy Transfer (FRET) biosensors for cAMP, PKC and ERK that are selectively targeted to the cytosol, nucleus or membrane of the cell.

Results. 100 nM GCRP triggers β-arrestin2 recruitment, receptor desensitisation and internalisation. Stimulation of the CGRP receptor with 1 nM CGRP generates the following signalling profile; cytosolic and plasma membrane cAMP production, cytosolic PKC activation and cytosolic and nuclear ERK activation. Inhibition of CGRP receptor internalisation using genetic and pharmacological approaches (dynamin dominant negative or Dyngo4a, respectively), or lipidated antagonists, inhibited nuclear ERK activity. (Inset, ***, ^^^, p<0.001 vs vehicle control or CGRP control, respectively, two-way ANOVA with Sidak's multiple comparison test).

Discussion. Together, these data suggest that, like the NK$_1$R, the CGRP receptor elicits compartmentalised signalling once internalised. The consequences of this signalling for pain transmission are currently under study.
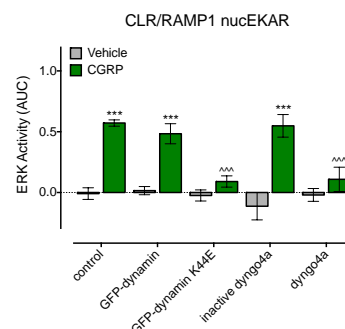
149

Site-directed mutagenesis at the dopamine D$_2$ receptor reveals key residues involved in receptor activation and biased agonism

Carmen Klein Herenbrink[1], Christopher Draper-Joyce[1], Laura Lopez[1], Arthur Christopoulos[1], J Robert Lane[1]. Drug Discovery Biology[1], Monash Institute of Pharmaceutical Sciences, Monash University, VIC, Australia.

Introduction. Agonists and partial agonists that target the dopamine D$_2$R are used clinically to treat Parkinson's disease and schizophrenia, respectively. Furthermore, we and others have provided evidence that some clinically used antipsychotics display biased agonism at the dopamine D$_2$ receptor compared to other dopaminergic agonists. Thus, it is important to understand the molecular determinants of agonist efficacy at this therapeutically important drug target.

Aims. The aim of this study was to identify residues within the dopamine D$_2$ receptor that are important for the ability of clinically relevant dopaminergic agonists to bind to the receptor, induce receptor activation and for biased agonism.

Methods. SNAP-tagged wild-type and mutant dopamine D$_2$ receptors were stably expressed in CHO-FlpIn cells. ERK1/2 phosphorylation and cAMP accumulation assays were performed and data analysed with an operational model of agonism to measure the effect of selected ligands at the wild-type and mutant receptors, and to quantify biased agonism between these two pathways. Competition binding experiments were conducted to determine binding affinity. These data in parallel with molecular dynamics simulations were used to gain more insight into the binding pose of the selected ligands within the receptor, and the receptor conformations induced upon ligand binding.

Results. We have identified key residues around the orthosteric binding site important for binding, efficacy and biased agonism at the dopamine D$_2$ receptor. Furthermore, we identify a cluster of residues below the orthosteric site in TM III and TM IV that, while not important for binding affinity, play an important role in receptor activation.

Discussion. This study is the first to systematically evaluate the dopamine D$_2$ receptor residues are important for agonist binding, receptor activation and biased agonism. These results further our understanding of the determinants of agonism at the D2R and will aid the design of new therapeutics with a desired efficacy or biased profile.

150

"Use with caution in renal impairment" – implications for drug dosing in the general practice setting

Margaret Jordan[1,2], Adele Stewart[1], Julie Blaze[2], Jeff Hall[2], Judy Mullan[3]. Woonona Medical Practice[1], NSW; Bulli Medical Practice[2], NSW; School of Medicine, Univ of Wollongong[3], NSW.

Introduction. Many medicines prescribed by GPs require altered management as kidney function declines. A research project was undertaken involving a clinical pharmacist integrated into a general practice in the management of oral anticoagulants. The results highlighted the implications of using readily available estimates (Glomerular Filtration Rate, or eGFR) for dosing of the non-vitamin K antagonist oral anticoagulants (NOACs). The outcomes informed a second project on the management of medicines in the presence of impaired kidney function.

Aims. To describe the methods used to assess kidney function in two general practices; how these influence the management of renally-excreted medicines and the impact on management of these by a clinical pharmacist.

Methods. Two research projects were undertaken in separate general practices. Study 1 was to investigate the impact of a clinical pharmacist on the management of oral anticoagulants compared to the outcomes measured in a retrospective review. Study 2 was to assess the appropriateness of medicine management in patients over 65 with impaired kidney function, before (stage 1) and after (stage 2) the input of a pharmacist. Medicines most commonly prescribed were targeted in stage 2. Estimates of kidney function were recorded (uncorrected eGFR) or calculated (creatinine clearance [eCrCl] using Cockcroft-Gault for actual and ideal body weights [ABW, IBW]) in both studies.

Results. Study1: before the pharmacist input, eCrCl was not used to guide drug-dosing of NOACS. Appropriate monitoring and adjustment was seen in only 50% usage compared to 100% at the study close (p = 0.0245). Study 2: the appropriateness of management of the target medicines (pregabalin, NOACS, antidiabetic agents, statins, and digoxin) improved from 43% to 92% (p<0.0001) despite similarities in all measures of kidney function between both stages (P=0.4872). The metrics used to assess kidney function (eGFR vs eCrCl IBW) however, differed significantly from each other in both stages; means of stage 1 were 63mL/min/1.73m^2 vs 47mL/min; (P <0.001.)

Discussion. There were significant differences in the estimates of kidney function which led to discrepancies in drug dosing in both studies, particularly for the NOACs. GPs have an ever-increasing range of medicines to manage with competing sources of information for guidance. The input of a clinical pharmacist improved the management of medicines in these general practices.

151

Clinicians' attitudes and perceptions regarding stroke prevention in atrial fibrillation

Bryony E Scott[1], Colin M Curtain[1], Leanne Chalmers[1], Luke RE Bereznicki[1]. Pharmacy, School of Medicine, University of Tasmania[1], Hobart, TAS.

Introduction. While appropriate oral anticoagulation in atrial fibrillation (AF) can significantly reduce the risk of stroke and is supported by evidenced-based guidelines, evidence suggests that it remains underused, or is used inappropriately. There is currently little data regarding clinicians' perspectives on stroke prophylaxis in AF since the introduction of the non-vitamin K antagonist oral anticoagulants (NOACs).

Aims. To evaluate Australian clinicians' attitudes in their prescribing of anticoagulants for stroke prevention in AF, including their perceptions of the safety and effectiveness of the NOACs and preferred agents.

Methods. A vignette-based survey was distributed to a random sample of Australian cardiologists and general practitioners (GPs) (n=500 each), and an online version of the survey was distributed via email to all clinicians affiliated with the three major Tasmanian public hospitals. Clinicians were asked to indicate their preferred anticoagulant treatment for seven fictional patients with AF. Prescribing choices were compared against expert consensus. Additional multiple-choice and Likert-item questions addressed their use of guidelines and risk stratification tools, attitudes and perceptions.

Results. Of 174 respondents (39 GPs, 40 cardiologists and 95 other hospital-based clinicians), 88% reported routinely using an AF stroke risk stratification tool but only 44.5% routinely used a bleeding risk score. Fifty-three per cent of cardiologists, 49% of GPs and 32% of other hospital-based clinicians agreed with the expert panel's prescribing choices for four or more vignettes; this was unrelated to use of scoring tools and guidelines (p>0.05). Cardiologists were significantly (p = 0.005) more likely to favour a NOAC, with apixaban being the preferred agent. Choice of anticoagulant was also influenced by clinicians' knowledge and confidence.

Discussion. This study demonstrated that prescribing patterns varied between cardiologists, GPs and other hospital-based clinicians. Concerns over the safety of NOACs were identified as potential barriers to their utilisation by GPs and other hospital-based clinicians. Results from the vignettes demonstrated sub-optimal prescribing choices amongst all clinicians. Further education is required to ensure all clinicians are educated and confident prescribing stroke prophylaxis in AF.

152

SMART-AF: Development of a decision support smartphone app to improve antithrombotic prescribing in atrial fibrillation

Leanne Chalmers[1], Luke RE Bereznicki[1], Ritam Prasad[2], Nicole Hancock[2], Paul D MacIntyre[2], Duncan S McKenzie[2], Camille M Boland[2], Bridget E Frain[1], Paris Buttfield-Addison[3]. Pharmacy, School of Medicine, University of Tasmania[1], Hobart, TAS; Royal Hobart Hospital[2], Hobart, TAS; SecretLab[3], Hobart, TAS.

Introduction. Appropriate thromboprophylaxis in patients with atrial fibrillation (AF) is highly effective in reducing the risk of stroke. Previous studies have demonstrated underuse of anticoagulants in high-risk patients, and while the introduction of non-vitamin K antagonist oral anticoagulants (NOACs) may increase access to anticoagulation, these agents are associated with a new range of clinical problems necessitating improved prescriber support.

Aims. To develop and validate a decision support smartphone application ('app') to support prescribers in the choice of thromboprophylaxis in patients with AF.

Methods. The app was developed via collaboration between a haematologist, stroke physician, cardiologist, specialist clinical pharmacists, pharmacy academics and information technology experts to increase use of contemporary evidence-based guidelines and stroke and bleeding risk stratification tools, and address poor understanding of the clinical use of NOACs. The app provides assessment of patients' stroke and bleeding risk using entered patient data, a recommendation for the need for anticoagulation, and suggestions and practice points regarding the most suitable antithrombotic agent(s). Validation was undertaken by comparing the app's prescribing recommendations with the consensus opinion of an expert panel of clinicians for seven hypothetical case studies.

Results. Initial testing identified the need to make minor amendments to the app logic and interface to ensure consistency with the experts' recommendations. These were addressed in the final build, and the app is due for launch in late September 2015.

Discussion. Multidisciplinary collaboration has resulted in the development of a validated, readily accessible decision support tool for prescribers initiating thromboprophylaxis in patients with AF. Initial prescriber feedback has been positive. Future research will investigate the usability of the app, and its effect on prescriber confidence and prescribing choices, with the aim of optimising the clinical outcomes for patients with AF.

153

Home Medicines Review following acute coronary syndromes: Preliminary results from a randomised controlled trial

Daniel DL Bernal, Leanne Chalmers, Ronald L Castelino, Luke RE Bereznicki, Gregory M Peterson. Pharmacy, School of Medicine, University of Tasmania, Hobart, TAS.

Introduction. Following acute coronary syndromes (ACS) guidelines recommend use of four classes of medication (antiplatelet agents, statins, beta-blockers, and angiotensin-pathway inhibitors) to improve survival and quality of life. Sub-optimal prescribing by clinicians and poor adherence by patients are common and lead to worse outcomes.

Aims. To evaluate the effect of a directed Home Medicines Review (dHMR) performed by trained accredited pharmacists on adherence to guideline-medications at six months following ACS.

Methods. A randomised controlled trial was conducted at two Tasmanian hospitals with all patients admitted with ACS from April 2012 to April 2013 screened for enrolment. An online education package was developed to tailor the HMR towards detecting drug-related problems and improving adherence following ACS, including instructions on how to safely initiate guideline medications if they were not previously prescribed in hospital. Intervention patients received a dHMR two months following discharge. Patient adherence to guideline medications was measured as the primary outcome, using medication refill records at six months. Patients were considered adherent if they had a continuous medication supply of all four guideline medications and an average medication possession ratio of 0.8 or higher.

Results. Three hundred and sixty-seven patients were screened for enrolment; complete data was available for 154 patients (77 per group). The intervention group had a higher rate of non-ST-elevation myocardial infarction as their final discharge diagnosis (39.0% versus 20.7%, p=0.02); there were no other differences between the groups. Thirty-two (41.6%) control and 24 (31.2%) intervention patients were prescribed and adherent to all four classes of medication at six months post-discharge (p=0.18).

Discussion. A dHMR offered to patients two months post-ACS did not appear to influence prescribing and adherence at six months. The overall rate of concordance with prescribing guidelines and patient adherence with medication was low and will be further investigated.

154

Hospital readmission for major bleeding or thromboembolic complications in patients with atrial fibrillation

Durga Bista, Leanne Chalmers, Gregory M Peterson, Luke RE Bereznicki. Pharmacy, School of Medicine, University of Tasmania, Hobart, TAS.

Introduction. The Tasmanian Atrial Fibrillation (TAF) Study was established to provide ongoing Australian data on the clinical management and outcomes of people with atrial fibrillation (AF). The initial phase of the study involved the collection of retrospective data in the period prior to the introduction of the novel oral anticoagulants in Australia.

Aims. To examine the patient characteristics, antithrombotic prescribing patterns and rates of hospital readmission due to bleeding or thromboembolism (TE).

Methods. We reviewed the medical records of patients admitted to Tasmanian public hospitals between January 2011 and June 2012 with a diagnosis of AF. We documented risk factors for stroke, major bleeding and prescribed medication during the index (i.e. first) admission. Patients were followed for three months from the discharge date of their index admission to identify any subsequent readmissions due to bleeding or TE complications of AF.

Results. A total of 1469 of 2502 patients (≥ 18 years) with AF were eligible for inclusion. The mean ± SD age and $CHADS_2$ score of the patients overall were 76 ± 12.3 years and 2.1 ± 1.3, respectively. According to guideline recommendations, 64.1% of patients had a $CHADS_2$ score ≥2 and were eligible for anticoagulant therapy. An anticoagulant (or anticoagulant plus antiplatelet) was prescribed for 55.6% of these patients at discharge. Antiplatelet agents were prescribed in 34.5% of patients while 9.9% received no antithrombotic therapy. In contrast, 51.4% of those at low risk (score 0) were discharged on an anticoagulant. The rates of bleeding and TE-related readmissions within three months were 2.4% (95% CI 1.6-3.3%) and 1.3% (95% CI 0.7-1.9%) respectively.

Discussion. The rates of bleeding and TE are consistent with other 'real-world' study data, where higher rates of bleeding have been reported as compared to clinical trials data. Poor anticoagulation control after hospital initiation of warfarin and lack of guidelines adherence in prescribing antithrombotic therapy might have contributed partially to our findings. There still exists a gap between the evidence-based risk stratification and antithrombotic management patterns among hospitalised patients with AF in Tasmania.

155

Association of antihypertensive medication class and falls in the elderly: systematic review and meta-analysis

H Reza Kahlaee[1], Mark Latt[1,2]. Carl R Schneider[1], Faculty of Pharmacy, The Univ of Syd[1], Sydney, NSW, RPA Hosp[2], Sydney, NSW

Introduction: An association between antihypertensive medication use and falls in older persons has been reported in the literature. However, the differing effects of antihypertensive drug class on falls are less well characterised.

Aims: Our objective was to review the literature systematically to evaluate the effect of chronic antihypertensive medication use and antihypertensive drug class on the risk of falls in older persons.

Methods: Studies measuring the association between falls and antihypertensive medication use in older persons were identified through a systematic search of English language articles published from 2007 to 2014 in CINAHL, Cochrane, EBM, EMBASE and MEDLINE databases. Studies were included if they provided original data measuring the association between antihypertensive medication use (ATC Codes C to C09) and falls in people older than 65 years old. Of the 6208 retrieved articles, 15 studies met our inclusion criteria for meta-analysis. There was no statistically significant heterogeneity in the included studies. Pooled HR (hazard ratio) and OR (odds ratio) and 95% CI (confidence interval) were calculated to estimate the effects of antihypertensive medication use and antihypertensive drug class on risk of falls.

Results: The following pooled estimates were obtained: OR 0.96(95%CI 0.89-1.02) for any kind of antihypertensive drug use (ATC code C); OR 1.16(95%CI 0.82-1.49) for cardiac therapy drug use (ATC code:C01); OR 1.29(95%CI 1.09-1.48) for diuretic use (ATC code:C03); OR 1.02(95%CI 0.82-1.22) for β-blocker use (ATC code:C07); OR 1.12(95%CI 0.90-1.34) for calcium channel blocker (CCB) use (ATC code:C08); OR 1.16(95%CI 1.00-1.32) for angiotensin converting enzyme inhibitor/angiotensin II receptor blocker (ACEi/ARB)(ATC code :C09) use.

Conclusion: The chronic use of diuretics was significantly associated with increased risk of falls in older persons. ACEi/ARB use approached significance. Neither β-blocker nor CCB use was significantly associated with falls. Clinicians and patients should be aware of the increased risk of falls associated with chronic diuretic or ACEi/ARB use.

156

National survey of community pharmacy's capacity to screen for the risk of cardiovascular disease

Kevin P Mc Namara[1,2], Ines Krass[3], James A Dunbar[4], Josie Hughes[5], Gregory M Peterson[5]. Centre for Medicine Use and Safety, Monash University[1], Melbourne, Vic; Greater Green Triangle UDRH, Flinders University and Deakin University[2], Melbourne, Vic; Faculty of Pharmacy, University of Sydney[3], Sydney NSW; Faculty of Health, Deakin University[4], Melbourne, Vic; Unit for Medicines Outcomes Research and Evaluation, University of Tasmania[5], Hobart, Tas.

Introduction. Internationally, cardiovascular disease (CVD) screening is commonplace in community pharmacy but inadequate health policy, national guidelines and funding arrangements may negatively impact on quality and capacity. Data about the capacity of the profession to address public health problems, and the needs of the profession to optimise service delivery are both necessary to promote supportive macro-level interventions.

Aim. To explore the nature and extent of community pharmacy screening in Australia today, capacity to increase screening, and barriers to increased capacity at an individual, organisational and environmental or policy level.

Methods. A link to the online survey was disseminated by email to all Quality Care Pharmacy Program (QCPP) – accredited pharmacy managers (i.e. more than 90% of Australian pharmacies) by QCPP on behalf of the researchers. Follow up occurred at four and six weeks. The survey examined current and anticipated future service delivery, service models, capacity to increase delivery, and barriers to service delivery.

Results. We received 294 responses from 5080 members (6%). Blood pressure testing was almost universal (96%), but other assessments were provided by a minority. One quarter (26%) offered a 'heart health check' with multiple risk factors assessed. A majority providing each service predicted increasing future provision of these services, and 59% indicated capacity to provide a multiple risk factor assessment. A majority of pharmacies (64%) indicated that they had the current capacity to dedicate between one and three hours per week to CVD screening and prevention activities, and 24% indicated in excess of four hours. Two thirds (67%) reported regularly serving at least one ethnic community at elevated CVD risk. Financial viability was the most common significant barrier to increasing screening provision. Other patient-level, pharmacy-level and policy –level factors were identified.

Discussion. Pharmacies provide a wide range of CVD-related services and many indicate extra capacity to screen. Lack of financial viability was the most common barrier to expansion.

157

Understanding healthcare professionals' perspectives on Nepalese patients' diabetes management

Sujata Sapkota[1], Jo-anne Brien[1], Parisa Aslani[1]. Faculty of Pharmacy, The University of Sydney, Sydney, NSW.[1]

Introduction. Patients with type 2 diabetes (T2D) visit a multitude of healthcare professionals, ranging from general physicians to specialists to allied healthcare professionals such as dietitians. Exploring healthcare professionals' perspectives is essential to understanding and addressing gaps in diabetes care.

Aims. To understand Nepalese healthcare providers' perspectives on diabetes management in patients with T2D; and to explore strategies to improve diabetes care in Nepal.

Methods. Semi-structured in-depth face-to-face interviews are being conducted with healthcare professionals involved in diabetes care in Kathmandu Valley, using an interview protocol addressing the study aims.

Results. Eleven healthcare professionals have been interviewed to date. The participants reported an overall rise in patients' awareness and understanding of their own health and personal wellbeing, and an improvement in how they managed their diabetes. This was observed mostly in city-based patients, particularly in Kathmandu. Nonetheless, this observation was perceived to be highly variable between patients. While some patients were considered to be more aware and to effectively engage in their diabetes management, others were reported as "careless" and/or lacking in understanding about their disease and its management. Most participants felt that this limited understanding was the major barrier to effective diabetes management. Awareness and information dissemination was, therefore, reported as the most important strategy to address diabetes management in Nepal. The services provided to patients with T2D varied between healthcare institutions, with the patient care model regarded by majority as not ideal. Services were mostly limited to providing patient- consultations; only a few health institutions provided additional diabetes education classes and dietitian services. Effective multidisciplinary inter-professional collaboration was considered lacking.

Discussion. Although there has been an observed improvement in patients' understanding about diabetes and health over time, participants felt that patients' knowledge and understanding of their condition and management was still inadequate. Educating patients and the general public in Nepal, implementing a multidisciplinary collaborative model of care, and providing a consistent service to patients with T2D across all settings were considered as key strategies to addressing diabetes management.

158

A mathematical model of the metabolic pathway for azathioprine and 6-mercaptopurine

Murray L Barclay[1,2], Aidin Jalilzadeh[3,4], Pavan Vajjah[4] and Stephen Duffull[4]. Depts of Clinical Pharmacology and Gastroenterology, Christchurch Hospital[1]; Dept of Medicine, University of Otago Christchurch[2]; Dept of Mathematics and Statistics[3], and School of Pharmacy[4], University of Otago, Dunedin, NEW ZEALAND.

Introduction. Azathioprine (AZA) and 6-mercaptopurine (6MP) are well established for the treatment of inflammatory bowel disease (IBD). However, many patients are resistant to treatment or are under-dosed, and up to 30% stop treatment due to severe toxicity. The metabolic pathway for AZA and 6MP is complex and many factors influence the concentrations of the active and toxic metabolites that determine patient response to treatment.

Aims. To develop a mathematical model to quantify the metabolic fate of AZA and 6MP in plasma and red blood cells in order to investigate abnormal metabolite profiles in patients and aid dose prediction.

Methods. A systems pharmacology approach centred on development of a mathematical model based on the known biochemistry of thiopurine catabolism was used. The model was formed assuming equilibrium enzyme kinetics and comprises two major compartments, extracellular (EC) and intracellular (IC). Enzyme activity was represented as maximum velocities and Michaelis-Menten constants. The model retains mass-balance for the extracellular fate of azathioprine.

Results. The model developed was able to simulate the EC and IC concentration-time profile of AZA and its thiopurine metabolites upon administering single or multiple doses of AZA. An overlay of the model predictions and observed data from clinical studies indicated that the model provided a good description of the data.

Discussion. The model performed well when correlated with reported thiopurine metabolite concentration profiles. Further evaluation of the model in different patient cohorts will be required to assess its clinical utility. Various what-if scenarios can be simulated using the model to answer questions related to abnormal thiopurine metabolite concentration profiles. The model may be useful for individualising thiopurine dosing regimens.

159

Impact of deprescribing of polypharmacy on adverse geriatric outcomes

Aniko Huizer-Pajkos[1], Alice Kane[1,2], John Mach[1,2], David G Le Couteur[2,3], Sarah N Hilmer[1,2]. Departments of Clinical Pharmacology and Aged Care, Royal North Shore Hospital and Kolling Institute of Medical Research, St Leonards, NSW[1]; Sydney Medical School, University of Sydney, Sydney, NSW[2]; Centre for Education and Research on Ageing, Concord Hospital and Anzac Research Institute, Concord, NSW[3]

Introduction. Polypharmacy (use of ≥5 different medicines) occurs in over half of older Australians and is increasing. In observational studies, polypharmacy increases the risk of functional impairment, falls, frailty and death. Evaluation of the effects of polypharmacy and deprescribing in older adults is ethically and feasibly difficult. In our recently developed mouse model of polypharmacy, short term polypharmacy impaired physical function in old male mice.

Aims. Investigate whether adverse outcomes of short term polypharmacy are reversed with medicine withdrawal (deprescribing).

Methods. Old (23 months) male C57BL/6 mice received either control diet (no drugs) for 11 weeks, or polypharmacy diet (deprescribe group) containing simvastatin (20mg/kg/day), metoprolol (350mg/kg/day), omeprazole (10 mg/kg/day), paracetamol (100 mg/kg/day) and citalopram (10 mg/kg/day) for four weeks, which was gradually withdrawn over the next 6 weeks. Mice were assessed for physical performance at 0, 4 and 11 weeks. Performance measures were transformed into Frailty Intervention Assessment Values (FIAV) to compare the effects of treatments. FIAV is the sum of the standardised values for locomotor activity, rotarod performance (balance and coordination) and front paw hang.

Results. After 4 weeks of polypharmacy, FIAV did not change significantly for controls and declined in the deprescribe group ($p < 0.05$). After deprescribing (week 11) the FIAV of the control group remained at baseline, and the deprescribe group did not return to baseline.

Discussion. Our previously developed polypharmacy mouse model was successfully used to assess the effect of deprescribing on geriatric outcomes in old age. Physical function declined with polypharmacy and did not return to baseline after deprescribing.
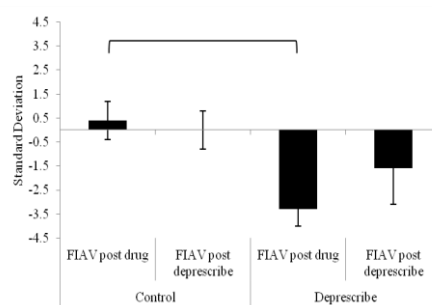
160

Medicine information exchange networks among health care professionals and prescribing in geriatric medicine wards

Bosco Chan[1], Sarah N Hilmer[1, 2], Emily Reeve[1, 2], Slade Matthews[2], Peter Carroll[1.2], Janet Long[3], Mark Latt[2], Vasi Naganathan[2]. Dept of Clin Pharmacol and Aged Care[1], Royal North Shore Hosp, Sydney, NSW: Sydney Medical School[2], Univ of Sydney, Sydney, NSW: John Walsh Centre for Rehabilitation[3], Faculty of Medicine, Univ. Of Sydney, Sydney, NSW

Introduction. Multidisciplinary care is a fundamental principle of prescribing and is particularly important in geriatric medicine, where patients are increasingly susceptible to high risk medicine use and adverse drug events.

Aims. The primary aim was to characterise the medicine information exchange networks of geriatric medicine wards in Sydney teaching hospitals. The secondary aim was to compare prescribing indicators between networks.

Methods. Social network analysis was conducted in three geriatric medicine wards. All ward staff (doctors, nurses, allied health, administration) identified those from whom they initiated discussion regarding use of medicines within the past two weeks. Retrospective data was collected on indicators of high risk prescribing (change in medicine use and potentially inappropriate medicines (PIMs)) for all patients discharged within the designated two week period.

Results. Hospital 1 had the highest overall observed network density (proportion of observed interactions/total possible interactions) of 21.7%, with Hospital's 2 and 3 having network densities of 17.3% and 16.1% respectively. All networks displayed the tendency to have more densely connected networks within professional disciplines (doctor-doctor) than between disciplines (doctor-nurse). Reciprocity (interactions identified by both respondents) was lowest in Hospital 2 at 38.9% of ties, compared to 41.7% and 48.5% in Hospitals 1 and 3 respectively. Individual analysis identified pharmacists as key players in Hospital 1 and 3, but not in Hospital 2, where pharmacists were not specific to geriatric medicine. The number of medicines on discharge for hospitals 1, 2 and 3 were 10.4±0.6, 8.2±0.5 and 10.7±0.8 respectively (p=0.004). The ratio of medicines started: stopped was 1.68:1 and 1.49:1 in Hospitals 1 and 3 respectively, compared to 3.29:1 in hospital 2. The ratio of PIMs (Beer's Criteria) started: stopped were 0.55:1 and 0.5:1 respectively in Hospitals 1 and 3, compared to 2.75:1 in Hospital 2.

Discussion: A variety of different network characteristics and prescribing outcomes were observed. Future studies implementing interventions to foster inter-professional relationships between health care disciplines may help utilise all skill sets, optimising the quality use of medicines in geriatric medicine.

161

Pharmacokinetics and efficacy of oral frusemide in decompensated vs. compensated heart failure

Maneesha Dedigama[1], Patrick Russell[2] Carmine De Pasquale[3] Arduino A Mangoni[1], Tilenka Thynne[1], Andrew Rowland[1] Department of Clinical Pharmacology, Flinders University and Flinders Medical Centre, Adelaide, SA[1]. Royal Adelaide Hospital, Adelaide, SA[2]. Flinders Medical Centre, Adelaide SA[3]

Introduction. Despite a paucity of evidence, the loop diuretic frusemide is commonly administered intravenously (iv) for the management of fluid overload in decompensated heart failure (HF). Expert opinion suggests that oral frusemide has poor bioavailability and efficacy in this setting, however this has not been confirmed by the small number of trials examining frusemide pharmacokinetics.

Aims. To compare the bioavailability and efficacy of oral frusemide in decompensated vs. compensated HF.

Methods. Patients (n=5) admitted to hospital with decompensated heart failure had serial bloods collected at t= 5min, 1, 2, 3, 4, 5 and 6hrs on three occasions: a) post iv frusemide (decompensated HF); b) post oral frusemide (decompensated HF); and c) post oral frusemide (compensated HF, at least 48 hours post decompensated blood tests). Serum frusemide concentrations and AUC for each dose were assessed, and bioavailability was calculated. Efficacy was assessed as the decrease in body weight and urine output within the first 6hrs post dosing.

Results. Average age and hospital stay were 74.8 years and 14 days, respectively. All patients had a diagnosis of congestive cardiac failure and clinical evidence of right heart failure. The oral bioavailability was 33.8% lower in (range 28.3 to 39.7) in decompensated vs compensated HF. In the decompensated state a lower Cmax and a longer Tmax with oral frusemide was observed. There was an increased diuresis with oral frusemide in decompensated HF compared to iv, for an equivalent exposure of serum frusemide in 4 of the 5 patients.

Discussion. A decrease in the bioavailability of oral frusemide was seen in decompensated HF. This is contrary to other published PK studies. This discrepancy may be due to a different patient population with the patients in our study all having predominant right heart failure. The use of oral frusemide in the decompensated setting was associated with a better diuresis than iv frusemide when corrected for AUC. These initial results suggest that an earlier switch from iv to oral frusemide may provide an effective diuresis whilst minimising adverse effects related to high exposure of loop diuretics.

162

Prescribing of oral anticoagulants in older inpatients: Has the availability of newer agents led to inappropriate prescribing practices?

Nicholas Farinola[1], Sepehr Shakib[1], J Simon Bell[2,3], Gillian Caughey[1], Des Colley[1], Dallas Grasby[1], Milka Hauta-aho[4,5], Sally Johns[1]. Department of Clinical Pharmacology, Royal Adelaide Hospital[1], Adelaide, SA; Sansom Institute, University of South Australia[2], Adelaide, SA; Centre for Medicine Use and Safety, Monash University[3], Parkville, VIC; Department of Pharmacology, Drug Development and Therapeutics, University of Turku[4], Finland; Unit of Clinical Pharmacology, Turku University Hospital[5], Finland.

BACKGROUND: Oral anticoagulation reduces stroke and venous thrombosis risks, but is associated with increased bleeding risk. The introduction of new oral anticoagulants (NOACs) provides treatment alternatives.
AIM: To examine if introducing the NOACs have changed the prescribing practice and appropriateness of oral anticoagulation in older inpatients.
METHODS: A prospective study was conducted between October 2012 and August 2015 of inpatients aged ≥60 years, initiated on an oral anticoagulant, in a large metropolitan teaching hospital. The cohort comprised patients prescribed an oral anticoagulant prior to (March 2014) and after (from April 2014) the introduction of NOACs on the hospital formulary. Descriptive statistics and significant differences in clinical characteristics and appropriateness of therapy between treatment groups were examined.
RESULTS: 289 patients were included; 153 in the pre-NOAC group and 136 in the post-NOAC group. There were significantly more patients with a history of stroke in the post-NOAC group (27.9%) by comparison to the pre-NOAC group (16.3%) (p=0.022). This was associated with a statistically higher $CHADS_2$ score in the post-NOAC group. No changes in HAS-BLED and frailty scores were observed between the groups. Despite this, 35% patients prescribed an oral anticoagulant for atrial fibrillation had higher HAS-BLED scores than $CHADS_2$ scores.
CONCLUSIONS: After the introduction of the NOACs, patients initiated on an oral anticoagulant had higher stroke risk but no increased bleeding risk, suggesting an appropriate change in prescribing practice. However, a large number of patients had major bleeding risks greater than their risk of stroke, warranting further improvements in prescribing.

163

What Is Polypharmacy Exactly (WIPE) study

Gao Jing Ong[1], Sepehr Shakib[2]. Department of Clinical Pharmacology, The Queen Elizabeth Hospital, Central Adelaide Local Health Network[1], South Australia; Department of Clinical Pharmacology, The Royal Adelaide Hospital, Central Adelaide Local Health Network, SA[2]

Introduction. Numerous studies have associated polypharmacy with significant harms, and various tools have been utilised with the aim of reducing polypharmacy showing mixed results. Consensus regarding the definition and determinants of polypharmacy also remain uncertain with, studies thus far stating various definitions.
Aims. We aim to better define and to determine the predictors of polypharmacy to improve patient outcomes.
Methods. We identified an expert panel of judges (n=3), who had a particular interest in polypharmacy and de-prescribing. We then presented them with 20 cases with a list of their medications and comorbidities, and asked each judge to rate each case on the topic of (a) the degree of polypharmacy, (b) the degree of ability of each medication regime to cause harm, and (c) the ability of each regime to be reduced. Their ratings for each question will be used as the "gold standard". We simultaneously analysed the list of medications in each case for the number of medications that meet the Beer's criteria, the STOPP criteria, medications that increases falls risks, calculated their drug burden index and also looked at the number of clinically relevant potential drug-drug interactions. Finally, judges' scores were analysed to determine which aspects of medication use correlate with polypharmacy.
Results. We found that the consistency of the scores between the judges regarding the 3 questions asked were high (Cronbach's alpha 0.91, 0.83 and 0.82 respectively). Analysis of the "gold standard" scores for polypharmacy revealed strong correlations with total number of drugs and drug:comorbidity ratio (r=0.77, p-value=0.0002; r=0.57, p-value=0.01 respectively), Beer's criteria (r=0.69, p-value=0.002), STOPP criteria (r=0.73, p-value=0.0006), Drug Burden Index (r=0.75, p-value=0.0004) as well as falls risk increasing drugs (r=0.73, p-value=0.0006).
Discussion. Our study showed that polypharmacy is not just related to a single aspect of medication use such as the number of medications alone, but it also relates to a range of other aspects including potentially inappropriate medicines as defined by the Beer's and STOPP criteria, as well as the amount of anticholinergic and sedative burden. Integration of these determinants will improve the definition of polypharmacy and allow easier identification and therefore treatment, to improve patient outcomes.

164

Drug-drug interaction alerts in an electronic prescribing system Philip Drennan[1], Paul Chin[1], Matthew Strowther[2], Christopher Lodge[1], Kathryn Dean[1], Matthew Doogue[1,2]. Department of Clinical Pharmacology, Canterbury District Health Board[1]; Department of Medicine, Otago University, Christchurch[2], NZ.

Introduction. Unintended drug-drug interactions (DDIs) are associated with adverse drug reactions and loss of drug efficacy. Electronic prescribing and administration (ePA) systems can include clinical decision support alerts to warn of potential DDIs. However proprietary DDI clinical decision support systems are associated with high alert burden (of the order of 70 to 360 per 1000 prescriptions) and risk of alert fatigue. Consequently, the DDI system integrated into MedChart™ has been switched off at Canterbury District Health Board. We hypothesised that restricting alerts to the DDIs most likely to be a) unintentional and b) cause patient harm, will reduce alert burden without compromising clinical outcomes.

Aims. To develop evidenced based rules for DDI alerts in an ePA system within the constraints of the software. To predict the alert rate due to these rules in hospital inpatients.

Methods. A literature search was undertaken and local "trigger tools" data were reviewed to develop DDI alert rules. Pharmacokinetic (PK) DDI alert rules were defined as single drug alerts to identify major perpetrators of DDIs for prescribers (Polasek et al, 2011). Initial pharmacodynamic (PD) DDI alert rules were defined based on bleeding risk. Alert burden was predicted by applying the DDI alert rules in a test environment to ePA drug administration data from older persons health inpatients, June-August 2015.

Results. 360 patients were prescribed 4242 medicines (mean 11.8 per patient). These generated 193 alerts (PK=123, PD=70), a rate of 45 alerts per 1000 prescription items and 50 alerts per 100 patients. These included 59 alerts for parenteral anticoagulants co-prescribed with oral antiplatelet drugs that were potentially intentional and/or low risk.

Discussion. The DDI alert rules developed have a lower alert burden than most current proprietary systems. System constraints preclude sophisticated alerts, e.g. considering drug doses or prescriber characteristics. Testing clinical decision support rules on real data sets prior to implementation should be routine practice. Future evaluations of clinical decision support systems should assess the effect on prescribing behaviour and patient outcomes.

Polasek TM, Lin FPY, Miners JO et al (2011). Br J Clin Pharmacol, *71*(5), 727–736.

165

What effect does methotrexate have on the liver in patients with a rheumatologic condition?
Andrew J Finch, Rheumatology and Clinical Pharmacology, Royal Brisbane Hospital, Brisbane, QLD

Introduction. The disease modifying anti-rheumatic drug (DMARD) methotrexate (MTX) has been used in the treatment of autoimmune conditions for many decades and is now it is used as a standard therapy world-wide. MTX is well known to cause an increase in liver enzymes in some patients and concern remains about the development of cirrhosis.

Aims. This review aims to determine the incidence of the raised liver enzymes as well as the development of cirrhosis in patients taking methotrexate for an autoimmune condition seen in a rheumatology outpatients setting.

Methods. All patients seen in the Caboolture hospital rheumatology outpatients between 2011 and 2014 had their outpatient letter reviewed to determine if they were taking methotrexate. Charts were then reviewed to confirm current use of MTX as well as the dose and if available, initiation date as well as treating diagnosis. Patient demographics including age was collected. Pathology tests including liver enzyme levels, inflammatory markers, as well as liver biopsy were also obtained if these were performed. Patients with multiple visits during this time period had their last review with current use of MTX used for analysis.

Results. 323 patients (254 females) with an average age of 54 years (23-86) were identified during the audit period. An increase in the liver enzymes was seen in 50 (15%) of patients. 8 patients proceeded to liver biopsy investigating cirrhosis and all patients were found to have an alternative cause for the development of cirrhosis.

Discussion. MTX is highly effective in the treatment of a number of autoimmune conditions, and is generally well tolerated. An increase in liver enzymes is frequently seen in patients on MTX. In this cohort of patients, MTX did not lead to cirrhosis which adds to the literature suggesting that MTX is a relatively safe medication, when used appropriately with regular monitoring.

166

NOX2 oxidase expressed in endosomes exacerbates viral pathogenicity

Eunice E To[1], Michelle Halls[2], Raymond Luong[1], Patrick Reading[3], Doug A Brooks[4], Grant R Drummond[1], Steven Bozinovski[5], Ross, Vlahos[5], Stavros Selemidis[1]. [1]Dept Pharm, Monash University, VIC; [2]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University. [3]Peter Doherty Institute, University of Melbourne, [4]Sansom Institute for Health Research, Uni of SA, Adelaide. [5]School of Health Sciences, RMIT, VIC.

Introduction. Reactive oxygen species (ROS) are crucial for the elimination of pathogenic bacteria, but the impact of ROS on viral pathogenicity is yet to be clearly defined.

Aim. To determine 1) the site of subcellular ROS generation, 2) the identity of the enzymes that generate ROS and 3) the impact of ROS on the pathogenesis of virus infection *in vivo*.

Methods. Confocal fluorescence microscopy was used to assess the subcellular distribution of viruses, Toll-like receptors (TLRs) and NOX enzymes, and to assess endosomal superoxide production in human and mouse macrophages that were infected with various ssRNA viruses (influenza A virus, HIV, Dengue, rhinovirus, respiratory synctitial virus, human parainfluenza virus, human metapneumovirus), dsRNA viruses (rotavirus) and dsDNA viruses (herpes simplex 2 and vaccinia virus). Mice (C57Bl/6) were infected intranasally with influenza A virus (Hong Kong X-31 strain, 10^5 PFUs/mouse) for assessments of airway inflammation, viral titers, cytokine expression and serum antibody levels. Site-directed mutagenesis was used to mutate cysteine residues to alanine on TLR7 for assessments of oxidatively modified receptor.

Results. NOX2 oxidase co-located with virus and TLR7 in early endosomes; providing a spatially targeted platform for ROS production that operated within minutes after the internalization of the single stranded RNA and DNA viruses into endocytic compartments. NOX2 oxidase activation was critically dependent on endosomal acidification and the engagement of TLR7 for ssRNA viruses or TLR9 for DNA viruses. NOX2-dependent endosomal H_2O_2 caused modification of crucial cysteine residues on the ectodomain of TLR7, resulting in suppression of innate anti-viral cytokine expression. Inhibition of NOX2 by genetic deletion or by the pharmacological inhibitor gp91ds-TAT resulted in a significant suppression in airway inflammation and viral titers following influenza A virus infection.

Discussion. Viruses activate endosomal NOX2 oxidase, which suppresses fundamental components of viral immunity, and this has major implications for the treatment of viral infections.

167

Urokinase-dependent plasminogen activation mediates increased interleukin-6 in lung fibrogenesis

Michael Schuliga[1], Trudi Harris[1], Asres Mitke[1], Jade Jaffar[2], Glen Westall[2], Alastair G Stewart[1]. Dept Pharmacology and Therapeutics, University of Melbourne[1], Parkville, VIC; Dept Allergy, Immunology and Respiratory Medicine, Alfred Hospital[2], Prahran, VIC. (Introduced by Michael Schuliga, Dept Pharmacology and Therapeutics University of Melbourne, Parkville, VIC).

Introduction. In lung diseases such as idiopathic pulmonary fibrosis (IPF), plasma-borne plasminogen enters the lung interstitium to be converted into plasmin, a protease with tissue remodelling actions. Extracellular urokinase plasminogen activator (uPA) and annexin A2 regulate plasmin formation and subsequent fibrogenic interleukin-6 (IL-6) production. There is evidence lung fibroblast uPA production is increased in IPF (Shetty *et al*, 1996).

Aims. To examine the role of uPA-generated plasmin in lung fibrogenesis.

Methods. IL-6 levels were measured in cultures of lung fibroblasts (LFs) from IPF and non-IPF patients incubated with plasminogen, or in bronchoalveolar lavage fluid (BALF) of mice following bleomycin-induced lung injury. Levels of uPA were also measured in the serum of IPF and non-IF donors.

Results. LFs from IPF patients, as compared to non-IPF donors, showed an uPA- and annexin A2- dependent increase in plasmin and IL-6 levels after plasminogen incubation. Plasmin-mediated IL-6 levels were reduced by siRNA and immuno-targeting of annexin A2. Plasmin(ogen)-induced mitogenesis was attenuated by targeting either annexin A2 or IL-6. Either annexin A2 gene deletion or treatment with the uPA inhibitor, B428, reduced bleomycin-induced increases in IL-6 levels BALF, and reduced the subsequent increases in BALF cell number and lung fibrogenic gene expression. Serum levels of uPA were higher in patients with IPF than controls.

Discussion. These data suggest that interstitial plasmin contributes to lung fibrogenesis. Extracellular uPA and annexin A2 may be targets and/or potential biomarkers in IPF.

Shetty S *et al* (1996) *Am J Respir Cell Mol Biol* **15**: 78-87.

168

Airway smooth muscle conditioned media protects airway epithelial cells from the viral-induced glucocorticoid insensitivity

Yuxiu C. Xia, Shenna Langenbach, Christine Keenan, Rosa Gualano, Alastair G. Stewart

Lung Health Research Centre, Depart of Pharmacology &Therapeutics, the Univ of Melbourne, Parkville, Vic 3010

Introduction: Airway smooth muscle (ASM) and epithelial cells are important airway structural cell targets for glucocorticoid (GC) action and resistance (Keenan 2012). Exacerbations of asthma are commonly associated with respiratory syncytial virus (RSV) infection, which responds inadequately to GC treatment (Fernandes 2013).
Aims: To explore the interaction between ASM and epithelial cells on GC activity during RSV infection.
Methods: Human bronchial epithelial cells (HBECs, Lonza) were differentiated in air-liquid interface on type I fibrillar collagen-coated transwell inserts. The cells were then non-contact co-cultured with human ASM (HASM) cells for 48 hours including 24 hours starvation. The cells were then inoculated with RSV at a multiplicity of infection of 0.1 for 1 hour, and incubated for 48 hours. GC-responsive gene expression was assessed by incubation of the cells with 100nM dexamethasone during the last 5 hours.
Results: Viral infection impaired GC-induced expression of glucocorticoid-induced leucine zipper protein (GILZ) and epithelial Na^+ channel alpha subunit (ENaCα) in HBECs. Impairment of GC activity is prevented by the selective ALK5 (TGFβRI) inhibitor, SB431542. The impairment is also prevented by co-culture with HASM cells. Viral infection increased the expression of transforming growth factor-β (TGF-β), a mediator of GC resistance (Keenan 2014). The increased expression of TGF-β in HBECs was abrogated by co-culture with HASM cells.
Discussion: RSV infection-induced GC insensitivity is partially caused by endogenous TGF-β. Co-culture of HASM cells with HBE cells demonstrated protective effects in the RSV-induced impairment of GC activity, which is likely to be mediated by attenuating the expression of TGF-β in HBE cells. Better understanding the interaction between airway smooth muscle and epithelial cells provide new targets for treating GC resistance.

Keenan C (2012) Drug Discov Today 17 (17-18): 1031-1038
Fernandes RM et al. (2013) Cochrane Database Syst Rev. doi: 10.1002/14651858
Keenan CR et al, Respir Res. 2014 15:55 doi: 10.1186/1465-9921-15-55

169

Inhibition of NOX2 oxidase via a novel endosome specific inhibitor reduces influenza A virus (IAV)- induced lung inflammation.

Raymond Luong[1], Eunice E To[1], Christopher JH Porter[2], Tim Quach[2], Ross Vlahos[3], Stavros Selemidis[1]. Monash Biomedicine Discovery Institute, Dept Pharmacol, Monash Univ[1], VIC. [2]Monash Institute of Pharmaceutical Sciences, Monash University, VIC. [3]School of Health Sciences, RMIT, VIC.

Introduction. Endosome-expressed NOX2 generates superoxide in response to IAV entering the endocytic pathway (To *et al.,* unpublished observations). To clarify the role of endosomal NOX2 in IAV pathogenesis, we have modified the NOX2 inhibitor, gp91ds-TAT by conjugating it to a lipid anchor (cholestanol) via a flexible PEG linker to facilitate endosome delivery.
Aim. To determine whether endosome-targeted delivery of a NOX2 inhibitor is superior to a non-targeted approach at suppressing superoxide production and IAV-induced lung inflammation.
Methods. Immunohistochemistry was used to localize IAV, endosomes, NOX2 and a cholestanol fluorophore. Superoxide was quantified via L-012-chemiluminescence in RAW 264.7 and alveolar macrophages. WT mice were infected intranasally with the Hong Kong X-31 (H3N2) IAV (10^5 PFU/mouse) in the absence or presence of unconjugated gp91ds-TAT (Ugp91) or cholestanol-gp91ds-TAT (Cgp91; 0.02mg/kg) delivered intranasally either preventatively (one day pre-infection) or therapeutically (one day post-infection). Three days post-infection, airway inflammation was assessed by cell counting, superoxide by chemiluminescence and cytokine expression by quantitative PCR.
Results. The cholestanol-PEG linker fluorophore co-located with IAV, early endosomes and NOX2, which was suppressed by the endocytosis inhibitor Dynasore (100μM). Cgp91 (0.1-30μM) inhibited superoxide production with ~10-fold more potency than Ugp91 (P<0.05). Both pre or post treatment of mice with Cgp91 reduced airway inflammation (P<0.05) and increased antiviral cytokine expression to IAV infection, whereas Ugp91 had no effect.
Discussion. The potency increase in gp91ds-TAT, as a result of cholestanol conjugation, provides proof-of-principle that endosomal NOX2 exacerbates IAV pathogenesis and highlights endosomal targeting compounds like Cgp91 as novel drugs for IAV therapy.

170

Assessing changes in airway and vascular remodelling and contraction in a mouse model of bronchopulmonary dysplasia

Maurice A.G.M. Kroon[1], Christine Bui[2], Maggie Lam[1], Chantal Donovan[1], Simon G. Royce[1], Marcel Nold[2], Claudia Nold[2], Jane E. Bourke[1]. Biomedicine Discovery Institute and Dept of Pharmacology, Monash University[1], Hudson Institute of Medical Research[2], Clayton, VIC.

Introduction. Higher survival rates of extremely preterm babies are associated with an increasing incidence of bronchopulmonary dysplasia (BPD), leading to multiple complications including increased risk of asthma and pulmonary arterial hypertension. Perinatal inflammation and mechanical ventilation with supplemental oxygen are known to contribute to BPD. Their application to a mouse model could provide a validated setting for assessment of novel treatments to prevent BPD and/or overcome the limited efficacy of existing bronchodilators and vasodilators.
Aim. To assess changes in vascular and airway remodelling and contractile responses in a mouse model of BPD.
Methods: Pregnant C57BL/6J dams received 150μg/kg lipopolysaccharide or saline i.p. at E14 of gestation. Within 24h of birth, pups were randomized to normoxic (N, 21% O_2) or hyperoxic (H, 65% O_2) conditions for 28d. Lung sections were stained for collagen (fibrosis marker) and α-smooth muscle actin (α-SMA, contractile marker). Artery and airway contraction to U46619 or methacholine (MCh) was visualised by phase-contrast microscopy in precision cut lung slices.
Results. Increased collagen deposition was seen in both airways and arteries following hyperoxia, but α-SMA staining intensity was elevated only in airways. U46619 caused concentration-dependent contraction of arteries (pEC_{50} 7.8±0.2, maximum % reduction in lumen area 36±7%; n=7). MCh elicited contraction with similar potency in both normoxic and hyperoxic groups (pEC_{50}: N 7.4±0.4, n=6; H 7.2±0.2, n=7), but increased maximum following hyperoxia (% reduction in lumen area: N 44±10; H 89±10; P<0.05).
Discussion. This mouse model mimics key features of BPD, namely airway and vascular fibrosis, and increases in contractile protein expression and function in airways. Future studies are needed in this model to assess the efficacy of anti-inflammatory agents targeting these structural changes and dilators to oppose the increased airway contraction following hyperoxia. This may in turn identify new options for treating the development and symptoms of BPD in premature babies.

171

Serelaxin is an epithelial-dependent bronchodilator in rat precision cut lung slices and trachea

Maggie Lam[1], Simon G. Royce[1], Chrishan S. Samuel[1], Jane E. Bourke[1]. Biomedicine Discovery Institute and Dept of Pharmacology, Monash University[1], Clayton, VIC.

Introduction. Serelaxin (RLN) is used for the treatment of acute heart failure due to its ability to reduce fibrosis and induce vascular relaxation. RLN has been shown to abrogate lung fibrosis, but its effects as a bronchodilator and potential asthma treatment targeting small and large airways have yet to be fully characterised.
Aims. To explore the effects of RLN on airway contraction alone and in combination with isoprenaline (ISO, β-adrenoceptor agonist) and the novel dilator rosiglitazone (PPARγ-agonist) in rat intrapulmonary airways and trachea.
Methods. Responses to RLN, ISO and RGZ were assessed after MCh pre-contraction in lung slices and trachea (changes in area and force respectively). Inhibition of contraction after pre-incubation with these dilators was measured, as well as regulation of RLN-mediated inhibition by indomethacin (COX-inhibitor), L-NAME (NOS-inhibitor), SQ22536 (AC, adenylate cyclase inhibitor), ODQ (GC, guanylate cyclase inhibitor) or epithelial removal.
Results. RLN (100nM) elicited partial relaxation in lung slices (%relaxation 64±8%; time to plateau 9±1min) under static but not perfused conditions, suggesting a requirement for accumulation of secondary mediators of relaxation. RLN elicited similar relaxation in trachea at a slower rate (52±15%; 96±9min), consistent with dilution of released factors in the larger myograph volume. ISO (1μM) and RGZ (100μM) caused rapid partial or slow complete relaxation respectively. RLN and ISO in combination caused greater relaxation than either alone (ISO 50±9%; RLN/ISO 69±4%, P<0.05), while RLN co-treatment increased the rate of RGZ-induced relaxation (RGZ 52±4min; RLN/RGZ 27±2min, P<0.001). Inhibition of contraction by RLN was reduced by COX, NOS, AC, GC inhibition or epithelial denudation.
Discussion. RLN caused relaxation in small and large airways via the release of epithelial-derived mediators (nitric oxide, PGE_2). Since RLN potentiated dilator responses to ISO and RGZ, further investigations are required to define its therapeutic potential, particularly when dilator responsiveness to current therapy is limited.

172

GPR40 and GPR120 – Novel targets for the treatment of lung disease

Chantal Donovan[1,2], Jacqueline B Micallef[1], Mirjam Simoons[2], Jane E Bourke[1,2]
[1]Biomedicine Discovery Inst, Dept of Pharmacol, Monash University, Clayton, VIC. [2]Lung Health Research Centre, Dept of Pharmacol, The Univ of Melb, Parkville, VIC.

Introduction. Current bronchodilator therapy with β-adrenoceptor agonists is ineffective in severe asthmatics and the majority of chronic obstructive pulmonary disease patients. Therefore, there is a crucial need for novel therapeutic alternatives, particularly targeting the small airways implicated in these diseases. The G-protein coupled receptors, GPR40 and GPR120, have recently been identified in human and mouse lungs, however their function is unknown.

Aims. To assess the effects of the GPR40/120 agonist, GW9508 and the GPR120 agonist, TUG891, on mouse trachea and small airways in precision cut lung slices (PCLS).

Methods. Mouse tracheal rings (2mm) were mounted in a myograph for measurements of tone and PCLS (150μm) were prepared for visualising changes in airway area by phase contrast microscopy. The effects of GW9508 and TUG891 on the development of contraction to methacholine (MCh) or following MCh pre-contraction were assessed, for comparison with the β-adrenoceptor agonist, salbutamol (SALB).

Results. Pre-treatment with GW9508, TUG891 or SALB did not inhibit the development of MCh contraction. In precontracted trachea, TUG891 (30μM) caused sporadic additional contraction, whilst TUG891 (100μM) elicited complete relaxation (n=4). In PCLS, all agonists elicited concentration-dependent relaxation (maximum % relaxation: TUG891: 90.2±2.2 > GW9508: 71.4±3.8 > SALB: 40.9±7.8, n=4-5). The rank order of bronchodilator potency was SALB > TUG891 > GW9508.

Conclusions: The GPR120 agonist, TUG891, caused complete relaxation of mouse large and small airway preparations, albeit at lower potency than the partial agonist SALB. Identifying more potent GPR120 agonists based on the structure of TUG891, and demonstrating their superior efficacy to b-adrenoceptor agonists in the small airways, will support GPR120 as a novel target in lung diseases.

173

Regulation of PAR$_2$ trafficking and resensitization by Gβγ and PKD

Peishen Zhao[1], Dane Jensen[1], Nigel Bunnett[1,2,3]; [1]Monash Institute of Pharmaceutical Sciences, Parkville, Australia; [2]Department of Pharmacology, University of Melbourne, Australia; [3]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology.

Introduction. Proteases that are generated during injury and inflammation can signal to cells by cleaving protease-activated receptor-2 (PAR$_2$) at the plasma membrane. Activated PAR$_2$ traffics from the plasma membrane to lysosomes for degradation. Resensitization of protease signaling requires mobilization and plasma membrane trafficking of the prominent stores of PAR$_2$ in the Golgi apparatus, by unknown mechanisms.

Aims. Identify and characterizing signalling pathways and mechanisms that regulate PAR$_2$ resensitization.

Results. In HEK293 cells, NCM460 colonocytes and KNRK cells expressing human PAR$_2$, the PAR$_2$ agonists trypsin and 2-Furoyl-LIGRLO-NH$_2$ (2F, peptide tethered ligand analogue) stimulated rapid phosphorylation of protein kinase D1 (PKD1) in the Golgi apparatus, where PKD1 can regulate protein trafficking. PAR$_2$ activation resulted in translocation of Gβγ, a PKD1 activator, to the Golgi apparatus. The PKD1 inhibitor CRT0066101 (100 nM) and the Gβγ (10 μM) prevented PAR$_2$-stimulated activation of PKD1. Exposure of KNRK-PAR$_2$ and NCM460 cells to trypsin or 2F strongly desensitized subsequent PAR$_2$-mediated Ca^{2+} signals, which slowly resensitized within 90 mins for KNRK and 120 mins in NCM460 cells. CRT 0066101 significantly inhibited resensitization in both cell lines (P<0.05). Irradiation of the Golgi apparatus (405 nm) resulted in immediate and complete green-red photo-conversion of PAR$_2$-Kaede in KNRK cells. In cells treated with trypsin, photo-converted PAR$_2$-Kaede appeared at the plasma membrane within 15 mins. CRT 0066101 inhibited the translocation of photo-converted PAR$_2$ from the Golgi apparatus to the plasma membrane, consistent with the inhibition of resensitization.

Conclusions. PAR$_2$ activates PKD1, which colocalizes with PAR$_2$ in the Golgi apparatus. PKD1 inhibition prevents translocation of PAR$_2$ from the Golgi apparatus to the plasma membrane and suppresses resensitization of cell-surface PAR$_2$ signaling. Thus, PKD1 is a key intermediate by which PAR$_2$ activation at the plasma membrane stimulates Golgi mobilization and plasma membrane trafficking of PAR$_2$, leading to sustained proteases signaling.

174

An ecological study of the extent and factors associated with use of prescription and over-the-counter codeine in Australia

Natasa Gisev[1], Suzanne Nielsen[1], Elena Cama[1], Briony Larance[1], Raimondo Bruno[1,2], Louisa Degenhardt[1]. National Drug and Alcohol Research Centre, UNSW Australia[1], Sydney, NSW. School of Medicine, University of Tasmania[2], Sandy Bay, TAS.

Introduction: The extent and factors associated with codeine use in the community remain poorly understood despite the widespread global use of codeine.

Aims: to examine use of prescription and over the counter (OTC) codeine in Australia and identify the geographic and socio-demographic characteristics associated with prescription and OTC codeine use.

Methods: National sales data for prescription and OTC codeine (supplied by IMS Health) were used to estimate codeine utilisation (in pack sales and milligrams) in Australia during 2013, mapped to Australian Bureau of Statistics (ABS) Statistical Local Areas (SLAs) and Remoteness Areas. Socio-demographic characteristics and total population estimates of SLAs were obtained from the ABS. SLA-level data on sex, age distribution, income, occupations involving physical labour and number of pharmacies, were included in linear regression analyses to examine their association with total, prescription and OTC codeine use.

Results: In total, 27,780,234 packs of codeine were sold in Australia during 2013, equating to 12,376kg. OTC codeine preparations accounted for 15,490,207 packs (55.8%) or 4,967.30kg (40.1%). Nationally, an estimated 1.24 packs (or 554.10mg) of codeine were sold per person; utilisation was higher in more remote areas. SLAs with a higher percentage of low-income earning households had the highest rates of prescription codeine use (β 0.16, $p<0.001$), whereas SLAs with a higher percentage of males had the highest rates of OTC codeine use (β 0.22, $p<0.001$).

Discussion: Codeine use is common in Australia, with clear distinctions in the geographic and socio-demographic characteristics associated with prescription and OTC codeine use.

175

Spontaneously reported haemorrhagic adverse events associated with rivaroxaban in Australia

Esa YH Chen[1], J Simon Bell[1,2], Michael J Dooley[1,3], Carl M Kirkpatrick[1], Basia Diug[4], Kevin P Mc Namara[1,5], John McNeil[4], Gillian Caughey[2], Jenni Ilomäki[1]. Centre for Medicine Use and Safety, Monash University[1], Parkville, VIC; Sansom Institute for Health Research, University of South Australia[2], Adelaide, SA; Pharmacy Department, Alfred Health[3], Melbourne, VIC; Department of Epidemiology and Preventative Medicine, Monash University[4], Melbourne, VIC; Greater Green Triangle University Department of Rural Health, Flinders University and Deakin University[5], Burwood, VIC.

Introduction. Rivaroxaban uptake in Australia has been rapid. Clinicians in Australia have called for more local data on the safety of non-vitamin K antagonist oral anticoagulants.

Aims. Describe spontaneously reported haemorrhagic adverse events associated with rivaroxaban use in Australia.

Methods. Case reports from June 2009 to May 2014 of haemorrhagic adverse events where rivaroxaban was reported as a potential cause were extracted from the Australian Therapeutic Goods Administration (TGA) Database of Adverse Event Notifications database. Demographic and clinical characteristics of cases were analysed for those aged <75 and those ≥75 years.

Results. In the 240 reports included, 101 of 164 haemorrhagic events reporting age occurred in the ≥75 years group. Seventeen of the 101 known outcomes were fatal, mostly due to nervous system related haemorrhage (n=11). The gastrointestinal (GI) system was the most common organ system affected (n=105). Thirty-two events occurred within 6 days of rivaroxaban initiation.

Discussion. The majority of haemorrhagic events related to rivaroxaban administration occurred in people aged ≥75 years. GI haemorrhage accounted for nearly half of all haemorrhages, while nervous system haemorrhage accounted for most fatalities. Haemorrhagic events often occurred within the first six days of treatment. Clinicians should carefully consider concomitant medications when prescribing rivaroxaban and monitor for bleeding closely on initiation.

176

Association rule and frequent-set analysis: Potential adverse drug reactions (ADRS) pharmacovigilance method

Fabian Held[1], David Le Couteur[2], Fiona Blyth[2,3], Vasi Naganathan[2], Robert Cumming[2,3] and Danijela Gnjidic[4].
Charles Perkins Centre, Univ of Sydney[1], Sydney NSW; Centre for Education and Research on Ageing and the Ageing and Alzheimers Institute, Univ of Sydney and Concord Hospital[2], Sydney NSW; Sch of Public Health, Univ of Sydney[3], Sydney NSW; Fac of Pharmacy, Univ of Sydney[4], Sydney NSW.

Introduction. There is growing interest in applying novel techniques to perform drug prescription data analysis in routinely collected databases to monitor for adverse events. Association Rule and Frequent-Set analysis may be useful for detecting new unanticipated signals of adverse events, and may raise the scientific questions for subsequent pharmacoepidemiolgical studies.

Aim. To apply Frequent-Set analysis and novel means of data visualisation to ascertain patterns of medication use on person-level within clusters and medication combinations contributing to medication group clusters.

Methods. Participants were community-dwelling men (aged ≥70 years) enrolled in the Concord Health and Ageing in Men Project. Medication exposure was categorised at medication class level and data were analysed according to geriatric syndrome status. Association Rule and Frequent-Set analysis was performed to identify "interesting" patterns and combinations of medications that occur together.

Results. Frequent-Set Analysis demonstrated just one medication combination, antiulcer and antidiabetic medications (3.5% of participants) with "interestingness" threshold in the overall population (n=1687). Moreover, frequency of medication combinations was similar in men with (n=666) and without (n=1020) geriatric syndromes. For instance, among participants with geriatric syndromes, the most frequent combinations included antigout with lipid-lowering agents (5.7%) followed by angiotensin II and diuretics combination (22%).

Conclusions. This novel methodology can be used to easily detect potential adverse drug reactions (ADRs) by data visualisation of common medication combinations overall and against specific ADRs such as geriatric syndromes. This may be useful pharmacovigilance approach to identify targets for prescribing improvement interventions.

177

Drug overdose in the elderly

S Ilango[1], P Pillans[1], C Page[1]. The Princess Alexandra Hospital[1], QLD

Introduction: There is relatively little information on drug overdose in the elderly, yet elderly people are at increase risk of morbidity and mortality from self-poisoning. The aim of this study was to evaluate the pattern and reasons of overdose in elderly and compare this with a younger cohort of overdose patients.

Method: This was an observational study of prospectively collected data on patients aged ≥65 who presented between June 2014 and May 2015 to the Princess Alexandra Hospital Emergency Department and admitted under the Clinical Toxicology Unit. Comparison was made with a random selection of 150 overdose patients age <65.

Results: Of the 1618 overdose cases, 58 patients were elderly (≥65), of which 35 were female (60%). There was a steady decline of overdose with advancing age. The predominant substances were pharmaceuticals (95%), with benzodiazepines recorded most frequently (43%), followed by paracetamol, opioids and antidepressants. The median length of stay was 21.9 hours and the majority (73%) were discharged home. Intentional overdose occurred in 38 (65.5%), the major reason being family conflict (33%). Psychiatric illness was seen in 84.2%, 45% had ≥3 co-morbidities and 58% were living with a family member. Pain (40%) and accidental overdose (55%) were the main reasons for unintentional self-poisoning. In the younger cohort, 86 (57%) were females and the length of stay was shorter (12.1 hours). The main reason for intentional overdose was depression (62%) and for unintentional overdose, it was recreational use (68%).

Conclusion: Elderly patients formed the minority of overdose cases, but had a longer length of stay. Multiple co morbidities, psychiatric illness and family conflict were associated with elderly intentional overdosing. Pain and accidental poisoning were the main reasons for unintentional overdose.

178

Integration of multiple computational models of bioassays improve in vivo rodent carcinogenicity prediction

Davy Guan[1], Kevin Fan[1], and Slade Matthews[1]. Pharmacoinformatics Laboratory, Discipline of Pharmacology, University of Sydney[1], Sydney, NSW;

Introduction. *In vitro* genotoxicity bioassays are cost-efficient methods of assessing potential carcinogens. However, many genotoxicity bioassays produce a substantial number of false positive results to non-carcinogens. Additionally, genotoxicity bioassays are inappropriate for detecting chemicals eliciting non-genotoxic carcinogenicity mechanisms such as tumour promotion and progression, necessitating the use of *in vivo* rodent carcinogenicity (IVRC) assays. *In silico* models of bioassays, featuring equivalent predictive performance and expedient result output, are hypothesised to supplement current batteries.

Aims. In this study, we developed computational QSAR models of novel bioassays, followed by statistical integration alongside the *in vitro*-Ames bioassay and ToxTree SAR models to predict IVRC results (n=822).

Methods. Bioassays included: Ames (n=6512), Syrian Hamster Embryonic (SHE, n=410) and GreenScreen GADD45a-GFP (n=1125). The molecular descriptors of these compounds were calculated. The resulting physicochemical properties were mapped to each assay result using machine learning algorithms (k-Nearest Neighbours, C.45 Decision Tree, Multilayer Perceptron, Random Forest and Rotation Forest).

Results. QSAR model performance was assessed by accuracy (A) and ROC AUC from ten times 10-fold cross-validation, resulting in 80.96%, 0.876 AUC; 82.56%, 0.893 AUC; 62.84%, 0.684 AUC for the Ames, SHE and GreenScreen models, respectively, followed by IVRC prediction results of 58.94%, 0.635 AUC, 65.8213%, 0.489 AUC and 60.63%, 0.562 AUC. Logistic regression was used to integrate IVRC prediction results from QSAR models, alongside *in vitro*-Ames (A=66.35%) and SAR models (Ames [A=63.29%], genotoxicity [A=57.66%] and non-genotoxic carcinogenicity [A=15.57%]), producing an integrated model, which then predicted IVRC with 73.11% accuracy and 0.765 AUC.

Discussion. This study demonstrates an integrated approach with computational models is evidently more predictive of *in vivo* rodent carcinogenicity than individual models alone and shows great merit in carcinogenicity prediction, leveraging the speed, scale and cost advantages of computational methodologies to enhance *in vitro* bioassay performance.

179

NQO1-dependent Mito-Toxicity of Clioquinol

Jamuna Chhetri[1], Robert Gasperini[2], Glenn Jacobson[1], Lisa Foa[3], Nuri Gueven[1]. Pharmacy, School of Medicine, University of Tasmania[1] Menzies Institute for Medical Research[2], School of Medicine, University of Tasmania[3], Hobart, TAS.

Introduction. The antimicrobial clioquinol (CQ) is associated with neurotoxicity-induced blindness in 10 000 Japanese patients. Despite the restriction of neurotoxicity to Japan, CQ was withdrawn from the global market in 1970. The mechanism of CQ toxicity is still elusive. Despite its toxicity, CQ is currently under investigation for the treatment of neurodegenerative diseases with some promising preclinical and clinical results.

Aims. To explore the mechanism of CQ-induced neurotoxicity *in-vitro* and *in-vivo*.

Methods. A screen of selected drugs and drug-like compounds against the potential to induce mitochondrial dysfunction identified CQ and the closely related 8-hydroxyquinoline (8HQ). These results were confirmed by measuring ATP levels and lipid peroxidation in vitro and by measuring visual acuity *in-vivo* model (zebrafish).

Results. CQ profoundly reduced cellular ATP levels and induced lipid peroxidation only in rodent neuronal cells, while no toxicity was observed in human hepatic cells. These two cell lines differ drastically in their ability to respond to oxidative stress, which is associated with grossly different expression of the antioxidant gene, NQO1. We confirmed NQO1-dependent CQ toxicity in human embryonic kidney (HEK) 293 cell lines that either overexpress or do not express NQO1. Levels of NQO1 expression directly correlated with resistance to CQ-induced cellular toxicity. Comparable with CQ-induced pathology in human patients, CQ treatment led to a clear dose dependent loss of visual function in zebrafish larvae that lack retinal NQO1 expression.

Discussion. Given the significantly higher prevalence of the inactivating NQO1 polymorphism in the Japanese and Asian population compared to the European population our results can for the first time explain the geographic restriction of CQ-induced neurotoxicity to Japan. Therefore, the use of CQ or its derivatives for the treatment of neurodegenerative diseases should require a prior determination of the NQO1 status of patients. Our results represent a significant step towards personalized medicine to minimize the CQ-associated risks.

180

Ageing and drug induced liver injury: Insights from animal studies

John Mach[1,2,3], Aniko Huizer – Pajkos[1,2], Catriona McKenzie[4], Sarah J Mitchell[5], Leo Phillips[1], Alice E Kane[1,2,3], Brett Jones[1,2,3], Rafael de Cabo[5], Victoria Cogger[3,6], David G Le Couteur[3,6] & Sarah N Hilmer[1,2,3]. Kolling Institute, Sydney, NSW[1]. Royal North Shore Hosp, Sydney, NSW[2]. Univ of Sydney, Sydney, NSW[3]. Royal Prince Alfred Hosp, NSW[4]. National Institute on Aging, National Institute of Health, Maryland, USA[5]. Alzheimers Institute of Ageing and Centre for Education and Research on Ageing and Anzac Research Institute, Concord Hospital, NSW[6].

Introduction. Historically, older people (>65) were thought to be at increased risk of drug induced liver injury (DILI). This has not been well established empirically.

Aims. Determine the effect of ageing on DILI induced by paracetamol and isoniazid in male Fischer 344 rats. These drugs cause DILI and are taken by older people.

Methods. Young and old rats were treated via intraperitoneal injection with toxic regimens of either paracetamol (single dose of 800mg/kg) or isoniazid (4 doses daily: 100, 75, 75, 75mg/kg every 3 hours over 2 days) or vehicle controls. After rats were euthanized, sera and liver were collected to measure drug levels and assess for toxicity.

Results. Paracetamol treatment resulted in higher serum drug levels in old than in young animals, and elevated serum hepatotoxicity markers in young animals (ALT: saline 55±3U/L, paracetamol 109±14U/L and AST: saline: 112±11U/L, paracetamol: 350±34U/L, $p<0.05$) but not old. Incontrast, hepatic DNA fragmentation was increased in old animals treated with paracetamol when compared to all other groups (young saline: 100±8%, young paracetamol: 335±40%, old saline: 158±30%, old paracetamol: 1397±276%, $p<0.05$). Only minor necrosis was observed with paracetamol treatment in both age groups. Isoniazid treatment resulted in higher metabolite levels in old than in young animals, and elevated serum AST in young animals (vehicle 131±14U/L, isoniazid 200±19U/L, p<0.05), but not in old. Histological assessment showed a trend towards increased necrosis in young isoniazid treated rats, and an increase in hepatic microvesicular steatosis in old isoniazid treated rats, compared to corresponding age control groups ($p<0.05$).

Discussion. Our animal studies indicate that old age affects the pattern and risk of DILI from paracetamol or isoniazid differently. Future research is required to translate this in humans to guide dosing and diagnosis of DILI in patients of all ages

181

Optimising the logistics of life-saving drug procurement and supply in NSW and the ACT

Christa Lynch[1], Margaret Jordan[1], Alexandra Bennett[1]; NSW Therapeutic Advisory Group[1], Sydney, NSW.

Introduction. Life-saving drugs (LSDs) are antidotes, antivenoms and other drugs that may treat infrequent but potentially fatal illnesses. Their high cost and infrequent use means not all LSDs can be considered normal components of hospital formularies. NSW TAG maintains an on-line register of LSDs stock holdings in NSW and ACT hospitals, updated annually via a survey of individual hospitals. The register enables users such as hospital pharmacists and Poisons Information Centres (PICs) to identify hospitals holding stock required by critically ill patients, thereby facilitating supply in emergencies.

Aims. 1) To identify specific challenges relating to the procurement, stocking and supply of LSDs in NSW and ACT hospitals; 2) To develop potential solutions to address identified concerns.

Methods. Email invitations to 2 online surveys for antidote and antivenom stocks were sent to 189 hospitals in NSW and 2 hospitals in the ACT during 2014. After stakeholder consultation and piloting for clarity, additional questions relating to hospital demographics, local hazards, access to resources (e.g local toxicologist) and to existing local policies for LSD review were included in the antidote survey. Participants were also asked to identify challenges relating to acquisition and storing of LSDs, and for suggestions to improve their management.

Results. Representatives from 130 hospitals (69%) completed the additional questions in the 2014 survey. Three themes were identified: a) high costs associated with frequent wastage of stock; b) lack of formal policies to facilitate sharing of stock between hospitals; and c) problematic stocking and supply of non-registered LSDs. Seventy one hospitals (55%) provided suggestions to improve LSD management, including real time IT access to individual hospital stock levels; state policy development to facilitate sharing of stock between hospitals; and national policy changes to facilitate access to non-registered LSDs.

Discussion. Challenges to LSD stocking and supply were identified A working group of stakeholders, such as clinical toxicologists, PICs, retrieval services and hospitals of varying capacities and roles, will be convened to develop strategies to optimise LSD procurement, stocking and supply across NSW/ACT and, ideally, nationally.

200
APSA MEDAL ORATION

201
Overview of changes in drug disposition with impaired kidney function

Darren M. Roberts. Medical School, Australian National University and Renal Medicine, The Canberra Hospital, Canberra, ACT.

Drugs are commonly prescribed to patients with impaired kidney function but the dose regimen may differ to that used for patients with normal kidney function. The purpose of dose-adjustment is to reduce adverse drug reactions, but care is required to avoid a subtherapeutic exposure which may compromise outcomes, for example antibiotics.

In practise, drugs are commonly dosed to patients with chronic kidney disease (CKD) according to the "intact nephron theory" which asserts that each component of kidney function (filtration, secretion, reabsorption, metabolism) change in proportion to glomerular filtration rate (GFR). However, CKD is associated with other changes in pharmacokinetics; for example, limited data suggest that uremic toxins reversibly inhibit drug metabolism and this is not commonly considered by clinicians or in dosing guidelines.

Guidance regarding the selection of a dose regimen in patients with acute kidney injury (AKI) is more complicated given that AKI varies in severity, duration and treatment with ill-defined impacts on serum biochemistry, drug and electrolyte clearance, drug concentrations and patient response. Current data suggests that the intact nephron theory is an inappropriate representation of what occurs in AKI. For example, animal research note that expression of drug transporters may increase or decrease depending on the model of AKI. Acute changes in nephron mass may not induce a proportional change in GFR, for example unilateral nephrectomy is followed by hyperfiltration in the remaining kidney (but data on changes in secretory function are unavailable). Unfortunately, human data are sparse but some data are noteworthy. For example, a decrease in atenolol and amikacin clearance occurs one month post-unilateral nephrectomy, which largely normalised by 12 months for atenolol but not amikacin; mechanistic data explaining this difference are not available. The relationship between GFR and drug clearance is further complicated when GFR is estimated using plasma creatinine due to a delay between the change in this concentration relative to the actual change in kidney function. Further, the influence of uremic toxin accumulation on drug disposition in AKI is poorly defined.

Therefore, impaired kidney function exerts differing effects on pharmacokinetics and efforts to dose medications based on GFR as the sole measure of kidney function is problematic.

202
Optimising drug dosing in acute kidney injury and renal replacement therapies

Jason A Roberts[1,2]. Burns Trauma and Critical Care Research Centre, and School of Pharmacy, The University of Queensland[1], Brisbane, QLD; Department of Intensive Care Medicine and Pharmacy Department, Royal Brisbane and Women's Hospital[2], Brisbane, QLD.

Introduction. There are many types of renal replacement therapy (RRT) that may be used in patients with acute kidney injury (AKI), with each potentially having different effects on clearance of drugs from the body. Unfortunately the interplay between the various patient, RRT and drug factors mean that there is little likelihood that a guideline for adequate dosing in this clinical scenario can be successfully developed.

Aims. To review the patient, drug and RRT factors that can affect drug clearance in patients with AKI receiving RRT.

Methods. A critical review of the published literature was undertaken to identify relevant factors affecting drug clearance during RRT. Based on this, a framework for interpreting possible dosing requirements in individual clinical scenarios will be developed.

Results. RRT settings can have a significant effect on drug clearances. Increasing blood flow rates, RRT effluent flow rates, RRT filter surface size can each cause increased drug clearance. The use of haemodiafiltration can result in greater drug clearance than haemofiltration which can have greater clearance than haemodialysis modalities. For drugs, increasing molecular size, lipophilicity, protein binding and volume of distribution are all associated with decreased drug clearance by RRT. From a patient perspective, the level of residual renal function is important for drug clearance as may be other organ dysfunctions in the case that the drug has multiple clearance mechanisms.

Discussion. Drug dosing in patients with AKI reeiving RRT can be very challenging due to the difficult-to-predict interplay for RRT, drug and patient factors that can each affect drug clearance. Knowledge of prior pharmacokinetic data for individual drugs during RRT can help predict the relative influence of the various factors. Where possible, titration of drugs to observed clinical effects or measured concentrations should be undertaken to ensure optimal therapy for these challenging patients.

203

Novel imaging techniques for assessing renal disposition *in vivo*

Xin Liu. Therapeutics Research Centre, School of Medicine, The University of Queensland, Brisbane, QLD

Kidney is the key organ for elimination of xenobiotics and their metabolites from the body. Renal disposition (i.e. glomerular filtration, active tubular secretion and reabsorption) are mainly studied *ex vivo* using isolated perfused rat kidney model previously. In recent years, multiphoton microscopy (MPM), a fluorescence imaging technique, has proven to be a powerful tool in evaluating renal disposition of fluorescent compound. It allows detailed non-invasive real-time visualization of dynamic processes at subcellular resolution in the context of 3-dimensional structure of kidney. Compared to confocal microscopy, MPM has the advantage of less phototoxicity and photobleaching as well as deeper tissue penetration. Other conventional intravital imaging techniques such as magnetic resonance imaging and positron emission tomography have a lower resolution compared to MPM. Using this unique technique, we found that small fluorescent nanoparticles- quantum dots only distributed in the peritubular capillaries or glomerular arterioles without glomerular filtration in rat kidney.

204

Augmented renal clearance: Effects on drug disposition and clinical response.

Andrew A Udy[1,2]. Department of Hyperbaric and Intensive Care Medicine, The Alfred[1], Melbourne, VIC; Department of Epidemiology and Preventive Medicine, Monash University[2], Melbourne, VIC.

Sepsis continues to manifest an unacceptably high mortality rate in the critically ill. The use of an antibiotic of appropriate spectrum, in adequate dose, as early as possible, is likely to provide the greatest chance of clinical cure. Complicating this scenario is the inherent virulence of organisms often isolated in this setting, and the substantial changes in organ function encountered. Critical illness represents a state of significant homeostatic dysfunction, such that drug handling is drastically altered when compared with healthy volunteers. The underlying systemic inflammatory state, administration of large volumes of intravenous fluids, and altered drug-eliminating organ function, all underpin the marked changes in pharmacokinetics observed in these patients.

Augmented renal clearance (ARC) is a relevant example; a phenomenon characterized by enhanced renal elimination of circulating solute (including nitrogenous waste products, metabolites, and pharmaceuticals). This will promote sub-optimal drug concentrations (see figure), which in turn can lead to inferior clinical outcomes. Antibiotic prescribing needs to more accurately consider these changes, and may involve novel dosing strategies.
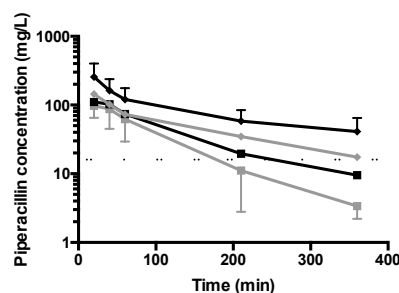
Mean piperacillin concentration (mg/L) on the y-axis (Log$_{10}$ scale), and time (min) on the x-axis, grouped according to CL_{CR} quartile: < 68mL/min (black diamond), 68-114mL/min (grey diamond), 115-170mL/min (black square), > 170mL/min (grey square). A dotted line at 16mg/L (MIC) is provided. CL_{CR} – creatinine clearance [1].

1. Udy AA, Lipman J, Jarrett P, Klein K, Wallis SC, Patel K, Kirkpatrick C, Kruger P, Paterson DL, Roberts MS, Roberts JA. Are standard doses of piperacillin sufficient for critically ill patients with augmented creatinine clearance? Critical Care, 2015; 19(1): 28.

205

Improving the lung exposure of therapeutic proteins following inhaled delivery via optimal PEGylation

Lisa M Kaminskas. Drug Delivery Disposition & Dynamics, Monash Institute of Pharmaceutical Sciences, Parkville, VIC.

Introduction. The inhaled delivery of various therapeutic proteins is being explored as a way of improving the treatment of a variety of lung-resident diseases, such as lung cancers, tuberculosis, viral infections and idiopathic pulmonary fibrosis. The utility of this approach, however, is limited by protein degradation in the lungs and rapid clearance. Whilst PEGylation can slow the rate of protein degradation and modulate absorption pathways and kinetics, it also typically reduces target binding affinity which can impact on in vivo activity.

Aims. Define whether the lung exposure of 'protein activity' after inhaled delivery of therapeutic proteins can be maximised by appropriate selection of PEG chain length (PEG 'loading') using human recombinant interferon α2b (Intron-A®) and approved PEGylated variants (PEGIntron® and PEGASYS®) as a model.

Methods. The pulmonary pharmacokinetics of native interferon α2b (Intron-A, 19 kDa), 12 kDa linear PEG-conjugated interferon α2b (PEGIntron, 31 kDa, 39% PEG loading) and 40 kDa branched PEG-conjugated interferon α2a (PEGASYS, 60 kDa, 67% PEG loading) was examined in rats after liquid instillation to the lungs.

Results. An inverse relationship was evident between PEG loading and bioavailability after pulmonary administration, and PEGylation increased lung exposure by 2.5 to 3 fold compared to Intron-A. When comparing the lung exposure of PEGylated and native interferon in terms of 'protein activity' however, lung exposure was only slightly higher for PEGIntron when compared to Intron-A. In contrast, lung exposure was reduced for PEGASYS as a result of the much lower activity of the highly PEGylated construct. Systemic exposure to 'protein activity' was also increased for PEGIntron, but not for PEGASYS. Similar results were observed for PEGylated (linear 10 kDa) human recombinant interferon γ.

Discussion. The data suggest that the lung exposure of therapeutic proteins can be maximised after inhaled administration by appropriate selection of PEG loading to minimise the loss of in vitro biological activity following PEGylation and slow protein clearance from the lungs. This is ultimately expected to optimise activity against lung-resident diseases and minimise systemic side effects that can result from the subcutaneous delivery of proteins.

206

Formulation of DNA for intramuscular administration

Joan K. Ho, Paul J. White and Colin W. Pouton, Monash Institute of Pharmaceutical Sciences, Melbourne, Australia

Cationic materials are often used to form complexes with DNA for transfection of cells in culture. However, in vivo, the high positive surface charge of the complexes results in aggregation and/or immobilisation of particles. A different approach is needed to realise the potential of DNA vaccines. Current understanding of the mechanisms of action of DNA vaccines is quite limited and more basic research is needed to facilitate improvements in efficiency.

A strategy to improve distribution has been to introduce a poly(ethylene glycol) (PEG) coating to reduce the high surface charge but there is limited knowledge of the fate and activity of complexes after intramuscular (IM) injection. We explored the effect of PEGylation on the distribution, transfection efficiency and immune response after IM injection of DNA complexes, using lipopeptides and PE-PEG to form complexes.

Biodistribution was investigated using qPCR and fluorescence microscopy after IM injection. Gene expression in muscle and draining lymph nodes were determined using the highly sensitive nanoluciferase reporter. The highest extent of gene expression was observed when DNA was administered in solution but extent of gene expression did not correlate with the strength of antibody or cell-mediated responses to ovalbumin DNA vaccination. Immune responses were examined primarily by assaying targeted cell killing in vivo by cytotoxic T cells. Both PEGylated complexes and naked DNA stimulated a significant cell-mediated immune response, with both eliminating a higher number of target cells (26.7% and 22.5%, respectively) (n=8, P < 0.001) than the cationic lipopeptide treated mice (14.3%). To test if this high response was due to the PEGylated complexes acting as an adjuvant *in vivo*, mice were also injected with naked ovalbumin plasmid DNA co-administered with PEGylated lipopeptide complexes with non-coding DNA. The lack of increase in cytotoxic T cell activity from this co-injected formulation suggested that either the activity of the PEGylated complexes was not due to an adjuvant effect, or the adjuvant effect of the particles was required at the site of transfection. Overall these results suggest that high levels of gene expression in muscle do not necessarily correspond to strong immune responses, and that PEGylation is a promising strategy for improving the activity of cationic DNA vaccine complexes.

207

Exploiting mucosal surfaces for delivery of antigens

Michael S. Roberts[1], Therapeutics Research Centre[1], School of Pharmacy & Medical Science, University of South Australia, Adelaide, S.AUST and School of Medicine, The University of QLD

The skin and the mucosal surfaces are the main physical and immune barriers to the entry of infectious agents into the body. Accordingly, there is a higher immune surveillance in the epithelial cells than in the subcutaneous and muscular sites most commonly used as sites for vaccination. Our studies have shown that Nanopatch delivery of antigen and adjuvant delivery to epithelial cells in the skin and buccal mucosa require less than 1/100th the dose required for delivery by conventional intramuscular injection using needle and syringe. In this overview, the physical and immune barrier properties of the skin and mucosa and the current research on interplay of innate and adaptive immune responses are first examined. This is then followed by a consideration of the potential delivery mechanisms for antigen delivery and adjuvant needs, noting along the way that the physical barrier function of the stratum corneum, the outermost "dead" layer of the skin, is difficult to overcome. Finally, these two aspects are combined to define the current state of the art on how mucosal surfaces may be exploited for antigen delivery.

208

Developing platforms for maximising immunisation responses

Sarah Hook. School of Pharmacy, University of Otago, Dunedin, New Zealand

Efficient delivery of large molecule biological therapeutics continues to be a barrier. Present approaches for overcoming this barrier in the field of vaccine delivery involve multiple parenteral administrations of the formulation. This is suboptimal in terms of cost and compliance. We have investigated a number of formulation strategies to enhance delivery and efficacy of vaccines. This includes parenteral delivery of the vaccine in immunogenic particles and sustained release formulations and needle-free delivery of vaccines through the oral and transcutaneous routes. This presentation will present recent data on the development and preclinical testing of a number of these formulations.

209

What outcomes do we want for our graduates?

Brian F Yates[1]. Faculty of Science, Engineering and Technology, University of Tasmania[1], Hobart, TAS.

Introduction. In 2010-2011 Professors Susan Jones and Brian Yates coordinated a nation-wide project to develop an agreed set of learning outcomes for graduates of science degrees.

Aims. To focus attention on the outcomes for students.

Methods. A nation-wide consultation was undertaken under the auspices of the Australian Council of Deans of Science, followed by the development and ratification of a set of learning outcomes for science.

Results. The science learning outcomes, together with explanatory notes, were released in a report to the Australian Learning and Teaching Council.

Discussion. I will discuss briefly the process we followed, the agreed set of desirable learning outcomes for our graduates, and the implementation in undergraduate degrees across Australia (including teaching guides, learning activities and assessment). Resources are now available on a national website.

Jones S M, Yates B F, Kelder J (2011) Science Learning and Teaching Academic Standards Statement, Sydney, Australian Learning and Teaching Council

Threshold Learning Outcomes for Science

Bachelor level

	Upon completion of a Bachelor degree in science, graduates will:
Understanding Science	1. *Demonstrate a coherent understanding of science by:* 1.1 Articulating the methods of science, and explaining why current scientific knowledge is both contestable and testable by further inquiry. 1.2 Explaining the role and relevance of science in society.
Scientific knowledge	2. *Exhibit depth and breadth of scientific knowledge by:* 2.1 Demonstrating well-developed knowledge in at least one disciplinary area. 2.2 Demonstrating knowledge in at least one other disciplinary area.
Inquiry and problem solving	3. *Critically analyse and solve scientific problems by:* 3.1 Gathering, synthesising and critically evaluating information from a range of sources. 3.2 Designing and planning an investigation. 3.3 Selecting and applying practical and/or theoretical techniques or tools in order to conduct an investigation. 3.4 Collecting, accurately recording, interpreting and drawing conclusions from scientific data.
Communication	4. *Be effective communicators of science by:* 4.1 Communicating scientific results, information, or arguments, to a range of audiences, for a range of purposes, and using a variety of modes.
Personal and professional responsibility	5. *Be accountable for their own learning and scientific work by:* 5.1 Being independent and self-directed learners. 5.2 Working effectively, responsibly and safely in an individual or team context. 5.3 Demonstrating knowledge of the regulatory frameworks relevant to their disciplinary area and personally practising ethical conduct.

210

Translating research experiences to employability skills: Using evidence to make a convincing case.

Kirsten Zimbardi[1], Kay Colthorpe[1]. [1]Educational Research Unit, School of Biomedical Science, University of Queensland, St Lucia, QLD; (introduced by Elizabeth Davis, Monash University, Clayton, VIC).

All graduates need the skills and habits of mind to solve the complex, unstructured problems they will face in the 21st Century workforce (Bybee & Fuchs, 2006). In science, analysing technical literature, identifying conflicts and gaps, developing relevant, testable hypotheses, collecting and analysing the evidence to test these hypotheses, and putting forward reasonable, specific and qualified conclusions, is our bread and butter – the basis of scientific reasoning (Kuhn & Pease 2008). Research experiences and inquiry-based curricula aim to help undergraduate students develop these habits of mind and cognitive skills (Zimbardi & Myatt, 2012). In our inquiry-based curricula we have documented the development of students' scientific reasoning skills (Zimbardi *et al.*, 2013) and their understanding of the contestable nature of scientific knowledge (Zimbardi *et al.*, in press). We have also developed a series of meta-cognitive assessment items which have revealed students' ability to translate these learning outcomes into employability skills. Specifically, undergraduate biomedical science students in their final semester are provided with a job interview scenario and asked behavioural questions (e.g "Tell me about a time when you successfully used your scientific problem skills") and hypothetical questions (e.g "Suggest a potential approach for investigating this issue…"). Students' responses to these open-ended questions have revealed the diverse skill levels amongst the cohort in translating educational experiences to workplace situations. Notably, we have found several underlying assumptions and misconceptions that hinder students' articulation of their employability skills, as well as useful models of specific, evidence-based, and convincing, approaches to answering such questions.

Bybee RW & Fuchs B (2006) J Res Sci Teach 43(4): 349–352.
Kuhn D & Pease M (2008) Cogn Instruct 26: 512–559.
Zimbardi K et al (2013) Adv Physiol Educ 37 (4): 303-15.
Zimbardi K et al (in press) IJISME
Zimbardi K & Myatt P (2012) SHE 39 (2): 233-250

211

Developing career ready graduates

Laurence Orlando, Faculty of Pharmacy and Pharmaceutical Science, Monash University, Melbourne, VIC

Introduction. Graduates nowadays face a more complex workplace and convoluted career path than ever before. Their capacity to tackle unknown problems with critical thinking, work in team, effectively communicate are now as valued as their didactic knowledge in the workforce and the education system needs to adapt to this new reality.

Aims. Developing teaching interventions and capstone units that efficiently prepare students for the workplace.

Methods. An innovative pedagogy based on constructive alignment and experiential learning has been developed and applied in five units in third year of pharmaceutical science students and the effect of these interventions has been evaluated during and after a four week placement in industry by the mentors and through oral presentation on site.

Results. The majority of students perform in placement beyond mentors' expectations and achieve in four weeks significant technical outcomes evidenced by the report and oral presentation in the organisation. Indicator used in this study are the number of summer scholarships that are proposed by Industry after placement and the number of students being recruited after placement and/or summer scholarship.

Discussion. The teaching approach involves five third year capstone units that follow the principles of problem based and team based learning as well as research skills development. The preparation of students for the workplace happens through semester-long problems or projects that are broken down in smaller in semester assessment tasks and the final examination is replaced by the production of industry relevant documents that are summatively evaluated. Students design their own scientific protocol, perform their own experiments and interpret their results as part of the assessment tasks. At the end of year 3 they go on a four week placement in industry. Mentors confirm that students are independent workers and are able to tackle challenging problems during placement. The number of summer scholarship places following placement has raised considerably over the years as mentors choose to prolong students as they find them valuable to the organization even after only 4 weeks of work. Industries also contact the faculty directly when they recruit, to access students from our course as one of their first preferences if not the only one.

212

Preparedness for prescribing: What do your medical students think

Claire Johnston and Gerry Corrigan, Australia National University Medical School, Canberra, ACT

Introduction. In July 2014, ASCEPT released the Standards for Basic and Clinical Pharmacology Education in Medical Graduates. Based on this, we were interested to see if current, postgraduate medical students think they are prepared for the challenge of prescribing once they graduate and how they felt in general about pharmacology.

Methods. Medical students were asked about their attitudes towards pharmacology and how well they thought they were prepared for prescribing once they graduate.

Discussion. In my opinion as a postgraduate medical student from a pharmacology background, there can never be enough pharmacology teaching. The ASCEPT standards published in 2014 are an excellent guide for medical schools, as well as for students to direct their learning. A focus on areas such as drug interactions, pharmacokinetics/pharmacodynamics/pharmacogenetics and adjusting doses for specific populations and disease states is crucial.

213

Ethnopharmacology: From drugs to medicines for all populations

Annette S Gross. Clinical Pharmacology Modelling & Simulation, CPQS, GlaxoSmithKline R&D, Ermington, NSW; Faculty of Pharmacy, University of Sydney, NSW.

A goal of drug development is the selection and confirmation of clinical doses with appropriate efficacy and safety profiles in the target patient population. During drug development it is consequently important to profile the inter-individual variation in response observed and to determine the factors which contribute to the differences noted. Intrinsic factors such as age, body weight, sex and disease state, as well as extrinsic factors such as co-administration with meals or other therapeutics are routinely investigated for their influence on pharmacokinetics and pharmacodynamics. When these factors differ across ethnic groups, inter-ethnic differences in average drug response can result. In 2015 evidence of the efficacy and safety of potential new medicines is obtained from multi-regional clinical trials which enrol patients in many countries and from many ethnic groups. Clinical data in local populations may also be required for successful drug registration in specific countries, such as Japan. It is therefore important to consider the potential for inter-ethnic differences in response when analysing the results of these studies, especially as clinical trial populations are increasing in ethnic diversity. An assessment of potential ethnic sensitivity during drug development considers relevant attributes of the disease being investigated, the drug under investigation and the ethnic groups being compared. Importantly this approach can potentially assess the suitability of the clinical doses selected in ethnic groups which may not have participated in the multi-regional clinical development programme. However, for many populations around the world, including the diverse ethnic groups in Africa, currently available data are insufficient to allow a robust assessment of ethnic sensitivity. Further research is required to increase our knowledge of the profile of the intrinsic factors such as genetics as well as the influence of extrinsic factors such as traditional dietary ingredients which could potentially contribute to variation in drug response in these populations.

214

***CYP2C19* genetics in Maori and Pacific peoples**

Nuala A. Helsby[1]. School Medical Sciences, University of Auckland[1], Auckland, New Zealand

There is considerable inter-ethnic variability in the incidence of *CYP2C19* homozygous null variant alleles. This ranges from ~3% of Europeans to ~20% in SE Asia. Colonisation of the Pacific Islands is believed to have involved migration from SE Asia, via Papua New Guinea, through Melanesia and Micronesia into Polynesia. The prevalence of *CYP2C19* homozygous null alleles is very high (36-48%) in Papua New Guinea (Masta et al 2003; Hsu et al 2008). However the highest frequency to date (68%) is reported for populations from the Melanesian island of Vanuatu (Kaneko et al 1997). An increased incidence of *CYP2C19*2* has been reported for Maori compared with Europeans (Lea et al 2008). In addition, we and others have also noted a low frequency of the gain of function *CYP2C19*17* allele in Pacific people (Helsby et al 2010; Larsen et al 2015). Recent reports suggest that the *CYP2C19* gene may be under positive evolutionary pressure (Janha 2014). My talk will highlight how both population history and the environment may have influenced the prevalence of null function *CYP2C19* in the Pacific region.

Hsu H-L (2008) Hum Genomics 3: 17–23

Helsby N (2010) NZ Med J 123: 37-41

Lea R (2010) N Z Med J. 123:37-41

Janha R (2014) BMC Evolutionary Biology 14:71

Masta A (2003) Pharmacogenetics 13: 697-700

Kaneko A (1997) Lancet 349:921-2.

Larsen PD (2015) Intern Med J 45:537-45

215

Personalised medicines for Aboriginal Australians

Andrew A Somogyi. School of Medicine, University of Adelaide, Adelaide, SA.

Personalised medicine means different things to different health professionals. In clinical pharmacology, pharmacogenomics studies have revealed new mechanistic insights into the causes of large intersubject variability into drug disposition, response and toxicity. A good understanding of such variability has been gained by studies into ethnicity as a factor and been translated in some cases into clinical practice, such as *HLA-B*1502* and carbamazepine-induced hypersensitivity in people of Asian ancestry. However, Australian Aborigines have been poorly served. Previous studies have shown differences in *CYP2D6*, *2C19* and *NAT2* genetic polymorphisms, but the data are old with many new and important SNPs not tested. In addition, statin-induced muscle pain and immune-mediated necrotizing myopathy seemingly having a greater incidence in Australian Aborigines has been reported by Gabb and colleagues, indicate that pharmacogenomic studies need to be conducted to "close the gap". At issue is the ethical constraint on genetic studies in Australian Aborigines who have been exploited for scientific but not health gain. Therefore new studies need to have substantial buy-in by the local communities who must be seen to be driving the research in order to improve the treatment of their poor health. We have been funded by NHMRC and have substantial community engagement through local health services, to conduct comprehensive pharmacogenomic studies in Australian Aborigines. Not only are we employing and training local health community workers but have in place, systems to ensure the knowledge in transferred in a practical way to improve the health of Aboriginal Australians by optimising their pharmacotherapeutics.

216

Interethnic differences in pain, analgesia and neuroimmunogenetics

Daniel T Barratt[1]. Disc Pharmacology, Univ of Adelaide[1], Adelaide, SA.

Opioids remain the first-line pharmacological treatment for the relief of moderate to severe acute postoperative pain, but pain severity and opioid dose requirements vary substantially between patients. There are major genetic components to acute pain and analgesia (up to 60%), and significant ethnic differences in pain reporting and opioid analgesic requirements.

For some opioids, pharmacokinetic factors may contribute to interethnic variability in dosage requirements due to differences in the prevalence of polymorphisms in important drug metabolism genes (e.g. *CYP2D6*). Pain phenotype and opioid pharmacodynamic factors are also major contributors. For example, frequencies of the *COMT* Val158Met (rs4680) variant allele (associated with increased pain sensitivity) range from 22-59% between ethnic groups, correlating with interethnic variability in post-operative pain scores. In addition, the association between the *OPRM1* A118G (rs1799971) variant allele and higher morphine doses is strongest and clinically important in ethnicities where it has higher frequency.

Innate immune system activation has been implicated generally in pain and opioid analgesia, and genetic variability in this pathway has recently been investigated for its role in postoperative pain and morphine requirements in patients of Chinese, Malay and Indian ethnicity. These ethnic groups differ significantly in the frequency of most polymorphisms investigated. More importantly, several polymorphisms are associated with postoperative morphine requirements overall (*TLR2* rs3804100) and within (*IL1B* rs1143634, *TGFB1* rs1800469) ethnic groups, with their relative frequencies also matching overall ethnic differences in post-operative morphine use. This suggests that innate immune genetics may also contribute to interethnic variability in postoperative opioid requirements.

The role of ethnicity *per se* in differences in pain and analgesia still remains controversial, likely due to difficulties differentiating genetic from psychological/cultural components. Reflecting this, genetic polymorphisms identified so far do not account for all interethnic variability in postoperative pain and opioid requirements. Nonetheless, knowledge that certain genetic factors affecting pain or opioid analgesia are more prevalent in certain ethnic groups can help to inform whether pharmacogenetic testing might assist with optimising pain management in different populations.

217

Pharmacological Inhibition of Insulin Regulated Aminopeptidase (IRAP) Completely Reverses Age-mediated Cardiac Fibrosis

HW Lee[1], V Suturin[2], I Spizzo[1], H Parkington[2], SY Chai[2], Robert E Widdop[1], Tracey A Gaspari[1]. Dept of Pharmacology[1], Dept of Physiology[2], Monash University, Clayton, VIC.

Introduction: We have previously demonstrated that inhibition of insulin regulated aminopeptidase prevents Angiotensin II-induced cardiac fibrosis.
Aims: (1) To investigate whether pharmacological inhibition of IRAP reversed established cardiac disease in aged mice; (2) To delineate the underlying mechanism/s associated with anti-fibrotic actions of IRAP.
Methods: (1) Aged WT (C57BL/6J) mice (18-22 months) were chronically treated with either synthetic IRAP inhibitor (HFI-419; 500ng/kg/min) or vehicle for 4 weeks to determine ability of IRAP inhibition to reverse age-induced cardiac fibrosis. (2) Primary human cardiac fibroblasts (HCFs) were stimulated with angiotensin (Ang) II or transforming growth factor (TGF-β_1) in the presence or absence of HFI-419 for 72 hours to investigate underlying mechanisms.

Results: (1) Aging resulted in marked cardiac fibrosis that was significantly reduced in hearts taken from 4 week HFI419-treated mice, supported by downregulation of collagen synthesis and enhanced collagen degradation. In addition, IRAP inhibition also significantly reduced cardiac oxidative stress and inflammation, shifting towards an anti-inflammatory cytokine profile. Furthermore, 4 week HFI-419 treatment also improved cardiac function and prevented infarction when the hearts were subjected to ischaemic/reperfusion (IR) cardiac injury via Langendorff heart preparation. (2) Ang II or TGF-β_1 induced myofibroblast differentiation and collagen deposition in HCFs was prevented by HFI-419 treatment. This effect was partially abolished when oxytocin (OT) receptors or AT_2 receptors were antagonised, indicating involvement of IRAP substrates that target these receptors.
Discussion: Pharmacological inhibition of IRAP completely reversed age-mediated cardiac fibrosis and exerted multiple cardiac improvements. These anti-fibrotic effects of HFI-419 were further replicated in HCFs, highlighting the multifactorial nature of IRAP inhibitors and their potential therapeutic role in combating cardiac fibrosis.
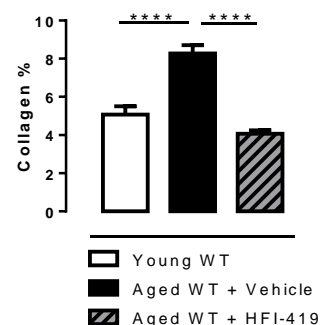
218

Implementation of the Drug Burden Index with Home Medicines Review in older Australians: a feasibility and utility study

Lisa Kouladjian[1,2], Danijela Gnjidic[1-3], Timothy F. Chen[3], Sarah N. Hilmer[1,2]. Dept of Clinical Pharmacology and Aged Care, Kolling Institute, Northern Sydney Local Health District[1], St Leonards, NSW; Sydney Medical School-Northern, Univ of Sydney[2], Sydney, NSW; Faculty of Pharmacy, Univ of Sydney[3], Sydney, NSW.

Introduction. The Drug Burden Index (DBI), a pharmacologic risk assessment tool that measures an individual's total exposure to anticholinergic and sedative medications, is associated with poor clinical outcomes in older adults. Home Medicines Review (HMR) is a collaborative medication review service that aims to enhance quality use of medicines, especially in older Australians with polypharmacy. The feasibility and utility of adding a report on a patient's DBI to HMR to inform medication management has not been assessed.
Aims. To assess whether addition of a report on DBI as a risk assessment tool for medication management is useful and feasible in the HMR setting.
Methods. An interventional implementation study was conducted. Pharmacists who regularly conduct HMRs were recruited to participate. Each pharmacist was educated on medication use in older adults and implementation of the DBI into practice, and given access to the Drug Burden Index Calculator© web-based software to generate a DBI report for inclusion in the HMR report for the General Practitioners (GP). Pharmacists recruited patients (≥65 years) who were referred to them for HMRs. Patients were sent a letter by the lead investigator with information about their DBI exposure, and a prompt to visit their GP to discuss their medication management options. GPs, pharmacists and patients were asked to evaluate the DBI report.
Results. All pharmacists (n=18, mean±SD 45.3±11.3 years) were able to generate and provide a DBI report together with the HMR report to the GP for each patient (n=47). Most pharmacists (88.9%; n=16/18) believe that the DBI report would be feasible in the HMR setting. Utility results indicate 81% of patients (n=38/47), 76.9% of GPs (n=10/13), and 88.9% of pharmacists (n=16/18) find the information in the DBI report very or somewhat useful.
Discussion. Implementation of the DBI report with HMR is feasible and is considered useful by pharmacists, GPs and patients. Further research will establish the impact of the DBI together with HMR service on prescribing and clinical outcomes in older adults.

219

Digoxin withdrawal in stable heart failure with reduced ejection fraction in sinus rhythm - a randomised controlled trial.

Ingrid Hopper[1], Marina Skiba[1,2]. Thomas G. von Lueder, Masataka Watanabe, Robyn Funston, Andrew Tonkin, Henry Krum. Clinical Pharmacology Department, Alfred Hospital[1], Melbourne, VIC; Centre of Cardiovascular Research and Education in Therapeutics, Monash University[2], Melbourne, VIC.

Introduction. The role of digoxin in heart failure (HF) patients with reduced ejection fraction, in sinus rhythm is unclear.
Aims. To investigate digoxin withdrawal in HF patients receiving digoxin with optimal ACE inhibitor/ARB and beta-blocker.
Methods. Prospective, randomized, single-blind, placebo-controlled, two-arm cross-over trial. Participants were randomized to digoxin continuation ("dig-on") or unmatched placebo ("dig-off") and crossed over after three months. Standard HF clinical status and quality of life endpoints were evaluated. Dig-on vs dig-off results were compared using a two-tailed paired t-test.
Results. The 16 participants were aged 61.3±11.0 years, 81% male, mean duration of HF 5.6±3.3 years and mean ejection fraction 33±10%. HF aetiology was ischemic (7) and non-ischemic (9). All participants completed the dig-on arm, and two withdrew from the dig-off arm early due to deterioration in HF (final assessments were included). Digoxin withdrawal resulted in a 50% increase in brain natriuretic peptide (dig-on: 405±587 vs dig-off: 604±843ng/L, p=0.019, 95%CI 39-361), reduced 6 minute walk distance (dig-on: 474±69 vs dig-off: 455±64m, p=0.015, 95%CI 4-34) but no worsening in quality of life measures including Cardiac Depression Scale (dig-on: 82±20 on vs dig-off: 72±22, p= 0.005, 95% CI 4-17), Minnesota Living with Heart Failure score (dig-on: 29±19 vs dig-off: 25±16, p=0.105, 95% CI -1-9), or Short Form-36 Health Survey (dig-on: 98±15 vs dig-off: 97±14, p=0.714, 95% CI -5–7). Echocardiographic parameters were unchanged.
Discussion. Withdrawal of digoxin in stable HF patients, in sinus rhythm on optimal contemporaneous therapy worsens HF clinical status with increased BNP and reduced submaximal exercise capacity but did not worsen quality of life.

220

Assessment of the potential remodelling of calcium signalling in human breast cancer-associated fibroblasts

Teneale A Stewart[1], Patsy S Soon[2,3,4], Sarah J Roberts-Thomson[1], Gregory R Monteith[1,5]. School of Pharmacy, The Univ of Queensland[1], Brisbane, QLD; Department of Surgery, Bankstown Hospital[2], Bankstown, NSW; South Western Sydney Clinical School, Univ of New South Wales[3], Sydney, NSW; Medical Oncology Group, Ingham Institute for Applied Medical Research[4], Liverpool, NSW; Mater Research, The Univ of Queensland[5], Brisbane, QLD.

Introduction. Cancer-associated fibroblasts (CAFs) play an active role in tumourigenic processes such as invasion and metastasis. Resident normal fibroblasts represent a potential source of CAFs in the breast tumour microenvironment. Proteins involved in Ca^{2+} signalling often display altered expression in pathologies including some cardiovascular disease states, such as the conversion of smooth muscle cells from a contractile to a proliferative state ("phenotypic-switching") and in some cancers. We assessed the transcriptional profile of selected Ca^{2+} pumps, channels and channel regulators in breast CAFs relative to normal breast fibroblasts using clinical samples and an *in vitro* model, and characterised the nature of calcium influx in activated breast fibroblasts.
Aims. To compare Ca^{2+} signalling in breast CAFs and non-activated breast fibroblasts.
Methods. Real time RT-PCR was used to assess the transcriptional profile of more than 30 proteins involved in calcium signalling in RNA isolated from primary cultured human breast CAFs, and their paired normal breast fibroblasts. To study the functional remodelling of the Ca^{2+} signal following acquisition of a CAF phenotype, an immortalised human breast fibroblast cell line was stimulated with TGFβ1 (0, 0.01, 0.1, 1 and 10 ng/mL) for 48 h and cytosolic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) measured using a fluorometric imaging plate reader (FLIPR^{TETRA}) in cells loaded with the Ca^{2+} sensitive indicator Fluo-4 AM.
Results. Patient derived breast CAFs displayed differential expression of specific Ca^{2+} channels relative to normal breast fibroblasts. Stimulation with TGFβ1 led to acquisition of CAF-like features in human immortalised breast fibroblasts and was associated with a functional remodelling of the Ca^{2+} signal.
Discussion. Breast CAFs differ from normal breast fibroblasts in their expression of specific classes of Ca^{2+} channels. Our results also suggest a potential functional remodelling of the Ca^{2+} signal in CAFs. Further studies are required to determine whether calcium signalling also contributes to the transition from a normal to CAF phenotype.

221

Understanding the mechanism of action of a novel class of allosteric modulator of the dopamine D2 receptor

Christopher J Draper-Joyce[1], Jeremy Shonberg[2], Mayako Michino[3], Lei Shi[3], Ben Capuano[2], Arthur Christopoulos[1], J Robert Lane[1], Drug Discovery Biology[1] and Medicinal Chemistry[2], MIPS, Monash University, Parkville, Australia. Computational Chemistry and Molecular Biophysics Unit[3], NIDA, National Institutes of Health, Baltimore, MD, USA

Introduction: SB269652 is the first drug-like negative allosteric modulator of the dopamine D2 receptor (D2R), an important target for the treatment of schizophrenia. We have proposed a novel mechanism of GPCR drug action whereby SB269652 adopts a bitopic pose at one protomer of a D2R dimer, to negatively modulate the binding of dopamine at the other protomer [1].

Hypothesis/Aims To validate the proposed bitopic mode of interaction of SB269652 at D2R, and probe the key ligand-receptor interactions that confer its novel allosteric action.

Methodology: Key ligand-receptor interactions were identified using molecular modelling, site directed mutagenesis and the generation of structural derivatives of SB269652. Radioligand binding was used in combination with a functional ERK1/2 phosphorylation assay to measure the effect of chemical modifications of the ligand and/or receptor mutations upon ligand affinity and allosteric cooperativity.

Results: We identified a putative allosteric pocket at the extracellular end of transmembrane domains 2 & 7, into which the indole moiety of SB269652 extends. Mutation of Glu95 to alanine caused a significant nine-fold ($p < 0.05$) (pKB = 5.14±0.28) decrease in affinity and 5-fold decrease in cooperativity of SB269652 (log$\alpha\beta$ = -0.32±0.14). Ligand docking experiments predicted that Glu95 forms a hydrogen bond with the NH of the indole heterocycle. Consistent with this prediction, an N-methyl derivative acted as an orthosteric antagonist (pKB = 7.28±0.09, Schild slope = 0.88±0.12).

Conclusions: These data provide validation of a bitopic mode of interaction for SB269652. In particular the interaction of the indole moiety of SB269652 with an allosteric pocket between transmembrane domains 2 and 7 is critical for allosteric pharmacology and distinguishes SB269652 from structurally related D2R antagonists. These studies reveal the mechanism of a novel class of drugs that target the D2R.

Lane JR et al (2014) Nat. Chem. Biol. 10(9): 745-752

222

Paracetamol hepatotoxicity in mice: Effect of age, frailty, N-acetyl cysteine and exposure type

Alice E Kane[1,2,3], Sarah J Mitchell[4], John Mach[1,2,3], Aniko Huizer-Pajkos[1,2], Catriona McKenzie[5], Brett Jones[1,2,3], Victoria Cogger[3,6], David G Le Couteur[3,6], Rafael de Cabo[4], Sarah N Hilmer[1,2,3]. Kolling Institute of Medical Research[1], St Leonards, NSW; Royal North Shore Hospital[2], St Leonards, NSW; Sydney Medical School, University of Sydney[3], Sydney, NSW; National Institute on Aging[4], Baltimore, USA; Royal Prince Alfred Hospital[5], Sydney, NSW; Centre for Education and Research on Ageing and ANZAC Research Institute[6], Concord, NSW.

Introduction. Paracetamol is a commonly used analgesic that can cause severe hepatotoxicity in overdose. Despite old age and frailty being associated with extensive use of paracetamol and a high prevalence of adverse drug reactions, there is limited information on the risks of toxicity from acute, chronic or sub-acute paracetamol ingestion in old age and frailty. N-Acetyl cysteine (NAC) is currently used to treat acute paracetamol toxicity, although there is little evidence for the use of NAC to treat non-acute paracetamol exposures.

Aims. This study aimed to assess changes in the risk and mechanisms of hepatotoxicity from acute, chronic and sub-acute paracetamol exposure with old age and frailty in mice, and investigate whether NAC was effective at preventing paracetamol toxicity induced by sub-acute exposure.

Methods. Young and old male C57BL/6 mice were exposed to either acute (300mg/kg via oral gavage), chronic (100mg/kg/day in diet for six weeks) or sub-acute (250mg/kg, t.i.d, for three days) paracetamol, or saline control. A sub-group of sub-acute exposed mice were then gavaged one or two doses of NAC (1200mg/kg in saline), or saline control. Pre-dosing mice were scored for the mouse clinical frailty index, and after dosing serum and liver tissue were collected for assessment of toxicity and mechanisms.

Results. There were no differences with old age or frailty in the degree of hepatotoxicity induced by acute, chronic or subacute paracetamol exposure as assessed by serum liver enzymes and histology. Neither a single nor double dose of NAC protected against subacute paracetamol toxicity in young or old mice. Age-related changes in the paracetamol toxicity pathways included increased liver glutathione concentrations, and an increased pro- and anti-inflammatory response to paracetamol in old age.

Discussion. There was no overall increase in paracetamol hepatotoxicity with old age or frailty in mice, and NAC did not protect against toxicity from sub-acute paracetamol exposure, which has significant clinical implications.

223

New horizons for male contraception: A non-hormonal approach via blockade of P2X1-purinoceptors and α_{1A}-adrenoceptors

Nicole T Eise[1], Kim Gundani[2], Nghi Nguyen[1], Jonathan Baell[1], Phillip E Thompson[1], Jamie S Simpson[1] & Sabatino Ventura[2]. Med Chem[1], Drug Disc Biol[2], Monash Institute of Pharmaceutical Sciences, Parkville, VIC.

Introduction. Safe, effective and reversible male contraceptives promise to be a valuable addition to the current choices for family planning. Both the α_{1A}-adrenoceptor and the P2X1-purinoceptor are essential in the male urogenital system for the transport of sperm from its storage site in the cauda epididymis into the ejaculate through contraction of the vas deferens. Previous studies have shown male α_{1A}-adrenoceptor knockout mice, and more markedly P2X1-purinoceptor knockout mice to be sub-fertile, due to lower numbers of sperm in the ejaculate resulting from a reduction in vas deferens contractility (Sanbe *et al*.,2007, Mulryan *et al*., 2000). Furthermore, simultaneous knockout of both receptors results in complete male infertility (White *et al* 2013) Antagonists for the α_{1A}-adrenoceptor are readily available and currently in use for the treatment of benign prostatic hyperplasia. However, currently available selective P2X1-purinoceptor antagonists are all large polyanionic molecules or acidic nucleotides that are broken down rapidly making them unsuitable for *in vivo* use.

Aims. To develop a novel, potent and selective small molecule P2X1-purinoceptor antagonist.

Methods. An initial starting compound and additional synthesised compounds were tested in isolated organ bath studies to determine their antagonist effects at P2X1-purinoceptors using $\alpha\beta$methylene adenosine 5'-triphosphate (ATP) (30 nM – 1 μM) as an agonist to induce contraction of the isolated rat vas deferens.

Results. Five compounds attenuated $\alpha\beta$methylene ATP induced contraction (n=4; p = 0.003 to p<0.0001) of the isolated rat vas deferens.

Discussion. Attenuation of $\alpha\beta$methylene ATP induced contraction implies that these compounds act as novel antagonists at the P2X1-purinoceptor ligand-gated ion channel.

Mulryan K et al (2000) Nature 403:86-89
Sanbe A et al (2007) Br J Pharmacol 152:332-340
White CW et al (2013) Proc Natl Acad Sc 110:20825-20830

224

Factors influencing patients' adherence to antidepressant medicines in unipolar depression: A qualitative study

Pornchanok Srimongkon, Parisa Aslani, Timothy F Chen. Faculty of Pharmacy, Univ of Sydney, Sydney, NSW.

Introduction. Non-adherence to antidepressant medicines is a major barrier to the successful treatment of unipolar depression. Whilst factors associated with medication adherence have been documented, how these factors relate to the different stages of adherence (i.e. initiation, persistence and discontinuation of treatment) are not known.

Aims. This study aimed to explore the positive influencing factors which promote medication adherence as well as the negative factors which reduce medication adherence, at all three stages of adherence (initiation, implementation and discontinuation) to antidepressant medicines used for unipolar depression.

Methods. A semi-structured interview guide designed to address the study aims was developed and pilot tested for face and content validity. Participants aged 18 years and over and taking antidepressant medicines for the management of unipolar depression were recruited via community pharmacies in the Sydney metropolitan area and a market research company. Semi-structured, face-to-face interviews were conducted and digitally audio recorded. Verbatim transcripts of the interviews were thematically content analyzed. Data were managed using N-Vivo software. This study was approved by the Human Research Ethics Committee of The University of Sydney.

Results. Twenty three interviews have been conducted and analyzed. Preliminary results indicate that a wide range of factors influence medication adherence. At initiation of therapy these positive factors included: severity of depressive symptoms, good support from family and friends, and self-management; negative factors included: fear of adverse reactions and negative information about antidepressant. The factors related to increasing persistence with therapy included: belief in antidepressants, clinical improvement, good relationships with and empathy of health care professionals; factors reducing persistence included: feeling better, and ineffective antidepressants. Discontinuation of therapy was triggered by: experiencing adverse drug reactions, feeling better and lack of support from family and health care professionals.

Conclusion. A range of factors influence patients' adherence to antidepressant medications, and these factors vary depending on the stage of adherence. Strategies to address medication adherence in patients with unipolar depression should firstly consider the stage of adherence, followed by the severity of depression.

225

Spontaneous adverse event reports associated with zolpidem

Carmen K. Wong[1], Nathaniel S. Marshall[2,3], Ronald R. Grunstein[2], David E. Hibbs[1], Romano A. Fois[1], Samuel S. Ho[1], Jane R. Hanrahan[1], Mary Chebib[1], Bandana Saini[1,2]. Faculty of Pharmacy, Univ of Sydney[1], Sydney, NSW; NHMRC Centre for Integrated Research and Understanding of Sleep (CIRUS), Woolcock Institute of Medical Research, Univ of Sydney[2], Sydney, NSW; Sydney Nursing School, Univ of Sydney[3], Sydney, NSW

Introduction. Stimulated reporting occurs when patients and healthcare professionals are influenced or 'stimulated' by media publicity to report specific drug-related adverse reactions, significantly biasing pharmacovigilance analyses. In early 2007, sustained negative media attention surrounding zolpidem in Australia caused a large stimulated reporting event in adverse event data (Ben-Hamou et al., 2011). Likewise, the United States experienced substantial media interest surrounding zolpidem and the development of bizarre idiosyncratic sleep-related behaviours. However the effect of this stimulated reporting on signal generation in the US Food and Drug Administration Adverse Event Reporting System (FAERS) has not been explored.

Aims. To investigate and determine if a stimulated reporting event is evident in the FAERS for zolpidem.

Methods. The FAERS database between January, 2003 and August, 2012 was analysed using case/non-case methods. Multivariate logistic regression was used to determine year-by-year reporting odds ratios for zolpidem exposure and the following adverse events; parasomnias, movement-based parasomnias, non-movement based parasomnias, amnesias, hallucinations and suicidality.

Results. The odds ratios increased significantly after the media publicity for parasomnias, movement-based parasomnias, amnesias and hallucinations. We also observed that zolpidem adverse drug reaction reports have higher odds for parasomnias, movement-based parasomnias, amnesias, hallucinations and suicidality compared to all other drugs, even before the media publicity cluster.

Discussion. Similar to Australia, our analyses indicates that a stimulated reporting phenomena occurred in the FAERS database. Clearly media induced panic as well as the "bandwagon effect" may have stimulated the reporting of adverse events. The effect of such reporting must be borne in mind when decisions around drugs which have been the subject of intense media publicity are being made by health professionals or regulatory bodies.

Ben-Hamou et al (2011) J. Sleep Res. 20:559-568

226

Exploring the visible components of organisational culture: what influences the use of psychotropic medicines in nursing homes?

Mouna Sawan[1], Yun-Hee Jeon[2], Timothy F Chen[1]. Faculty of Pharmacy, University of Sydney[1], Camperdown, NSW; Faculty of Nursing, University of Sydney[2], Camperdown, NSW (introduced by M Sawan, University of Sydney, Camperdown, NSW).

Introduction. Psychotropic medicines are associated with significant drug related morbidity and mortality yet they are commonly used in nursing homes. The influence of organisational culture on the use of psychotropic medicines in nursing homes has not been extensively studied. Schein's theory provides the framework for examining organisational culture which begins with the exploration of an outer layer called artifacts. Artifacts are observable objects of culture and include process and procedures.

Aims. To identify key artifacts related to the use of psychotropic medicines in nursing homes from the perspective of on-site and visiting staff and explore how these were adopted across nursing homes

Methods. A qualitative study was conducted with staff from eight nursing homes in Sydney, Australia. Purposive sampling was used to recruit 40 participants representing a broad range of disciplines and roles. The method of constant comparison was used to derive key concepts.

Results. Three artifacts were linked to the use of psychotropic medicines and how they were adopted varied across nursing homes. These were Medication committee (MAC) meetings, Multi-disciplinary Medication Management Review (RMMR) as well as resident and family participation. A few nursing homes utilised MAC meetings to communicate the need improve the use of psychotropic medicines. RMMRs usually meant a lever for the cessation of psychotropic medicines for a number of nursing homes. Also how nursing homes engaged with residents and their families influenced the cessation of psychotropic medicines.

Discussion. How nursing homes embrace key artifacts of organisational culture influence the use of psychotropic medicines in nursing homes. Nursing homes need to ensure the adoption of key artifacts by on-site and visiting staff ties with objectives to achieve quality use of psychotropic medicines.

227

Targeting health related quality of life (HRQoL) in pharmaceutical care: A systematic review and meta-analysis of the impact of pharmaceutical care services on quality of life

Mohammed A Mohammed[1], Rebekah J Moles[1], Timothy F Chen[1], The Uni of Sydney[1], Faculty of Pharmacy, Sydney, NSW.

Aim: To examine studies that have evaluated pharmaceutical care (PC) interventions impact on health related quality of life (HRQoL) with a focused meta-analysis to determine sensitivity of quality of life measures to PC interventions.

Methods: Published studies were identified from MEDLINE, EMBASE, International Pharmaceutical Abstracts, PubMed, Global health, PsychINFO, CINAHL and Web of Science (from January 2005 to March 2015).

Results: Of 5,302 studies, 48 met the inclusion criteria: 32(66.7%) randomized control trails, 4 controlled studies, 8 single group pre-post and 4 parallel group studies. The mean quality index score was 0.66 ± 0.12 implying a medium quality. Statistically significant PC impact on HRQoL ranged from 66.7% on one dimension to 27.1% on ≥ 3 dimensions of HRQoL. There was significant impact on at least one dimensions of HRQoL in 56.3 %(18/32) and 76.2% (16/21) of studies using generic, and disease specific measures respectively. Moderate impact were found for social functioning (Standardized mean difference/SMD 0.53, 95% CI 0.33, 0.73, P<0.001), general health (SMD 0.36, 95% CI 0.02, 0.59, P=0.005) and physical functioning (SMD 0.33, 95%CI 0.10, 0.55, P=0.004) dimensions of the SF-36 measure. While analysis of data on emotional role, vitality and bodily pain dimensions of the SF-36 measure tends to favor PC and those of physical role and mental health indicated no significant, a substantial heterogeneity was observed. Outcomes on heart-failure specific quality of life measure showed minimal impact (SMD -0.16, 95% CI -0.32, 0.01, P=0.04) whereas those of Asthma (SMD 0.17, 95%CI -0.03, 0.36, P=0.09) chronic obstructive pulmonary disease (COPD) (SMD -0.09, 95%CI -0.35, 0.17, P=0.48) specific measures indicated no significant impact of PC on both measures.

Conclusions: PC interventions can significantly impact at least one dimensions of HRQoL. Meta-analysis results indicated minimal to moderate sensitivity of dimensions HRQoL measures to PC services with evidences pointing more towards social functioning, general health and physical functioning of the SF-36 measure. However, evidences generated from current non-PC specific HRQoL measures are insufficient to judge the worth of PC service benefits for improving HRQoL. Future PC studies also likely to report variability in HRQoL outcomes, until the inconsistency in measuring HRQoL and reporting of PC is accounted for. Working towards developing specific and sensitive measure to PC help mitigate the inconsistency of HRQoL measures in PC studies. This may benefit future studies in using consistent and more suitable measure that may help generate better evidence on PC services impact on quality of life.

228

Potential medicine sharing risk reduction strategies: A qualitative analysis of patients' and health professionals' views using the Behaviour Change Wheel

Mr. Kebede Beyene, Dr Trudi Aspden, Associate Prof. Janie Sheridan. School of Pharmacy, The University of Auckland, Auckland, New Zealand.

Introduction. There is growing research evidence around non-recreational prescription medicine sharing behaviours. However, there is a need for systematic (theory led) interventions to reduce risks associated with sharing medicines. To address this gap, we adopted the Behaviour Change Wheel (BCW) framework to conceptualise factors which influence sharing behaviours and to identify intervention options. Part of the BCW includes the COM-B model, which hypothesises that human behaviour is the interaction between "Capability" (skill or knowledge to engage in the behaviour), "Opportunity" (environmental factors), and "Motivation" (the person's attitudes and beliefs).

Methods. Semi-structured, face-to-face interviews were conducted with purposively sampled doctors (n=4), nurses (n=6), pharmacists (n=8), and patients (n=17) from Auckland to identify factors influencing medicine sharing behaviours and potential interventions to reduce risks/harms of sharing. The data were coded using a general inductive approach. Themes which described factors influencing sharing, and described interventions to overcome those factors were mapped onto the COM-B system.

Results. Poor medication knowledge, forgetting to refill or carry around medications, and a lack of information about risks of sharing were examples of "capability"-related factors. Lack of access to health services, illness denial and embarrassment about medicines, having the same illness as the other person, linguistic/cultural barriers created "opportunity" for sharing. Altruism and fear of negative health consequences of missing regular doses of medicines "motivated" patients to share medicines. Education (e.g. providing information on safe disposal of unused medicines), enablement (e.g. helping patients to assess the risks of sharing in order to make educated decisions), environmental restructuring (e.g. removing unused medicines from households), and restriction (e.g. avoiding oversupply of pain medicines) were proposed as interventions.

Discussion. The BCW has provided important insights into personal (motivation or capability) and environmental (opportunity) factors influencing sharing behaviours, and a means by which theoretically underpinned interventions could be proposed.

229

Sedatives and safety: A matter of risk perception and communication between pharmacists and patients

Ibrahim Jomaa[1], Mariam Odisho[2], Keith Wong[3], Bandana Saini[1,3], Janet Cheung[1,3]. Faculty of Pharm, Univ of Sydney, Sydney, NSW[1]; Faculty of Pharm, Uppsala Univ, Uppsala, Sweden[2]; Sleep and Circadian Research Group, The Woolcock Institute of Medical Research, Sydney, NSW [3].

Introduction: A core role of the pharmacist is to ensure safe and effective medication use. Therapeutic classes that impair alertness (e.g. sedative hypnotics) can pose safety concerns for the patient when undertaking activities requiring psychomotor vigilance (e.g. driving). Standard labelling and counselling protocols have a longstanding history of use in the profession but little is known about how pharmacists perceive and/or assign medication-related risks, communicate risk related messages or indeed the impact of current practice in this area on patient safety.

Aim: This study aims to explore pharmacists' current perceptions and communication of risks related to alertness impairing medications in clinical practice and to determine the feasibility of implementing new clinical resources.

Method: In-depth semi-structured interviews explored pharmacists' perceptions of medication-related risks, current medication provision and the feasibility of new practice tools. Interviews were digitally recorded, transcribed verbatim and analysed using Framework Analysis to identify emergent themes.

Results: Synthesis of the qualitative dataset of 27 pharmacist interviews revealed three key themes: '*Different Perspectives of Risk*', '*Clinical Decision Making*' and '*Refining Risk Communication*'. Pharmacists were generally aware of the therapeutic classes associated with medication-related risks but were concerned about patients' level of understanding. Counselling approaches were largely dictated by perceived patient interest/experience with a medication. While the current reliance on labelling was deemed adequate, pharmacists also highlighted workflow limitations and the need to bring patients' attention to this resource during the clinical interaction to maximise impact. Concerns were also voiced about inter-individual differences, which could make the precise assignment of risk difficult. Most pharmacists were receptive to the possibility of new risk-assignment clinical tools.

Discussion: Medication-related risk communication is a complex clinical phenomenon dictated by patients' prior experiences and the pharmacists' practice environment. Extending pharmacists' clinical knowledge in this therapeutic area and refining clinical resources are key steps towards optimising safe medication use in patients.

230

Does Mental Health First Aid training affect MPharm students' literacy, knowledge and attitudes towards perinatal depression? A pre-test/post-test pilot study

Sarira El-Den[1], Claire L. O'Reilly[1], Timothy F.Chen[1] Faculty of Pharmacy, The University of Sydney[1], Sydney, NSW.

Introduction. Perinatal depression (PND) affects 10-15% of women, worldwide. Screening for PND is acceptable to most health professionals, but no studies involving pharmacists have been conducted (El-Den et al., 2015). Mental Health First Aid (MHFA) training has been shown to improve pharmacy students' attitudes towards mental health (O'Reilly et al., 2011), and was offered to final year MPharm students. Despite not explicitly covering PND, MHFA training provides background information on depression rates, symptoms and risk factors.

Aims. To pilot test a new survey instrument and explore the impact MHFA training has on literacy, knowledge and attitudes towards PND and screening.

Methods. A single group pre-/post- test design was used. An online survey instrument, consisting of two vignettes on PND, 6 knowledge questions, and 31 attitudinal items, was administered immediately before and after MHFA training.

Results. Forty MPharm students completed the survey. Before the training none of the students used the terms "antenatal", "prenatal" or "perinatal" depression to describe the first vignette; however, this increased to 60% after training. Answers to the knowledge questions improved for 4 out of 6 questions. Students attitudes regarding their comfort with PND screening almost doubled (28% vs 53%) and their acceptability of screening increased by 10%.

Discussion. PND is categorised as a form of major depressive disorder in the DSM V. Therefore, it is essential to explore if interventions that improve primary healthcare professionals' literacy, knowledge and attitudes towards depression extend to depression with peripartum onset.

El-Den S, et al. 2015 J Affect Disord. [Article in press] DOI: http://dx.doi.org/10.1016/j.jad.2015.06.015

Kitchener B, et al. 2013 Mental health first aid manual. 3rd ed. 12 Melbourne: Mental Health First Aid Australia.

O'Reilly, CL, et al. (2011). Impact of mental health first aid training on pharmacy students' knowledge, attitudes and self-reported behaviour: a controlled trial. Aust N Z J Psychiatry. 45:(7)549-557.

231

The role of medicinal cannabis in clinical therapy: pharmacists' perspectives

Sami Isaac[1], Bandana Saini[1] & Betty B Chaar[1]. Faculty of Pharm, Univ of Sydney[1], Camperdown NSW

Introduction. Medicinal cannabis has recently attracted much media attention in Australia and across the world. With the exception of a few countries, cannabinoids remain illegal – known for their adverse effects rather than their medicinal application and therapeutic benefit. However, there is mounting evidence demonstrating the therapeutic benefits of cannabis in alleviating neuropathic pain, improving multiple sclerosis spasticity, reducing chemotherapy induced nausea and vomiting, and many other chronic conditions. Many are calling for the legalisation of medicinal cannabis including consumers, physicians and politicians. Pharmacists are the gatekeepers of medicines and future administrators/dispensers of cannabis to the public, however very little has been heard about pharmacists' perspectives.

Aims. To explore pharmacists' views about medicinal cannabis; its legalisation and supply in pharmacy.

Methods. Semi-structured interviews with 34 registered pharmacists in Australia were conducted. All interviews were audio-recorded, transcribed ad verbatim and thematically analysed using the NVivo© software.

Results. Emergent themes included stigma, legislation, safety and collaboration. Overall the majority of pharmacists felt national legalisation of a standardised form of cannabis would be suitable, and indicated various factors and strategies to manage its supply. The majority of participants felt that the most suitable setting would be via a community pharmacy setting due to the importance of accessibility for patients.

Discussion. This study explored views of practicing pharmacists, revealing a number of previously undocumented views and barriers about medicinal cannabis from a supply perspective. There were several ethical and professional issues raised for consideration. These findings highlight the important role that pharmacists hold in the supply of medicinal cannabis. Additionally, this study identified important factors, which will help shape future policies for the successful implementation of medicinal cannabis in healthcare. We recommend that these views and strategies be incorporated in the development of policies and legislations.

232

Personalising busulfan in children; from assessment of new dose regimens to individualised limited sampling for therapeutic drug monitoring.

Sam Salman[1], Catherine H Cole[2,3], Sean O'Halloran[4], Fiona Kerr[2], David A Joyce[1,4] School of Medicine and Pharmacology, University of Western Australia[1], Perth, WA; Department of Haematology and Oncology, Princess Margaret Hospital[2], Perth, WA; School of Paediatrics and Child Health, University of Western Australia[3], Perth, WA; Clinical Pharmacology and Toxicology, PathWest[4], Perth, WA.

Introduction. Intravenous busulfan is commonly used in myeloablative regimens for haemapoietic stem cell transplantation in children and is the only such drug for which outcomes appear to be superior when therapeutic drug monitoring (TDM) is used. Current TDM monitoring requires multiple blood samples. A number of optimised dose regimens have been presented recently in the literature and are yet to be assessed in the Australian context.

Aims. i) Assess newly suggested dose regimens within an Australian population. ii) Develop a population pharmacokinetic (PPK) model using local data. iii) Investigate the reliability of limited sampling schedules, including those with optimised sampling, utilising the developed PPK model as well as PPK models in the literature.

Methods. Historical busulfan TDM data for children receiving QID (n=15) and OD (n=26) intravenous busulfan were obtained. The effect of alternative dosing regimens on achieving target concentrations was assessed assuming linear kinetics. NONMEM was used to develop a PPK model from the available data including size and maturation covariates and the results compared with the original TDM results. Limited sampling regimens using one to five of the current six time points for OD dosing and individually optimised sampling points were assessed within the context of a PPK model (local and published) for the historical data as well as in a simulated population.

Results. Newly suggested dose regimens did provide some small improvements over established regimens. A PPK model using the historical data was successfully developed with some support from prior information. Limited sampling regimens (with the support of an established PPK model) performed as well as full sampling schedules even with as little as two appropriately timed samples, regardless of the PPK model used as the backbone

Discussion. There is room to improve the use of busulfan in children. Newer dose regimens based on PPK models can be used to personalise the dose with some benefits, however, the need for TDM remains. Individualised limited sampling can be used successfully for TDM and are less intensive clinically.

233

Systemic inflammation predicts drug pharmacokinetics and clinical outcomes in advanced non-small cell lung cancer patients receiving paclitaxel and carboplatin chemotherapy

Benjamin Harris[1,2], Viet Phan[3], Vidya Perera[4,5], Anneliese Szyc[4], Peter Galettis[6], Philip Beale[3], Andrew McLachlan[4], Kellie Charles[*,1,2] and Stephen Clarke[*,3]. Discipline of Pharmacology[1] and Bosch Institute[2] and Central Clinical School[3] and Faculty of Pharmacy[4], University of Sydney, Sydney, NSW; School of Pharmacy and Pharmaceutical Sciences, SUNY at Buffalo[5], USA; School of Medicine and Public Health, University of Newcastle[6], Callaghan, NSW. [*], indicates shared senior author.

Introduction. Lung cancer patients with cancer-related inflammation have a 50 % shorter overall survival compared to patients without inflammation. The reason for this difference in survival remains unknown. However, inflammation has been previously shown to decrease the pharmacokinetics of hepatically cleared anti-cancer drugs whilst less is known about renally cleared drugs.
Aims. This study aimed to determine whether carboplatin and paclitaxel pharmacokinetics and clinical outcomes (patient toxicity, response and survival) are impacted by inflammatory status (neutrophil-to-lymphocyte ratio (NLR) > 5) in a prospective clinical trial of patients with advanced non-small cell lung cancer (NSCLC).
Methods. Seventy-two advanced NSCLC patients were recruited into the trial. Pharmacokinetics was measured using HPLC-MS (paclitaxel) and ICP-MS (carboplatin). Population pharmacokinetic modelling was performed using NONMEM. Univariate analysis and multivariable regression analysis was used to identify relationship between NLR status (NLR ≤ 5 and NLR > 5) and to pharmacokinetics, chemotherapy usage and clinical outcomes.
Results. Patient demographics were not different between NLR status groups. Univariate analyses identified NLR > 5 associated with increased carboplatin AUC, decreased number of chemotherapy cycles, decreased response and a trend for decreased survival. Multivariable regression analysis maintained NLR > 5 as predictive of altered carboplatin pharmacokinetics, decreased number of cycles and decreased response.
Discussion. The results suggest patients with elevated inflammation have altered drug pharmacokinetics and this may be negatively impacting patient toxicity, completion of planned chemotherapy cycles, response and survival. Future studies are currently investigating larger cohorts to confirm the findings in a community setting, which may determine applicability of NLR for dose optimisation and patient selection for this common chemotherapy regimen.

234

Utility of the OncoFOCUS™+*KIT* somatic cancer mutation screen in directing targeted pharmacotherapy

Thomas M Polasek[1], Karen Ambler[2], Hamish S Scott[2], Michael J Sorich[1], Peter A Kaub[2], Andrew Rowland[1], Michael D Wiese[3], Ganessan Kichenadasse[4], Dept of Clinical Pharmacology, Flinders University[1], Adelaide, SA; Dept of Genetics and Molecular Pathology, SA Pathology[2], Adelaide, SA; School of Pharmacy and Medical Science, University of South Australia[3], Adelaide, SA; Dept of Medical Oncology, Flinders Centre for Innovation in Cancer[4], Adelaide, SA.

Introduction. OncoFOCUS™+*KIT* (Agena Bioscience) is a multi-gene panel screening test for clinically significant mutations in the *KRAS*, *NRAS*, *BRAF*, *EGFR* and *KIT* genes. Targeted pharmacotherapy is indicated for patients with *BRAF*-mutant (MT) malignant melanoma, advanced non-small cell lung cancer (NSCLC) with activating *EGFR* mutations and metastatic colorectal cancer (mCRC) that is *RAS* wild-type (WT).
Aim. To describe the utilisation rates of OncoFOCUS™+*KIT* results for directing targeted pharmacotherapy according to mutation status among patients at the Flinders Centre for Innovation in Cancer (FCIC).
Methods. A retrospective chart-based audit of OncoFOCUS™+*KIT* results and targeted pharmacotherapy use was conducted. Inclusion criteria were: ≥ 18 years old; diagnosis of malignant melanoma, advanced NSCLC or mCRC; attended FCIC in 2014; OncoFOCUS™+*KIT* results reported in 2014. Information was obtained from all available patient medical records (hardcopy and electronic).
Results. A cohort of 149 patients met the inclusion criteria and 143 (96%) had sufficient medical records to audit. All patients were *KIT*-WT. In malignant melanoma, 44% were *BRAF*-MT. Mutations that activate EGFR were present in 18% of patients with NSCLC. Patients with mCRC had tumours that were *RAS*-MT (46%) or *RAS*-WT (54%). In total, 99% (100/101) of patients not indicated for targeted pharmacotherapy did not receive a targeted drug. Of the patients indicated for targeted pharmacotherapy, 48% (20/42) received such treatment according to their mutation status after OncoFOCUS™+*KIT* testing; 71% (5/7) with *BRAF*-MT malignant melanoma, 60% (6/10) with *EGFR*-positive NSCLC, and 36% (9/25) with *RAS*-WT mCRC.
Discussion. OncoFOCUS™+*KIT* results are applied well when targeted pharmacotherapy is not indicated and in *BRAF*-MT malignant melanoma. Targeted drugs are less widely used following genetic testing in *EGFR*-positive NSCLC and *RAS*-WT mCRC. This is due largely to PBS restrictions on first-line treatment.

235

Cerebrospinal Fluid Pharmacokinetics of Tobramycin Following Intraventricular Administration

Pamela J Buffery[1], Sarah Metcalf[2], Matthew P Doogue[3]. Dept. Clinical Pharmacology[1], Dept. Infectious Diseases[2], Canterbury District Health Board, University of Otago[3], Christchurch, NEW ZEALAND

Introduction. A 62 year old woman with recurrent Pseudomonas stutzeri ventriculitis was treated with 2 weeks of intraventricular tobramycin. A literature search located only a few papers that described the use of intraventricular antimicrobials. None of these papers reported drug concentrations or described pharmacokinetics.

Aims. To describe the cerebrospinal fluid (CSF) pharmacokinetics of tobramycin following intraventricular administration.

Methods. Following an initial intraventricular dose of 20 mg samples of CSF were taken 1, 5 and 24 hours post dose for the measurement of tobramycin concentrations and repeated after subsequent doses. Tobramycin was measured by immunoassay on the Abbott c series analyser and data were analysed in Microsoft Excel. CSF concentration targets were selected based on plasma concentration targets for infections at other sites.

Results. Tobramycin concentrations are shown in the figure. Following the daily 20 mg doses CSF tobramycin the average Cmax was 81 mg/L, Cmin 18 mg/L, AUC 1440 mg.hr/L and T1/2 15 hours. The dose was reduced to 10 mg every three days with average Cmax 45 mg/L, Cmin 1.1 mg/L, AUC 960 mg.hr/L and T1/2 15 hours. The ventriculitis was effectively treated and the patient did not experience any adverse effects of tobramycin. In this patient the estimated CSF Vd for tobramycin was 240 mL, CSF CL 0.014 L/hr and CSF T1/2 15 hours.

Discussion. The average adult volume of CSF is 150 mL slightly less than the apparent volume of distribution of tobramycin in this case. The CSF clearance of tobramycin was low with a consequent half-life much greater than that in plasma. As tobramycin has concentration dependent effects these results favour an extended dosing interval of 3 to 4 days when tobramycin is administered directly into the CSF. The low CSF clearance suggests low bidirectional aminoglycoside movement between CSF and plasma. This is consistent with the relatively poor responses of CNS infections to intravenous aminoglycosides.
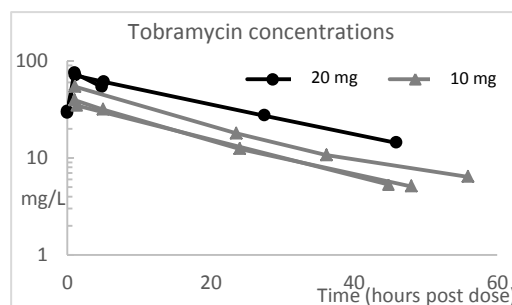
236

Flucloxacillin: total concentrations poorly reflect unbound concentrations in hospitalised patients

Paul K L Chin[1], Philip G Drennan[1], Simon C Dalton[2], Mei Zhang[2], Evan J Begg[1]. Dept of Clinical Pharmacology, Christchurch Hospital[1], Christchurch, NZ; Dept of Infectious Diseases, Christchurch Hospital[2], Christchurch, NZ.

Introduction. Total concentrations of flucloxacillin are measured to guide its dosing in hospitalised patients with *S. aureus* bacteraemia at Christchurch Hospital. Flucloxacillin has a low fraction unbound in plasma (fu) of ~ 0.07.

Aims. To determine the flucloxacillin fu in a) healthy volunteers and b) hospitalised patients. To examine the performance of a model, assuming a single protein binding site on albumin, using total flucloxacillin and plasma albumin concentrations, for predicting unbound concentrations (Musteata 2012).

Methods. Data from healthy volunteers (262 samples) and patients (61 samples) were examined.

Results. The correlation plot shows the data for unbound vs

total flucloxacillin concentrations for both cohorts. Median (range) flucloxacillin fu for healthy and hospitalised individuals were 0.04 (0.02, 0.07) and 0.10 (0.05, 0.37), respectively. The model predicted unbound flucloxacillin concentrations to within 15% of the measured value in 13% of patient samples.

Discussion. The fu of our cohort of hospitalised patients on flucloxacillin for *S. aureus* bacteraemia was greater than the value typically quoted in the literature, with a wide range of fu values. Unbound flucloxacillin concentrations were predicted poorly by the single protein binding site model. Until an improved prediction model is developed, unbound flucloxacillin should be measured to guide dosing.

Musteata FM (2012) Clin Pharmacokinet 51:55-68
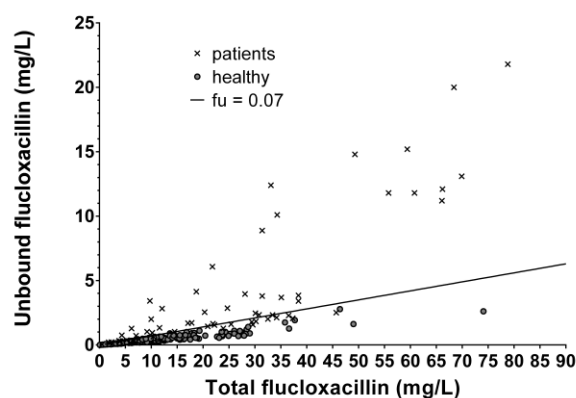
237

Antibiotics prescribed to patients receiving haemodialysis in both the community and hospital settings.

Katrina Hui[1], Michelle Nalder[2], Kirsty Buising[3], Eugenie Pedagogos[4], Craig Nelson[5,6], Carl Kirkpatrick[1], David Kong[1], Centre for Medicine Use and Safety, Monash Univ[1], Parkville, VIC; Dept of Pharmacy, Royal Melb Hosp[2], Parkville, VIC; Victorian Infectious Diseases Services, Royal Melb Hosp[3], Parkville, VIC; Dept of Neph, Royal Melb Hosp[4], Parkville, VIC; Dept of Neph, Western Health[5], Footscray, VIC; North West Academic Centre, Univ of Melb[6], Parkville, VIC.

Introduction. Inappropriate use of antibiotics contributes to antimicrobial resistance, a global public health threat. Patients receiving haemodialysis are at risk of developing infections and consequently, are likely to receive antibiotics. Little is known about the types and appropriateness of antibiotics prescribed to these patients.

Aims. To assess the type and appropriateness of oral and iv antibiotics prescribed to haemodialysis patients.

Methods. A prospective, observational study involving four community outpatient and two hospital inpatient haemodialysis units was conducted in Melbourne, Victoria. Data were collected from July 2014 to January 2015 from participants. The antibiotic regimens prescribed were compared with available national guidelines, and then the appropriateness of the regimens were classified according to the recommendations in the National Antimicrobial Prescribing Survey tool.

Results. Overall, 114 participants consented. A total of 235 antibiotic regimens (110 oral and 125 iv antibiotic regimens) were prescribed with 63/114 (55.3%) participants receiving at least one antibiotic during the study period. The most common oral antibiotics prescribed were amoxycillin/clavulanic acid (18.2%, 20/110) and cephalexin (12.7%, 14/110). The most common iv antibiotics were vancomycin (25.6%, 32/125), piperacillin/tazobactam (18.4%, 23/125), cephazolin (14.4%, 18/125) and ceftriaxone (12.8%, 16/125). In the community and hospital haemodialysis settings, the percentage of inappropriate antibiotic regimens prescribed were 34.9% (15/43) and 22.1% (40/181), respectively. Additionally, 29.4% (30/102) of oral antibiotics and 20.5% (25/122) of iv antibiotic regimens prescribed were classified as inappropriate. Incorrect dose or frequency was the primary reason for inappropriate prescribing.

Discussion. This is the first study to describe the extremely high antibiotic exposure that patients receiving haemodialysis experience, and highlights inappropriate dosing as a major issue requiring attention.

238

Febuxostat: an analysis of its dose-response relationship with serum urate

Diluk RW Kannangara[1,2], Garry G Graham[1,2], Kenneth M Williams[1,2], Elizabeth Corbett[3], Peta Pentony[3], Neil W McGill[3], Michael Doherty[4], Richard O Day[2,5]. School of Medical Sciences, Univ of New South Wales[1], Kensington, NSW; Dept of Clin Pharmacol, St Vincent's Hosp[2], Darlinghurst, NSW; Dept of Rheumatol, Royal Prince Alfred Hosp, Sydney, NSW; School of Medicine, Univ of Nottingham[4], Nottingham, UK; St Vincent's Clinical School, Univ of New South Wales[5], Darlinghurst, NSW

Introduction. Febuxostat is an alternative xanthine-oxidase inhibitor indicated for gout following resistance to, failure of or contraindications to allopurinol treatment. While the potency of febuxostat versus allopurinol is apparent from clinical studies, the dose-response relationship of febuxostat has not been evaluated in detail in comparison to allopurinol.

Aims. To examine the dose-response relationship of febuxostat in patients with gout.

Methods. Patients with gout (n=14 [Australia], 51 [UK]) were given increasing doses of febuxostat up to 120 mg/day. Serum urate was recorded at each dose titration. A modified Emax model (Graham et al, 2013) describing the hypouricaemic action of allopurinol was then fitted to the febuxostat and urate data using the program R (v2.15.2). Estimates of the ID_{50} and the 'resistant' urate (U_R) concentration were calculated and the goodness of fit was measured using the coefficient of determination (R^2).

Results. The model performed best with the inclusion of the U_R term (R^2=0.22 versus 0.10 without U_R, P=1.1x10^{-4}). Mean (95% CI) ID_{50} and U_R values were 27 mg (13-48 mg) and 0.19 mmol/L (0.12-0.24 mmol/L), respectively.

Discussion. There are some similarities between the allopurinol and febuxostat dose-response models. The model indicates that, like allopurinol, the baseline urate concentration strongly influences the urate concentration obtained on febuxostat. In addition, like the allopurinol relationship, there is a significant febuxostat 'resistant' urate concentration. However, the dose-response models differ with respect to their goodness of fit (R^2=0.22 versus 0.74, Graham et al, 2013). A febuxostat concentration-response model might better describe the data.

Graham G et al (2013) Br J Clin Pharmacol 76:932-938

239

Metformin and weight loss: An exposure-response relationship.

Shaun S Kumar[1,2], Carl M Kirkpatrick[3], Kenneth M Williams[1,2], Robin Bell[4], Susan Davis[4], Ric O Day[1,2,5].
Dept of Clin Pharmacol, St Vincent's Hosp Sydney[1], Darlinghurst, NSW; School of Medical Sciences, Univ of New South Wales[2], Kensington, NSW; Centre of Medicines Use and Safety, Monash Univ[3], Parkville, VIC; School of Public Health and Preventative Medicine, Monash Univ[4], Melbourne, VIC; St Vincent's Clin School, Univ of New South Wales [5], Darlinghurst, NSW.

Introduction. Metformin is the first line pharmacotherapy for type 2 diabetes mellitus. One of the positive benefits of metformin is that it can cause weight loss in individuals with obesity. To date the relationship between metformin plasma concentration and weight loss has not been examined.

Aims. To examine the relationship between metformin concentration and weight loss.

Methods. Data was available from a double-blind placebo controlled trial of metformin in women with obesity (n=118). The women (n=59) were dosed with 1700 mg/day for 6 months. Plasma samples were collected on 3 separate occasions and assayed by a validated HPLC-UV assay. Concentration and weight loss data were analysed NONMEM (Version 7.2). Utilizing our previous population PK structural model (Duong et al, 2013) in combination with the sparse sampling strategy (3 samples) the cumulative exposure represented by the AUC per week was predicted.

Results. Overall the mean weight change in the metformin group was -2.16 kg (95% CI -3.0 to -1.3). A slope and intercept model with cumulative AUC (range: 16-45 mg/L.week^{-1}) provided the best fit and took the form: Weight = Base − Slope*Cumulative AUC. The mean value (CV %) of the parameters were Base = 84.2 kg (12.4%) and Slope = 0.069 (79.1%). Insulin resistance was correlated with increasing body weight (r^2 = 0.18).

Discussion. In conclusion a relationship between cumulative metformin exposure (AUC) and weight loss was established. More work is required to determine if this model can be used to individualise dosing to achieve a desired weight loss. The relationship between weight and insulin resistance needs to be explored further.

Duong et al (2013) Clin Pharmacokinet 52:373-384.

240

Styrene maleic acid micelles as a nanocarrier system for oral delivery of paclitaxel

Neha N Parayath[1], Samuel Norton[2], Sebastien Taurin[1], Roslyn Kemp[2], Khaled Greish[1]. Department of Pharmacology and Toxicology[1], Department of Microbiology and Immunology[2], University of Otago, Dunedin.

Introduction: Oral route is the preferred mode of drug administration by patients. However, most anticancer agents are administered intravenously due to their low oral bioavailability and severe gastrointestinal (GI) toxicity. A nanocarrier system for oral delivery of anticancer drugs would help decrease the toxicity to the GI tract and take advantage of the wide fenestrations of tumor vasculature to selectively accumulate in the tumor tissue, improving the therapeutic efficacy and safety of these drugs.

Aim: We demonstrate the efficacy of styrene maleic acid (SMA) micelles as a nanocarrier-system for oral anticancer drug delivery.

Methods: SMA-micelles encapsulating epirubicin or paclitaxel (PTX) were synthesized in a pH dependent process. An *in-vitro* and *ex-vivo* model of intestinal epithelium were used to predict the ability of SMA-epirubicin to traverse the intestinal epithelium. PTX was encapsulated in SMA micelles to determine the maximum tolerated dose (MTD) *in vivo*. The anticancer activity of the SMA-PTX was evaluated in, orthotopic-colon-cancer mice model (n=7).

Results: SMA-epirubicin was efficiently transported across the *in vitro* enterocytes-like monolayer (12.7±0.9 µM/cm^2) and across *ex-vivo* model of rat intestine (19.2±1% of total treated micelles (n=5)). The single MTD for SMA-PTX was 120mg/kg, compared to 60mg/kg of the commercially available PTX (PTX-Ebewe). The repeated MTD for SMA-PTX was 60mg/kg in contrast to 30mg/kg of PTX-Ebewe. A significant reduction in colon tumor weight was observed following oral administration of SMA-PTX 60mg/kg (126.4±27.8mg) as compared to the tumors from control group (416.4±53.3mg) or PTX-Ebewe 30mg/kg group (344.5±76.7mg) (p<0.05).

Discussion: Following oral delivery, SMA-PTX is absorbed through the small intestinal epithelium and passed into the systemic circulation. SMA-PTX accumulates at the tumour site due to the wide fenestrations of the tumour vasculature [1]. In addition, the colon tumours increase the permeability of the intestinal epithelium allowing SMA-PTX accumulation in the tumour tissue located beneath the epithelium. Thus, SMA micelles could provide an effective strategy for safe oral administration of paclitaxel in cancer therapy for gastrointestinal tumors.

Greish, K et al (2004) Journal of controlled release, 2004. 97(2): p. 219-230.

241

Development of a physically stable high dose powder for inhalation by spray-drying

Lily Chen, Shyamal C Das.
School of Pharmacy, University of Otago, Dunedin, New Zealand

Introduction. Spray-drying is being investigated to develop high dose powders for inhalation intended for treating lung infections. In order to offer a high delivery dose, the drug is spray dried alone or with minimal amount of excipients. A major problem with spray dried powders is that the powders are amorphous. During storage they convert to the crystalline form which may have different aerosolization property. Therefore, it is important to develop strategies to prevent or at least slow the amorphous to crystalline transition so that the product remains unchanged during its storage life.

Aims. The objective of this study was to develop a physically stable spray-dried powder, containing a small amount of excipients with high glass transition temperature, for delivery of a high dose of drug by inhalation.

Methods. A model drug SS was spray dried alone or with different concentrations of L-leucine. The powders were characterized for morphology, crystallinity, moisture content and glass transition temperature by Scanning Electron Microscopy, X-ray Powder Diffractometry, Thermogravimetric Analysis, and modulated Differential Scanning Calorimetry, respectively, immediately after preparation and periodically during storage at 15% and 75% relative humidity at 25 ºC. In vitro aerosolization performance was assessed using a Next Generation Impactor.

Results. The fine particle fraction (FPF) of SS significantly increased in the presence of L-Leucine. For example, the FPF of SS alone was 45% which was increased to 54% in the presence of 10% Leucine. When stored at 15% RH for two months, the FPF did not change in the SS powders containing leucine while the FPF significantly decreased for SS spray-dried alone. When stored at 75%RH, the FPF of any spray dried powder did not significantly change for one week; however, the FPF of SS decreased in greater extent in SS-alone powder at two months than the powders containing leucine. The glass transition temperatures of SS-leucine powders were higher than SS alone. The formation of hydrogen bonds between SS and leucine was also found. XRPD studies revealed the amorphous nature of freshly spray dried powders and the crystalline nature of the powders whose aersosolization was decreased.

Discussion. Co-spray drying of SS with leucine can improve physical stability by decreasing amorphous-crystalline transition by increasing glass transition temperature and by forming hydrogen bonds between SS and leucine.

242

Development of Localized Stent Drug Delivery System for Esophageal Cancer

Sanjay Garg[1], Mohsin Shaikh[1], Namita Choudhury[2], Jagat Kanwar[3], Yunmei Song[1], HongYuan Wang[4], Huihui Zhang[4], Xiuli Guo[4]. CPID[1] and Ian wark Research Institute[2], University of South Australia, Adelaide, SA. NLIMBR[3], Deakin University, Geelong, VIC. School of Pharmacy[4], Shandong University, PR China.

Introduction. Esophageal cancer (EC) is the sixth most common cause of death due to cancer. Poor prognosis coupled with late presentation of disease leads to advancement of EC. At the time of diagnosis more than half of the patients are in advanced stage when 50% of the lumen is compromised leading to dysphagia. Stent is the medical device often used for palliation because of easy administration and immediate relief of dysphagia. But, reintervention rate after stenting is high due to several reasons like tumor over/in growth, food impaction and stent migration.

Aims. To develop a drug delivery system which can be incorporated into a stent to produce a drug eluting stent (DES) with the ultimate aim of reducing tumor mediated re-occlusion.

Methods. Non-biodegradable polymers *viz*., Silicone and Polyurethane were selected for Docetaxel (DTX) delivery. Film formulations were prepared using solvent casting and evaluated for material/pharmaceutical properties, permeation characteristics, stability and biology evaluation *in-vitro* and *in-vivo*.

Discussion. Silicone elastomer exhibited chemical incompatibility with DTX but polyurathane polymer exhibited chemical and physical compatibility with DTX. Polyurethane film formulations delivered DTX over one month. Permeation characteristics were found rate limiting in DTX delivery to esophagus. Ambient temperature and humidity (25°C/60% RH) were found suitable for formulation storage. *In-vitro* assessment in EC cell lines showed weak but sustained activity of DTX/formulation. *In-vivo* analysis in mouse xenograft model showed skin toxicity necessitating formulation modification. Although high dose modified formulation was toxic, low dose formulation showed modest activity compared to commercial IV formulation. For enhancing DTX efficacy and reducing toxicity, combination with a drug having complementary mode of action and non-overlapping toxicity is recommended in future
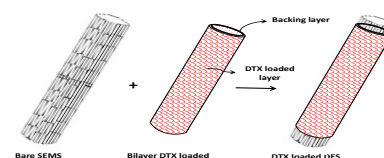
243

Enhancing topical delivery of glutathione by utilizing chemical modification and niosomal delivery systems

Murad A Gailani[1], Naibo Yin[1,2], Margaret A Brimble[2], Paul WR Harris[2], Shuo Chen[1], Jingyuan Wen[1.] School of Pharmacy, UoA[1], Auckland, School of Chemical Sciences, UoA[2], Auckland.

Introduction. Glutathione, the mother of antioxidants is able to protect the skin from oxidative damage. The challenge with topical delivery of glutathione is its poor stability and permeability across biological membranes. In this study, pro-drugs of glutathione and their niosomal delivery systems have been developed to overcome these limitations.

Aims. To develop a promising niosomal delivery system for glutathione and its prodrugs as well as evaluating their antioxidation effects, drug permeation and release characteristics.

Methods. Glutathione and several pro-drugs have been incorporated into niosomes, utilising an optimised thin-film hydration method. 24 h *In vitro* release and skin permeation studies have been performed using the Franz diffusion cell apparatus. Cytotoxicity of glutathione and pro-drugs were evaluated utilising a Thiazolyl blue formazan (MTT) assay and the antioxidation effect was determined after UV irradiation.

Results. Development of a novel niosomal delivery system with an entrapment efficiency of 90.7%±0.6 ($P<0.05$) achieving a higher topical antioxidation effects when compared to the parent drug. Glutathione niosome and prodrugs significantly enhance cell viability when compared to glutathione alone; showing up to a 31% improvement during an UV irradiated fibroblast study. Controlled release properties were also observed for niosomal encapsulated glutathione.

Discussion. Both niosomal delivery systems and glutathione prodrugs showed improved antioxidation activity and cell viability than that of glutathione alone. This is because, the drug was able to enter fibroblast cells, reducing oxidative stress. This niosomal controlled release system is ideal for including glutathione and its prodrugs into sunscreen or cosmetics.
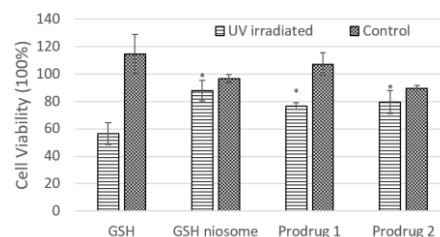
Gul M et al (2000) Indian J Exp Biol, 38:625-634

244

Characterization of Gloup: Is it suitable for medication delivery in dysphagic patients?

Lucia Crino, Yady J Manrique-Torres, Julie AY Cichero, Kathryn J Steadman. School of Pharmacy, The University of Queensland, Brisbane, QLD.

Introduction. A medication lubricant, Gloup, designed to facilitate the swallowing of solid oral dosage forms, has recently been introduced into the Australian market. Gloup is intended to help those who have a psychological aversion to swallowing whole tablets and capsules. Dysphagic patients have a physiological reason for swallowing difficulties, and the question arises as to whether Gloup would be safe and useful for this patient group.

Aims. To investigate the physicochemical properties of Gloup and evaluate its influence on drug release in oral medication delivery with respect to its potential use in patients with dysphagia.

Methods. Rheological characterisation of Gloup was performed using a Rheometer TA Instrument hybrid with peltier and parallel plates at 37°C. Flow curves using shear rates up to $10000\ s^{-1}$ and viscosity at $50\ s^{-1}$ were obtained. Viscoelasticity was assessed in the linear viscoelastic region with frequency sweeps between 1 to 100 Hz. The effect of Gloup on dissolution of paracetamol 500 mg tablets was assessed following standard USP and BP guidelines. Micro-release from crushed tablets in static conditions was also measured using vertical diffusion cell apparatus.

Results. The viscosity of Gloup at $50\ s^{-1}$ was 464 cP, as measured in a peak hold test, which is similar to yoghurt (459 cP). Rheological profile indicated that Gloup is a viscoelastic fluid, with storage modulus (G') values greater than loss modulus (G") and both G' and G" being constant. Paracetamol dissolution was unaffected by the presence of Gloup, in fact the tablet was observed to separate from the gel immediately on entry to the dissolution vessel.

Discussion. Gloup is consistent with Level 400 in the dysphagia-oriented product classification[1] and has no effect on drug dissolution, unlike some gum-based thickeners designed to ensure safe fluid delivery in dysphagia[2]. However, due to Gloup's slipperiness and propensity for the tablet to slide out of the gel, further work is required to determine whether it will safely transport medications without aspiration before Gloup is recommended for use in dysphagics.

[1]Jukes S, Cichero JAY, Haines T, Wilson C, Paul K, O'Rourke M. Int J Speech Lang Pathol. 2012; 14(3):214-225
[2]Manrique YJ, Lee DJ, Islam F, Nissen LM, Cichero JAY, Stokes RJ, Steadman KJ. J Pharm Pharm Sci. 2014; 17(2): 207-21

245

Solubility and stability improvement of Curcumin using solid dispersion approach

Ankit Parikh, Krishna Kathawala, Xin Fu Zhou, Sanjay Garg
School of Pharmacy and Medical Sciences, University of South Australia, SA

Introduction: In recent years, Curcumin has been reported to have significant therapeutic potential in cancer, inflammation, infections including HIV and HPV, and a range of neurological, cardiovascular, pulmonary, and psychological disorder. Poor physicochemical properties like limited water solubility (0.4 mcg/mL), susceptibility to degradation in alkaline pH and light, and dissolution behaviour limits the therapeutic applications of this natural compound.

Aims: To boost solubility, stability and dissolution of Curcumin by using Solid dispersion (SD) based strategy.

Methods: A number of pharmaceutical carriers were screened on the basis of enhancement in solubility and stability of curcumin at pH 1.2, 6.8 and 7.4 buffers, and light. The optimum drug to polymer ratio was finalised based on the kinetic solubility of the SD. The optimised SD was further evaluated for in-vitro dissolution study and characterised using Fourier Transform infrared spectroscopy, differential scanning calorimeter, X-ray diffraction, and scanning electron microscopy.

Results: From preliminary screening, Soluplus was identified as the best carrier for Curcumin, providing 1024 fold improvement of solubility and greater stabilizing effect in alkaline pH and light. SD also exhibited improvement in dissolution by displaying 100% drug release within 2 hours. These unique results may be due to amorphization and micellization.

Dissolution profile of Curcumin Drug and Solid Dispersion

Discussion: Soluplus based Solid dispersion system has great potential to mitigate limitations associated with Curcumin's physico-chemical characteristics and can pave the way for its clinical development.
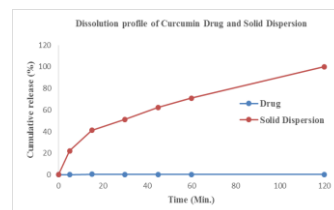
Reference: Seo S W et al (2012), Preparation and pharmacokinetic evaluation of curcumin solid dispersion using Solutol(R) HS15 as a carrier, International journal of pharmaceutics, 424: 18-25

246

A semi-physiologically based pharmacokinetic model and a time-to-event model to explore leflunomide disposition and cessation due to toxicity

Ashley M Hopkins[1,2], Michael D Wiese[2], Susanna M Proudman[3], Catherine E O'Doherty[2], Richard N Upton[1,2], David JR Foster[1,2]. Australian Centre for Pharmacometrics[1] and the Sansom Institute for Health Research[2], UniSA, Adelaide, SA; Department of Rheumatology, Royal Adelaide Hospital[3], Adelaide, SA.

Introduction: Leflunomide is used in rheumatoid arthritis treatment, yet approximately 40% of patients cease due to toxicity.

Aim: To develop a semi-physiologically based pharmacokinetic (semi-PBPK) population model describing free teriflunomide concentrations, which have not been investigated previously. Furthermore a time-to-event model describing leflunomide cessation due to toxicity was developed.

Methods: A semi-PBPK population model describing key physiological properties of leflunomide disposition was developed in NONMEM®. This model was used to predict steady-state teriflunomide concentrations. A time-to-event model was used to describe the time until leflunomide cessation and the influence of teriflunomide exposure and pharmacogenetic variants were assessed.

Results: Data from 105 patients was analyzed, with 34 ceasing due to toxicity. Teriflunomide concentrations were measured in 69 individuals. A 15 compartment model was able to predict teriflunomide concentrations; only fat free mass and liver function (ALT) improved concentration prediction. Within the time-to-event model, dropout hazard and random censoring hazard were best described by step functions. No statistically significant associations of cessation with predicted steady-state teriflunomide concentrations were identified. Carriers of the C allele of *CYP1A2* rs762551 had a 2.29 fold increase in cessation hazard compared to non-carriers (95% CI 2.24 - 2.34, p=0.016).

Discussion: A semi-PBPK model effectively evaluated the multiple covariates which may influence leflunomide pharmacokinetics. The C allele of *CYP1A2* rs762551 was once again linked to increased leflunomide toxicity, however no association with teriflunomide exposure was identified. Future research should continue to investigate exposure-toxicity relationships

247

Towards Point of Care Analysis of Ampicillin from Whole Blood

Rosanne M Guijt[1], ,Aliaa I Shallan[1,2] and Breadmore, Michael[2] School of Medicine and Australian Centre for Research on Separation Science (ACROSS), University of Tasmania[1], Hobart TAS 7001; ACROSS, School of Physical Sciences, University of Tasmania[2], Hobart TAS 7001

Introduction. Personalised dosage of medication is gaining interest as result of the move towards a more personalized approach in medicine.
Aims. The development of a novel approach enabling affordable point of care analysis
Methods. Two nanopore junctions were formed between microchannels by dielectric breakdown in poly(dimethyl) siloxane (PDMS) devices produced using soft lithography. Creating pores of decreasing diameter allows for the creation of a size mobility trap, enabling the simultaneous extraction and trapping of the target analyte from whole blood based on size and mobility (Fig 1).
Results. Fluorescently labeled ampicillin was detected directly from whole blood within 5 minutes. The signal increased linearly across therapeutically relevant levels (2.5 – 20 mg/mL).
Discussion. A simple device integrating diverse electrokinetic steps enables the extract and enrich analytes directly from blood followed by electrophoretic separation. Here, relatively small junctions were used to demonstrate the analysis of ampicillin from blood within 5 min, but the method could also be applied to DNA or proteins.
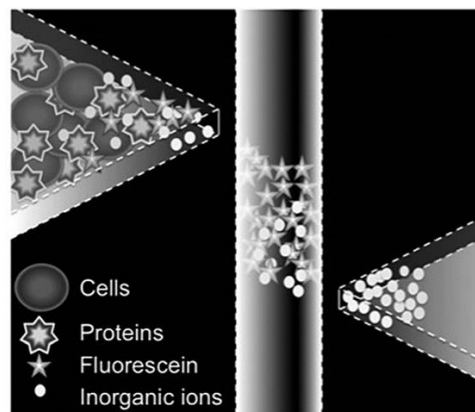
Fig. 1 *Schematic of Size Mobility Trap (SMT), with nanojunctions controlling electrophoretic transport between the V-shaped and central channels based on size and mobility*

Shallan, Guijt and Breadmore, Angewandte Chemie, 2015, DOI: 10.1002/anie.201501794

248

ASCEPT MICHAEL RAND MEDAL

300

Transporters in pharmacology and molecular target drug discovery

Yoshikatsu Kanai; Department of Bio-system Pharmacology, Osaka University Graduate School of Medicine, Osaka, JAPAN.

Transporters are membrane proteins mediating permeation of organic and inorganic solutes through the plasma membrane and membranes of intracellular organella. They play essential roles in the epithelial absorption and cellular uptake of nutrients, and the regulation of neurotransmitters and autacoids, as well as the absorption, distribution, metabolism and excretion of drugs. They have, thus, been regarded as important drug targets and controlling factors of pharmacokinetic profiles, thereby occupying an important position in pharmacodynamics and pharmacokinetics. Because transporters contribute to determining the distribution of compounds in the body in concert with metabolic/synthetic senzymes, the drugs that affect the functions of transporters are expected to alter the distribution of compounds in the body and to ameliorate disrupted homeostasis. In this context, drugs targeting transporters have been used clinically. Such drugs include antidepressants targeting monoamine transporters, diuretics targeting inorganic ion transporters of renal tubules, and uricosuric agents targeting renal urate transporters. Now, the genome-wide pictures of transporter families have been obtained, providing the extensive basis of comprehensive analysis of the transcriptomes and proteomes regarding transporters. Furthermore, the structural bases of molecular functions dependent on the crystal structures of transporter proteins and the roles of transporters in bio-homeostasis and diseases deduced from metabolome data have been revealed on each transporter. Based on such intensive knowledge currently accumulated on transporters, drug developments targeting identified transporter molecules have been attempted on some transporters. The newly developed anti-diabetic drug targeting a glucose transporter SGLT2 in renal proximal tubule is one of such successful examples. We are now focusing on cancer-type amino acid transporter LAT1 for cancer diagnosis and therapeutics. In the lecture, I will introduce our recent trials to develop cancer-specific PET probes targeting LAT1 for diagnosis and anti-tumor agents inhibiting LAT1 to suppress tumor growth for therapeutics. I will also present the possible application of newly found renal organic anion transporter to excreting keton bodies in diabetes. Finally, the impact of transporters on molecular target drug discovery will be discussed and summarized.

301

Morphine modulates breast cancer metastatic potential

Marie-Odile Parat[1]. School of Pharmacy, University of Queensland[1], Woolloongabba, QLD

Introduction. Appropriate pain management in the perioperative period may play a role in prevention of tumor recurrence and metastasis. Opioids are proven to be highly effective perioperative analgesics and are widely used in cancer surgery patients.

Aims. We are studying the effect of morphine on tumor growth and metastasis using a variety of *in vitro* and *in vivo* models.

Methods. The level of extra-cellular matrix (ECM) degrading enzymes is measured in cell-conditioned media using in-gel zymography. The expression of enzymes or markers is measured at mRNA level using RT-PCR.

Results. Administration of morphine caused a reduction in breast tumor growth and tumor cell dissemination to the lungs in a mouse model. Morphine treatment also caused a reduction in circulating proteolytic enzymes of extracellular matrix (ECM), matrix metalloproteinase-9 (MMP-9) and urokinase-like plasminogen activator (uPA). Morphine treatment of co-cultures of breast cancer cells with accessory cells found in tumour stroma also altered the overall proteolytic profile. Morphine affected co-cultures but not cells grown individually. This suggests that anti-tumor effects of morphine are mediated through modulation of paracrine communication between cancer cells and infiltrating cells. Because macrophages display a range of activation states in pathological contexts and alternatively activated (M2) macrophages can promote tumour aggressiveness, we tested whether morphine can modulate the activation of macrophages induced by interleukin-4, the prototypical M2 polarization-inducing cytokine, or coculture with 4T1 breast cancer cells to induce a tumour-associated macrophage phenotype *in vitro.* Our results indicate that morphine may modulate tumour aggressiveness by regulating macrophage protease production and M2 polarization.

Discussion. These results must be considered in the context of morphine modulating tumour growth and metastasis via multiple mechanisms, some of which are likely to promote, others likely to prevent, cancer aggressiveness. The results from our laboratory are in favour of a protective role for morphine, via modulation of cellular interactions in the tumour microenvironment.

302

Inflammation resolution mediators in breast cancer

Alastair G Stewart[1], Yan Tu[1], Nuha Al-Zaubai[1], Ebony Fietz[1], Cameron Johnston[2]
Dept Pharmacology and Therapeutics, University of Melbourne[1], Parkville, VIC; Peter MacCallum Cancer Centre[2], East Melbourne, VIC.

Virchow viewed inflammation as a key feature of solid tumours. Dvorak regarded "the tumour as a wound that would not heal" (as reviewed by: Srikrishna and Freeze, 2009). Inflammation-resolution is an active process set in train by inflammation: "the beginning programmes the end" (Serhan and Savill, 2005). We set out to explore the persistent inflammation in the tumour microenvironment as a persistent trigger to the inflammation-resolution process. This interest triggered studies on inflammation-resolving mediators, including Lipoxin A_4 (LXA_4), Resolvin D_2 (RvD_2) and Annexin A1, in the breast tumour setting. LXA_4 and RvD_2 promote proliferation of estrogen receptor positive MCF7 breast tumour cells via an indirect action through the ER pathway Al-Zaubai et al (2014). These pro-tumour effects may be offset by RvD_2 suppression of epithelial mesenchymal transition (EMT) responses. The roles of annexin A1 were examined using murine tumour cell lines in wild-type and annexin A1 deficient mice, and in immunodeficient annexin A1+/+ mice using human cell lines that are either annexin A1 intact or depleted by stable expression of siRNA. In a murine breast tumour model using polyoma middle T tumour derived cell line in which annexin A1 knockdown was achieved by stable transfection of two annexin A1-targetting siRNA sequences, the appearance of tumours was delayed by at least 4 months compared with lines bearing a non-silencing siRNA construct. These findings are consistent with the suggestion that annexin A1 and its peptide have net pro-tumour activity (Khau et al., 2011), as further supported by a large study demonstrating that annexin A1 positive tumours were associated with lower breast cancer survival rates (Sobral-Leite et al., 2015).

Al-Zaubai et al (2014). JPET 351:172-80.
Khau et al (2011) FASEB J 56:23-33
Serhan and Savill (2005) Nat Immunol 6:1191-1197.
Srikrishna and Freeze (2009). Neoplasia 11:615-628.
Sobral-Leite et al (2015) BMC Med 13:156

303

UDP-glucuronosyltransferases (ugts) and steroid hormone regulation in breast cancer

Robyn Meech, Dhilushi D Wijayakumara, Apichaya Chanawong, Nurul Mubarokah, Dong Gui Hu, Peter I Mackenzie, and Ross A McKinnon, Department of Clinical Pharmacology and Flinders Centre for Innovation in Cancer, Flinders University School of Medicine, Flinders Medical Centre, Bedford Park SA

The superfamily of human UDP-glycosyltransferases includes 19 enzymes that conjugate sugars to small lipophilic chemicals such as drugs, toxins, dietary compounds, hormones/signalling molecules, and byproducts of metabolism. Differential UGT activities are relevant to interindividual differences in drug metabolism, as well as drug-drug interactions, and cancer risk. We have focused on defining transcriptional and posttranscriptional mechanisms that regulate UGT gene expression in various contexts including steroid responsive tissues. This presentation will cover our recent work on the regulation of various UGT family members, including regulation of UGTs by steroid hormones and cancer anti-cancer drugs that are also UGT-substrates, thus generating regulatory loops through which these ligands can induce their own metabolism. We will also present work on post transcriptional regulation of UGT mRNAs. The clinical significance of these studies includes the control of local and systemic levels of cancer promoting steroid hormones and anti-cancer therapeutics.

304

Development of a novel class of ω-3-fatty acid epoxide analogues with in vivo activity against breast cancer

Michael Murray[1], Herry Dyari[1], Kirsi Bourget[1], Hassan Choucair[1], Julie Dwyer[1], Sarah Allison[1], Yongjuan Chen[2], Colin R. Dunstan[2], Tristan Rawling[3]. Discipline of Pharmacology[1] and Department of Biomedical Engineering,[2] University of Sydney, Sydney, NSW; Graduate School of Health, University of Technology Sydney, NSW[3]

Introduction. ω-3 Polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) decrease tumorigenesis, whereas ω-6 PUFA enhance tumor growth. In cells, cytochromes P450 (CYPs) oxidize ω-3 and ω-6 PUFA to generate isomeric epoxides. Epoxides of the ω-6 PUFA arachidonic acid stimulate tumor proliferation and survival. In contrast, the epoxide formed by oxidation of the unique ω-3 bond in EPA (ω-3-epoxy-EPA) decreased proliferation and activated apoptosis (Cui et al., 2012).

Results. Saturated C20-C22 fatty acid ω-3 epoxide analogues of ω-3-epoxy-EPA (C20-C22-epoxides) decreased ATP production and cell cycle progression, and increased caspase-3 activity and annexin V-staining (Dyari et al., 2014); the C20-epoxide was more effective than ω-3-epoxy-EPA. The apoptotic mechanism of the C20-epoxide involved death receptor and downstream Jun-N-terminal kinase signalling, Bid cleavage and mitochondrial disruption. We prepared urea isosteres as metabolically stable C20-epoxide analogues. Simple ω-alkyl- and monosubstituted ω-aryl-ureas were inactive but further structural modification produced the analogue CTU that markedly impaired breast cancer cell viability. From JC-1 staining the mitochondrial membrane potential was impaired in cells within 5 min of CTU addition. Further, the growth of MDA-MB-231 xenografts in nude mice was strongly decreased after CTU administration. Increased TUNEL staining in excised tumours indicated CTU-mediated apoptosis in vivo, while decreased Ki-67 staining indicated decreased tumour proliferation.

Discussion. CTU is the first member of a novel class of potential antitumour agents based on ω--epoxy-EPA and the C20-epoxide. CTU rapidly targets the mitochondrion in tumour cells and impairs the membrane potential, which decreases viability and activates cell death pathways in vitro and in vivo.

Cui PH et al (2012) Br J Pharmacol 162:1143-1155.
Dyari HRE et al (2014) J Med Chem 57: 7459-7464.

305

The burden of adverse drug events in older people

Simon Bell, Monash University, Melbourne, VIC

Medicine-related problems are considered a leading cause of morbidity and mortality in older people. There are an estimated 230,000 medicine-related hospitalisations each year in Australia. However, as with other complications of clinical care, the overwhelming majority of adverse drug events (ADEs) may go unrecognised and unreported by consumers and clinicians. Only five of over 4,000 deaths by external causes in residential aged care reported to the Victorian Coroners Court from 2000-2012 were classified as medicine errors. There is a growing body of evidence to suggest that important geriatric syndromes such as frailty, delirium, falls, and incontinence may be medicine-related. However, because these geriatric syndromes typically have a multifactorial aetiology the contribution of medicines to these syndromes may go unrecognised or under-appreciated. Medicines defined as potentially inappropriate for older people using popular explicit criteria (e.g. Beers Criteria) account for a small percentage of ADEs causing hospitalisations after emergency department visits. Conversely, research suggests four commonly prescribed medicines (warfarin, insulin, oral antiplatelets and oral hypoglycaemics) account for more than two-thirds of hospitalisations in the United States. Deintensifying medicine regimens and redefining treatment targets for older people at high risk of ADEs may help address the high burden of ADEs.

306

The misdiagnosis of adverse drug reactions

Matthew P Doogue[1,2]. Department of Medicine, University of Otago[1], Christchurch, NZ; Department of Clinical Pharmacology, Canterbury District Health Board, Christchurch, NZ.

Introduction. The diagnosis of an adverse drug reaction (ADR) is a relevant to clinical care for the life of the patient. An ADR not recorded can lead to potentially avoidable harm, while an ADR incorrectly recorded can lead to withholding potentially beneficial drug treatment. Health systems are moving rapidly from multiple institutionally based health records to single shared electronic health records. Accurately diagnosed ADRs are a core element of a shared patient health record.

Aims. To describe the ADR process and the elements of a shared electronic health record necessarily for a functioning ADR system. To describe clinical and technological barriers to a single national ADR record.

Methods. Data from local and published studies were used to describe the state of ADR records in hospitalised patients. A clinical process map was developed and tested. ADRs descriptions were obtained retrospectively from clinical coding data. The accuracy of the ADR records of a cohort of hospitalised patients was studied prospectively.

Results. The prevalence of previous ADRs in hospitalised patients is about 50%. In Canterbury there were 20,826 new ADRs coded in 4 years, approximately 23 per 100 hospital admissions. The majority of ADRs are due to expected drug effects compromising patient function (e.g. hypotension, delirium). ADRs were recorded on current medication charts less than 50% of the time. Of the patients with a previously reported ADR 7% were prescribed a drug with a known ADR during the study admission. About 15% of documented ADRs were not verifiable from clinical records or patient interview. ADR standards are poorly defined and software vendors are inconsistent with terminology and design

Discussion. Diagnosing ADRs is difficult, poorly done and under resourced. Clinical guidelines and processes to support ADR diagnoses are weak and in urgent need of standardisation. Technical standards to document ADRs are immature and constrained by the lack of agreed clinical standards. The use case for ADR alerts is usually focused on unexpected extreme events, whereas ADR diagnoses usually fall on the spectrum of expected potential drug effects. ADRs alerts are better recorded at the time of the event than retrospectively. To inform prescribing ADR alerts should be linked to information from the index event.

307

Adverse drug events: the cost-effectiveness of moving from paper to electronic systems

Johanna I Westbrook Centre for Health Systems and Safety Research, Macquarie University, Sydney, NSW

Introduction. Electronic medication management systems (eMM) are being adopted by hospitals around the world as a strategy to improve the safety, quality and efficiency of medication management. Such systems provide the foundation for delivering benefits beyond hospitals with electronic medication information able to follow patients as they move between health sectors and providers. As such these systems are expected to deliver substantial returns on investments in terms of more effective medicine management and reduced adverse drug events both in hospital and in the community. Internationally evidence of the effectiveness of eMM to deliver benefits has been largely generated from US hospitals, but with very limited data on cost-effectiveness.

Aims. To measure the cost-effectiveness of an eMM in an Australian teaching hospital

Methods. We compared costs and benefits of paper-based prescribing with a commercial e-MM (CSC MedChart) on one cardiology ward in a major 326-bed teaching hospital.

Results. The rate of potential ADEs following e-MMS fell from 0.17 per admission to 0.05; a reduction of 71%. The annualised e-MM implementation, maintenance and operating costs for the cardiology ward were A$61,741. The estimated reduction in ADEs post e-MMS was approximately 80 actual ADEs per year. The reduced costs associated with these ADEs were more than sufficient to offset the costs of the e-MMS.

Discussion. e-MM within this setting was more effective and less expensive than paper-based prescribingthe heading and without additional line spacing.

Westbrook JI, Gospodarevskaya E, Li L, Richardson R, Roffe D; Heywood M, Day RO, Graves N (2015) Journal of the American Medical Informatics Association, 22(4): 784-793.

308

eSystems decision support, a panacea for ADRs?

Dr Sepehr Shakib, Department of Clinical Pharmacology, Royal Adelaide Hospital, University of Adelaide

Electronic health records (eHR) are increasingly being introduced into clinical practice, to improve legibility of documentation, improve access to health records, but also to improve medication safety. Whilst general practitioners have been using electronic records and electronic prescribing for many years, they are increasingly becoming common in specialist private practice, as well as in public and private hospitals. One of the important benefits of electronic medication management (eMM) is the decision support provided for drug allergy, drug-drug interaction, as well as drug-disease, drugs in pregnancy/lactation alerting. Whilst these tools are seen by many as essential safety features which can provide a panacea for ADRs, clinicians express dissatisfaction with over alerting, which may potentially make the system less safe. Data will be demonstrated from SA Health's eHR program for allergy and drug drug interaction decision support. Summary: outcomes from any eHR system are dependent on a combination of the users, the system, the user interface, as well as the governance and culture within the organization. Hence, improving ADRs requires a multifaceted implementation project that includes eMM, rather than one that simply relies on it

309

Balancing safety and quality of life. Listening to what consumers want.

Lyntara S Quirke, Consumer Representative, Consumer Dementia Research Network and the Cognitive Decline Partnership Centre, Alzheimer's Australia, Canberra, ACT.

Introduction. In 2010, Alzheimer's Australia established a national Consumer Dementia Research Network (CDRN) comprising both individuals who are living with dementia and family carers of people with dementia. Members of the CDRN are actively involved in research, not as 'subjects' but as active participants in the research process.

Aims. The aim of the CDRN is to ensure that research has relevant and meaningful outcomes for consumers.

Methods. Members of the CDRN are actively involved in prioritising areas for research, reviewing research proposals and ensuring research projects meet established criteria. Research projects funded through Alzheimer's Australia and the Cognitive Decline Partnership Centre have ensured all projects have consumer representation and participation.

Results. Researchers are valuing consumer expertise and including consumers as equal members of the research team.

Discussion. As the ageing population in Australia reaches unprecedented numbers, consumers are increasingly concerned with the end-point of research. Are we prioritising what really matters to consumers and are we really improving the quality of life for older people? This presentation will consider what is important as people age, and demonstrate how researchers can work collaboratively with consumers to ensure research is meaningful and measurable and making a difference to the quality of life of consumers. Tara will use examples of where the CDRN has contributed to medication research projects and the influences these projects have had on knowledge translation.

310

Genetic screening for people at risk of adverse drug events: costs vs benefits from the individual and societal perspective

Danny Liew[1,2]. Monash University, Melbourne, VIC[1]; University of Melbourne[2], Melbourne, VIC.

Introduction. In the era of precision medicine, there is growing interest in genetic screening to identify patients at high risk of adverse events and/or most likely to benefit from a particular therapy. This includes pharmacogenetic screening to inform drug therapy. While the benefits of genetic screening are intuitive, costs also need to be considered, as well as harms that may arise.

Aims. To discuss the issues relevant to genetic screening from a health economic perspective.

Methods and Results. A brief overview of the principles of health economics will be provided, followed by a discussion of key considerations in the economic evaluation of genetic screening to inform 'more precise' therapy. An example will be provided of pharmacogenetic screening for HLA-B*15:02 among patients at risk of carbamazepine-induced severe skin reactions.

Discussion. Genetic screening offers opportunities to deliver on the promises of precision medicine, but there are potential limitations related to significant costs, potential inefficiencies and unintended harm. These need careful consideration before genetic screening is recommended in policy and practice.

311

Non-experimental evaluation of medical products: Challenges and established approaches

M. Alan Brookhart, Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, USA.

Large existing healthcare databases are increasingly being used to evaluate the safety and effectiveness of medical products as they are used in routine care. Such studies can provide valuable information about real world drug effects in patient populations that are often excluded from RCTs, such as the elderly, children, pregnant women, or patients with significant polypharmacy or comorbid disease. Yet conduct of such studies can be challenging and inappropriate design and analysis can lead to results with substantial bias. We discuss some common sources of bias in non-experimental studies, with a focus on confounding. We describe how many of sources of bias can be mitigated by a new user design (or more generally a treatment decision design) and appropriate analysis based on propensity scores or multivariable outcome models.

312

Register-based pharmacoepidemiology – Insights from research using the Nordic prescription databases

Jenni Ilomäki, Monash University, Melbourne, VIC.

Prescription claims databases have become an increasingly important data source in pharmacoepidemiology. Most pharmacoepidemiological studies published in high impact journals now utilise prescription claims data. Research conducted using these databases complement evidence from randomised controlled trials (RCTs), particularly in relation to adverse drug events among populations often excluded from RCTs. The Nordic countries have a long history of register-based pharmacoepidemiology. Nordic prescription claims databases are a source of patient-level data for the 25 million inhabitants of Denmark, Finland, Iceland, Norway, and Sweden. By using each resident's unique identifier, data from prescription databases can be linked to data in other government and non-government registries. These include the hospital discharge register, clinical quality registries, and large prospective cohort studies. A literature review published in 2013 identified that there were 515 studies that used the Nordic prescription claims databases published between 2005 and 2010. A key advantage of Nordic prescription databases is that they provide sufficient statistical power to permit investigation of rare outcomes. A disadvantage is that unless the databases are linked to other data sources they lack many important clinical and demographic details of individual patients.

313

A small-molecule formyl peptide receptor (FPR) agonist limits myocardial reperfusion injury in vivo: Unmasking ligand-biased agonism as a novel cardioprotective mechanism

Cheng Xue Qin[1,2,], Lauren T. May[3,], Renming Li[1,2], Nga Cao[1], Sarah Rosli[1], Minh Deo[1], Amy E. Alexander[1], Jane E. Bourke[4], Yuan H. Yang[5], Xiao-Jun Du[1], Patrick M. Sexton[3], Arthur Christopoulos[3], Xiao-Ming Gao[1], and Rebecca H. Ritchie[1,2,6]. [1]Baker IDI Heart & Diabetes Institute; [2]Dept of Pharmacology, University of Melbourne, [3]Drug Discovery Biology & Dept of Pharmacology, Monash University, Parkville; [4]Dept of Pharmacology, Monash University, [5]Centre of Inflammatory Diseases, Monash University & Monash Medical Centre, Clayton, [6]Dept of Medicine, Monash University, Vic.

Introduction. FPRs are integral to inflammation regulation and are thus attractive therapeutic targets for myocardial ischemia-reperfusion (I-R) injury. Dual FPR1/FPR2 agonists potentially offer FPR1-mediated cardiomyocyte preservation together with FPR2 inflammation-limiting actions.

Aims. To investigate the cardioprotective potential of two small-molecule FPR agonists on myocardial I-R injury *in vivo* and their FPR1/FPR2 signalling fingerprints *in vitro.*

Methods. Mice subjected to coronary artery occlusion were administered the pyridazin-3-(2H)-one compound-17b (Cmpd17b), Amgen compound-43 (Cmpd43), or vehicle commencing just prior to reperfusion, to assess their cardioprotective action. FPR signalling pathways were investigated *in vitro* in CHO cells stably transfected with human FPR1 or FPR2.

Results. Significant cardioprotective effects of Cmpd17b (but not Cmpd43) were evident on cardiac necrosis (infarct size and plasma levels of cardiac troponin I after 24h), circulating leukocytes and neutrophil infiltration (after 48h) and adverse cardiac remodelling (after 7-days reperfusion); Cmpd17b similarly exhibited superior cardioprotection in isolated cardiomyocytes and cardiac fibroblasts *in vitro.* Both agonists elicited concentration-dependent activation of multiple intracellular signaling pathways, including Ca^{2+} mobilization and phosphorylation of ERK1/2, Akt1/2/3(Thr308) and Akt1/2/3(Ser474). Statistical evaluation of the signal transduction established that, relative to Cmpd43, Cmpd17b exhibited a significant 30-fold bias away from intracellular Ca^{2+} mobilization.

Discussion. These findings reveal ligand-selective cardioprotection with the dual FPR1/FPR2 agonist Cmpd17b both *in vitro* and *in vivo,* with significant limitation of cardiac necrosis, inflammation and remodelling up to 7-days post-I-R. The biased signalling profile of Cmpd17b is a possible mechanism for its superior cardioprotection, providing a new approach for development of small-molecule FPR pharmacotherapies for myocardial infarction.

314

Adrenoceptors promote glucose uptake into adipocytes and muscle by an insulin-independent signalling pathway involving mTORC2

Masaaki Sato[1,2], Jessica Olsen[2], Anna Sandstrom[2], Linzi Lim[1], Saori Mukaida[1], Dana Hutchinson[1], Bronwyn Evans[1], Tore Bengtsson[2] and Roger J. Summers[1]. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University[1], Parkville, VIC; Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University[2], Stockholm, Sweden.

Introduction. Stimulation of adrenoceptors (ARs) causes glucose uptake in brown adipocytes and skeletal muscle by a mechanism involving glucose transporter (GLUT) translocation to the plasma membrane (Sato et al., 2014; Olsen et al., 2014). However the detailed signalling pathways downstream of AR activation that link to GLUT translocation still remain to be identified.

Aims. To evaluate the signalling pathways utilised by α_{1A}-, β_2-, β_3-AR to promote glucose uptake.

Methods. Signalling pathways mediating glucose uptake were investigated using selective kinase inhibitors, siRNAs and specific assays for signalling proteins and measured by 2-deoxy-D-[^{3}H] glucose uptake, western blots, α-screen assays and confocal microscopy.

Results. α_{1A}-, β_2-, and β_3-AR agonists concentration-dependently increased glucose uptake in cardiac myocytes, skeletal muscle, and brown adipocytes respectively. Unlike insulin, AR-mediated glucose uptake was not affected by Akt inhibition. The mTOR inhibitor, KU0063794, and mTORC2 component rictor siRNA significantly inhibited AR-mediated glucose uptake. Western blots and α-screen assays showed that AR agonists had no effect on Akt phosphorylation but increased mTOR phosphorylation at Ser2481. Confocal analysis showed increased GLUT translocation following AR stimulation.

Discussion. ARs activate mTORC2 that has a key role in glucose uptake mediated by translocation of glucose transporters to the plasma membrane and involving actin reorganization.

Sato et al (2014) Diabetes. 63(12):4115-29
Olsen et al (2014) J. Cell Biol. 207(3):365-74

315

Understanding biased signalling and allosteric modulation at the glucagon-like peptide-1 receptor

Denise Wootten, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC

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 four variants of GLP-1 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 novel ligands, including small molecules that can be administered orally and drugs that can overcome side effect profiles associated with the current therapeutics.

The GLP-1 receptor is a class B GPCR and, like most GPCRs, is pleiotropically coupled with the physiological impact of receptor activation dependent on the spectrum of signalling and regulatory events initiated by ligand binding. Understanding how this receptor binds both natural and synthetic ligands, and the impact of binding on downstream signalling is crucial to development of better therapeutics. We are beginning to unravel how peptides agonists and small molecule ligands interact with the receptor and the complexity of signalling and regulatory events that are engaged by interactions with individual ligands. We have identified evidence of peptide and small molecule engendered signal bias, 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. The recent solution of the transmembrane structures of two class B GPCRs are enabling us to start to uncover molecular mechanisms linked to these behaviours of individual ligands, and how engagement of ligands with distinct regions of the receptor can control signalling to different pathways, in a ligand-specific manner. Collectively, this work is starting to provide novel insights into GLP-1 receptor structure and function and how this might be exploited for the development of novel therapeutics.

316

Essential roles of the store-operated calcium channel ORAI1 in the mammary gland

Felicity M Davis[1],*. [1]Signal Transduction Laboratory, National Institute of Environmental Health Sciences, NC, USA. *Present address: Department of Pathology, The University of Cambridge, Cambridge, UK.

The recently identified store-operated calcium channel pore-forming subunit ORAI1 is a pharmacological target for the treatment of autoimmune and inflammatory diseases, and for specific cancers, including melanoma and breast cancer. ORAI1 is widely expressed in human and mouse tissues; however, the precise roles of these channels in the normal development and function of many organs, including the mammary gland, is not fully understood. During pregnancy, the mammary gland undergoes massive epithelial expansion to transform a relatively simple ductal epithelium into a functional, milk-producing gland that is densely populated by lobuloalveolar structures. Milk production by alveolar secretory cells involves a complex interplay of substrate-specific transport pathways. The transport of calcium ions (Ca^{2+}) into milk poses a significant biological challenge to these cells, which move huge quantities of Ca^{2+} across their cytoplasm for milk-Ca^{2+} enrichment, whilst evading cell death pathways associated with excess cytosolic Ca^{2+} accumulation. Using genetically-modified mouse models, we demonstrate that the store-operated channel ORAI1 delivers 50% of the Ca^{2+} present in milk. We also uncovered an unanticipated signaling role for these channels in oxytocin-mediated milk ejection. We reveal that activation of the oxytocin receptor produces slow, asynchronous Ca^{2+} oscillations in contractile mammary myoepithelial cells, which are mediated exclusively by ORAI1. To characterize the importance of ORAI1-dependent Ca^{2+} oscillations in milk expulsion, we developed a novel, 4D confocal imaging strategy to visualize live alveolar unit contractions in the intact *ex vivo* mammary gland. Using this technique, we demonstrate that milk is ejected by way of ORAI1-dependent, pulsatile contractions of mammary alveoli, an observation that redefines the process of milk ejection. These findings reveal that Ca^{2+} is not just a substrate for nutritional enrichment in mammals, but is also a master regulator of the spatiotemporal signaling events underpinning mammary alveolar unit contraction during lactation. ORAI1-dependent Ca^{2+} oscillations may represent a conserved language in myoepithelial cells of other secretory epithelia, e.g., sweat and salivary glands, potentially shedding light on other ORAI1 channelopathies and pharmacological targeting strategies for other glandular disorders.

317

Calcium sensing receptor dysregulation in induced pluripotent stem cell derived podocytes from patients with Alport Syndrome

John M Haynes[1], Wai-Ling Lieuw[1], Sheetal Saini[2], Sharon D Ricardo[2]. Monash Institute of Pharmaceutical Sciences, Monash University [1], Parkville, VIC; Faculty of Medicine, Nursing and Health Sciences, Monash University[2], Clayton, VIC.

Introduction. Alport syndrome (AS) is a genetic disorder where mutations in the genes coding for collagens α3, α4 or α5 lead to changes in composition of glomerular basement membrane, podocyte death and kidney failure. The calcium sensing receptor (CaSR) is a Family C G-protein coupled receptor present on podocytes, where it is likely to play roles in podocyte homeostasis, slit diaphragm maintenance and cell viability.

Aims. We compare CaSR signalling in induced pluripotent stem cell derived podocytes from patients with AS and from healthy individuals (NHMC).

Methods. Podocytes were differentiated from human iPSCs and loaded with Fura-2AM (10 μM, 30min) prior to calcium imaging.

Results. In calcium free media NHMC podocytes responded to extracellular calcium ($[Ca^{2+}]_o$) with an acute dip in intracellular calcium ($[Ca^{2+}]_i$) followed by a sustained elevation in $[Ca^{2+}]_i$. The acute dip in $[Ca^{2+}]_i$ could be blocked by the IP$_3$ receptor inhibitor 2-aminoethoxy diphenyl borate (2APB, 2μM) and by the large conductance calcium activated potassium channel (BKCa) blocker, iberiotoxin (100nM). In contrast, podocytes derived from patients with AS only responded to $[Ca^{2+}]_o$ with elevations of $[Ca^{2+}]_i$. In addition, the BKCa opener NS1619 reduced intracellular calcium in NHMC, but not in AS podocytes. Resting calcium in AS and NHMC podocytes was ~94 nM and ~84nM, respectively.

Discussion. These data indicate that AS podocytes show a profound reduction in BKCa activity that may correlate with the clinical signs of disease.
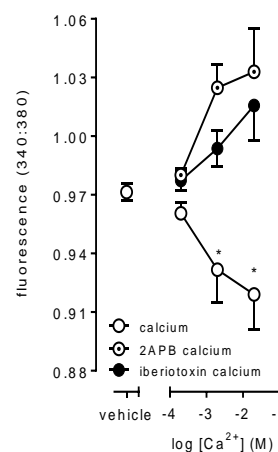
318

The 5-HT$_3$ receptor C subunit modulates receptor function

Nor S Yaakob, Ray L Fam, Dan-Thanh Nguyen, Cameron Nowell, Betty Exintaris, Helen R Irving. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC.

Introduction. 5-HT$_3$ receptors are ligand-gated cation channels present in both central and peripheral nervous systems. Five different subunits of the human 5-HT$_3$ receptor exist and these each contain polymorphisms that have been associated with clinical conditions. 5-HT$_3$ receptor antagonists are used to treat various conditions such as diarrhea predominant-irritable bowel syndrome (IBS-D), chemotherapy induced nausea and vomiting (CINV) and depression. Both the polymorphisms and the receptor subunit arrangement may contribute to differences in efficacy observed with the 5-HT$_3$ receptor antagonists (Yaakob et al. 2011).
Aims. To characterise the effect of the C subunit on 5-HT$_3$ receptor function.
Methods. 5-HT$_3$ receptor subunit expression in human intestinal tissue was determined by qPCR. HEK293T cells were transiently transfected with constructs of tagged 5-HT3 receptor subunits and used for whole cell patch clamp recording and visualization of receptor location to compare heteromers containing containing the C and A subunits with homomers containing only the A subunit.
Results. Expression of the C subunit was similar to the A subunit throughout the human colon and ileum. The 5-HT$_3$ receptor C subunits contributed subtle changes in the electrophysiological responses to 5-HT. The common single nucleotide polymorphism (N163K) in the C subunit significantly altered the electrical properties of the AC heteromer. The profiles of responses to the 5-HT$_3$ receptor antagonists, palonsetron and ondansetron, varied where for instance odansetron exhibited reduced efficacy on both wild-type and N163K AC heteromer.
Discussion. Patch-clamp experiments indicate that the presence of C subunits in heteromeric receptors alter the efficacies of clinically used antagonists, which could contribute to the inadequate responses observed in up to 40% of patients treated with these antagonists for CINV and IBS-D.

Yaakob N et al. (2011) Current Molecular Medicine 11:57-68

319

The involvement of pannexin-1 channels and purinergic P2X7 receptors in cytokine-induced colitis in human colonic mucosa

Erica F Diezmos[1], Shaun L Sandow [1,2], Paul P Bertrand[1,3], Lu Liu[1]. Dept of Pharmacology[1], School of Medical Sciences, UNSW Australia, Sydney, NSW; Faculty of Science[2], Health, Education and Engineering, University of the Sunshine Coast, Maroochydore DC, QLD; School of Medical Sciences[3], RMIT University, Bundoora, VIC.

Introduction. Pannexin-1 (Panx1) is a membrane spanning protein channel which, in conjunction with the purinergic P2X7 receptor (P2X7R), is involved in inflammation and apoptosis (Locovei et al, 2007). The Panx1-P2X7R complex may play a role in inflammatory bowel disease, where the aetiology is not well defined.
Aims. In this study, we used a colitis model (Harvey et al 2013) to observe whether block of Panx1 channels or P2X7R will prevent cytokine-induced inflammation in the mucosal layer of the human colon.
Methods. Human colon mucosal explants (4 × 10 mm) were incubated in 3 ml RPMI 1640 media containing 1% foetal calf serum for 16h. Cytokines TNFα and IL-1β (both 10 ng/ml) were used to induce colitis. The involvement of Panx1 channels and P2X7R in colonic inflammation was investigated in cytokine-treated tissue explants in the presence of putative Panx1 blocker [10]Panx1 (100 μM), and P2X7R antagonist A438079 (100 μM). One-way ANOVA with multiple comparisons was used for statistical analysis.
Results. Histological analysis of mucosal structures showed that cytokine incubation induced crypt and epithelial damage. The crypt damage between control and cytokine-treated group was statistically significant (n = 4; **P=0.0098). Luminal epithelial damage appeared to increase in the cytokine-treated group compared to control, however, this was not statistically significant (n = 4). The drug-treated groups showed a reduction in mucosal damage, particularly in the presence of A438079, where the reduction in crypt damage was statistically significant (n = 4; *P=0.0232). No differences in lymphocyte infiltration were observed between groups.
Discussion. The present study showed a clear reduction of mucosal damage induced by cytokines in the A438079-treated groups, suggesting that inflammation in colitis may involve P2X7R activation.

Locovei S (2007) FEBS letters 581(3):483-488
Harvey B (2013) Cytokine 63(2):209-217

320

Distinct Effects of Zolpidem and Dual Orexin Receptor Antagonists in Rats Sleep-Wake Profile and EEG Power Spectrum.

Laura H. Jacobson[1,2], Ludovic Perrot[1], Markus Fendt[1], Dirk Behnke[1], Simona Cotesta[1], Grit Laue[1], Silvio Ofner[1], Eric Legangneux[1], Samuel Hintermann[1], Claudia Betschart[1], Christine E. Gee[1], Daniel Hoyer[1,2,3]
[1]Novartis Institutes for Biomedical Research, CH 4002 Basel Switzerland, [2]Florey Institute of Neuroscience and Mental Health, [3]University of Melbourne, VIC 3010 Australia

Introduction. The orexin (OX) system plays a prominent role in sleep-wake regulation. Dual OX_1R/OX_2R antagonists (DORAs) are validated in the treatment of insomnia. The advantages of DORAs over $GABA_A$ receptor hypnotics (benzodiazepines, Z drugs), may include lesser side effects, e.g. tolerance, withdrawal, interactions with ethanol and addictive potential. Yet, the detailed effects of DORAs on sleep architecture remain to be investigated.

Aims. To compare the effects of the four clinically effective DORAs: SB-649868 (50 mg/kg), almorexant (50, 150 mg/kg), MK-6096 (filorexant; 50,100 mg/kg) and MK-4305 (suvorexant; 30 mg/kg) with those of zolpidem (10 mg/kg), on sleep-wake profile and EEG power spectrum in rats.

Methods. Male Sprague-Dawley rats were implanted with 4 electrocorticogram electrodes. Pizoelectric detectors were used to monitor activity and define vigilance states. EEG signals were acquired using Harmonie and analysed with Somnologica. Relative spectral power was analysed in REM, selected wake and NREM epochs.

Results. All drugs reduced wake: the four DORAs had similar effects on sleep-wake, increasing total sleep and REM, whereas Zolpidem reduced REM. SB-649868, almorexant (150 mg/kg), filorexant (50 mg/kg) and zolpidem increased NREM, while filorexant (100 mg/kg) and suvorexant had no effect on NREM despite potent REM-enhancing effects. Wake and NREM spectral ratios were unaffected by DORAs, in contrast to zolpidem which increased ß / γ / 5Hz power, but decreased theta power during wake. During NREM, zolpidem enhanced delta, but reduced theta power and higher frequencies. Filorexant and Suvorexant both enhanced theta power within REM.

Discussion. In summary, the DORAs investigated increased sleep in rats by preferentially or exclusively enhancing REM sleep similar to their effects in insomnia patients, suggestive of a class action. Further studies are needed to investigate the effects of DORAs on enhanced REM sleep theta power and its consequences, especially in insomnia patients, to assess translation from rodent to human sleep.

321

Does chronic morphine treatment alter endogenous opioid function in the amygdala?

Gabrielle C Gregoriou[1], Bryony L Winters[1], Oliver A Wells[1], Elena E Bagley[1]. Dept of Pharmacol, School of Med. Sci., Univ of Sydney[1], SYDNEY, NSW.

Introduction. Endogenous opioid signalling in the brain is critical in a variety of physiological and pathophysiological states, including drug dependence. However, the conditions under which they act and the precise mechanisms by which they influence synaptic transmission remain poorly understood. The recent discovery that endogenous opioids are robust regulators of glutamate release in the intercalated (ITC) cells of the amygdala provide a system in which their cellular effects can be further characterised. The actions of some endogenous opioids, such as enkephalin, are terminated through cleavage by extracellular peptidases. Previous biochemical experiments showed that chronic morphine treatment (CMT) increases the activity of these peptidases. A possible consequence of this would be to reduce the cellular effects of endogenous opioids but it is not known whether this occurs. Hence, we used CMT to determine whether peptidase activity alters endogenous opioid regulation of ITC cells.

Aims. To investigate the effects of CMT on peptidase regulation of both exogenous and endogenous enkephalin in ITC cells.

Methods. Rats were injected, subcutaneously, with morphine base (100mg/kg) in a slow release emulsion three times over five days to produce profound opioid dependence. Synaptic currents were measured using whole-cell voltage-clamp in acute brain slices containing the amygdala from CMT-treated and vehicle-treated animals. A stimulating electrode was placed in the basolateral amygdala (BLA) nucleus to evoke AMPA excitatory postsynaptic currents (eEPSCs).

Results. At the BLA-ITC cell synapse, CMT induced changes in opioid regulation of synaptic glutamate release. The exogenous application of met-enkephalin (ME, 100nM) reduced eEPSC amplitudes to a lesser extent in CMT animals (16±3%, n=6) than in vehicle-treated animals (25±3%, n=7, Students t-test, p=0.04). However, when peptidases were inhibited (10µM thiorphan, 1µM captopril, 10µM bestatin), ME was able to inhibit eEPSCs to a similar level in both groups. This suggests increased peptidase activity in ITC cells following CMT.

Discussion. CMT alters peptidase breakdown of ME in the ITC region. This indicates that prolonged exposure to morphine induces neural adaptations, specifically peptidase upregulation, in amygdala networks.

322

Receptor dwell time determines bias towards internalisation of the μ opioid receptor

Alexander J Gillis[1], Arsalan Yousuf[1], Setareh Sianati[1], Meritxell Canals[2], Michael Kassiou[1], MacDonald J Christie[1]. [1]Discipline of Pharmacology, University of Sydney, SYDNEY, NSW, [2]Monash Institute of Pharmaceutical Sciences, Monash University, PARKVILLE, VIC.

Introduction. The μ-opioid receptor (MOPr) is the major target of opioid analgesics via its G-protein signalling activity. Multiple additional pathways, such as ß-arrestin-2 dependent signalling and regulation, contribute to opioid actions. These signalling pathways are differentially engaged by various agonists (biased signalling) but the mechanisms underlying bias have not been elucidated. Ligand receptor dwell time is increasingly recognised as a key property in drug design and may contribute to ß-arrestin-2 recruitment to the MOPr.

Aims. We use a novel series of oxymorphone analogues with extended aliphatic substitutions in the 3-position from 7 to 16 carbons/amines long to systematically investigate the contribution of structure and receptor dwell time to G-protein/ß-arrestin-2 bias at the MOPr.

Methods. Studies were conducted on AtT20 cells stably transfected with the murine MOPr. Perforated patch clamp electrophysiology of agonist-induced MOPr-mediated potassium channel (GIRK) activation and rate of channel deactivation. A resonance energy transfer assay was used to determine degree of ß-arrestin-2 recruitment to the MOPr and similarly rate of ß-arrestin-2 dissociation. MOPr internalisation was quantified with immunohistochemistry. Direct agonist affinity was quantified by displacement of [^{3}H] DAMGO binding.

Results. Increasing substituent length in the 3-position of oxymorphone had little effect on agonist affinity in binding or GIRK assays and did not alter efficacy in GIRK assays, but systematically slowed decay of both MOPr-mediated potassium channel activation and dissociation of ß-arrestin-2, indicating increased agonist dwell time. Increasing substituent length increased efficacy of ß-arrestin-2 recruitment and internalisation of the MOPr.

Discussion. Increasing substituent length in the 3-position of oxymorphone shifts bias toward ß-arrestin-2 recruitment and internalisation in a length dependent manner. Rate of agonist unbinding from the MOPr was also slowed with increased tail length. There was a strong correlation between receptor dwell time and ß-arrestin-2 bias. Receptor dwell time is therefore a factor determining G-protein/ß-arrestin-2 bias at the MOPr. This series of oxymorphone analogues will provide a useful tool to systematically study the contribution of G-protein/ß-arrestin-2 bias to analgesia and side effects.

323

Optimal time course of antibiotic concentrations in combination dosage regimens is critical to combat difficult-to-treat 'superbugs'

Cornelia B Landersdorfer[1], Vanessa E Rees[1], Jurgen B Bulitta[2], Rajbharan Yadav[1], Antonio Oliver[3], Roger L Nation[1]. Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University[1], Parkville, VIC; Department of Pharmaceutics, University of Florida[2], Orlando, FL, USA; Hospital Son Espases[3], Palma de Mallorca, Spain.

Introduction. Chronic respiratory infections by *Pseudomonas aeruginosa* (Pa) are the main driver of mortality in cystic fibrosis (CF). Hypermutable Pa strains are highly prevalent in CF and clearly linked to significantly increased multidrug-resistance and worse outcomes in patients. Antibiotic combinations are currently chosen empirically and combination dosage regimens have never been optimised against these strains.

Aims. To evaluate clinically relevant meropenem (MER) + tobramycin (TOB) regimens against hypermutable Pa.

Methods. The hypermutable PAO$\Delta mutS$ was assessed in a 10-day dynamic hollow fibre infection model (HFIM, initial inoculum $10^{8.6}$ colony forming units (CFU)/mL) simulating the pharmacokinetics in CF for: A: MER ($t_{1/2}$ 0.8 h) 1g q8h as 0.5 h infusions; B: MER as 3g/day continuous infusion; C: TOB ($t_{1/2}$ 2.5 h) 5 mg/kg q24h as 1 h infusions; combinations of A+C and B+C. Counts of total and resistant bacteria and minimum inhibitory concentrations (MIC) of resistant populations were determined. A mechanism-based model (MBM) was developed.

Results. Both MER, even at 8 mg/L constant conc. of (8×MIC), and TOB failed in monotherapy with rapid regrowth to >$10^{8.7}$ CFU/mL and extensive resistance by 48 h. For A and B, complete replacement by resistant bacteria (with MIC_{MER} up to 64 mg/L) was observed on day 9. For C, MIC_{TOB} of the resistant population was increased to 32 mg/L (64-fold compared to before treatment). A+C achieved extensive synergistic killing initially, but failed with regrowth to >6 log10 CFU/mL at 4-5 days and high-level resistance (day 9-10: MIC_{MER} 32 mg/L; MIC_{TOB} 8 mg/L). At the same dose/day, B+C achieved >8 log_{10} CFU/mL killing and near eradication (<0.4 log_{10} CFU/mL, day 10) over the clinically relevant duration of 10 days. The MBM successfully described bacterial killing and regrowth.

Discussion. An optimised MER+TOB regimen achieved synergistic bacterial killing and suppression of antibiotic resistance against a difficult-to-treat hypermutable Pa. For the combination to be maximally effective, it was critical to achieve the optimal shape of the concentration-time profile for MER.

324 - withdrawn

325

Investigation of salbutamol enantiomer deposition in epithelial lining fluid in horses

Glenn A Jacobson[1], Kate Robson[2], Sharanne Raidal[2]. School of Medicine, University of Tasmania[1], Hobart, TAS; School of Animal and Veterinary Sciences, Charles Sturt University[2], Wagga Wagga, NSW.


Introduction. Beta2-agonists are chiral compounds, typically administered as 50:50 racemic mixtures of two enantiomers (stereoisomers denoted by R- and S- descriptors); molecules with non- superimposable mirror images analogous to right and left hands. Despite salbutamol (SALB) being used for over 40 years, there is surprisingly little information known about the enantioselective bronchopulmonary pharmacokinetics.

Aims. To investigate the enantioselective bronchopulmonary distribution of SALB in epithelial lining fluid (ELF) via direct swab sampling in a horse model using an enantioselective UPLC-MS/MS assay. Physiological advantages of horses include their large size and poorly developed airway protective reflexes.

Methods. Horses (n=12 per treatment group) underwent dosing using racemic (*rac*-) SALB 1000 microgram (10x100 microgram inhalations) administered via an Aerohippus® equine aerosol chamber. ELF sampling (2, 5, 10, 15 min) was undertaken using cotton tip swabs attached to the distal end of a nasogastric tube, sheathed in PVC tubing (1.2 cm external diameter), inserted blindly into the sedated horse until it was wedged in the airway. The swab was then advanced a further distance (~ 5 cm) to press against the airway wall for 60 seconds, before retrieval via the sheath. SALB enantiomers were then analysed by an enantioselective UPLC-MS/MS assay.

Results. Mean (±SD) levels of R- and S-SALB in ELF are shown in the figure, with reduction indicating a likely combination of pulmonary uptake into and through the epithelial tissue, and mucociliary clearance.

Discussion. Direct ELF sampling offers advantages over bronchoalveolar lavage (BAL); with BAL there is no reliable indicator to calculate the amount of ELF recovered from BAL. There was relatively consistent deposition between horses and for both enantiomers, ELF R- and S-SALB concentration decreased by approximately 25% between 2 to 15 min (~370 ng/g ELF to ~ 290 ng/g ELF) and did not appear to be stereoselective.
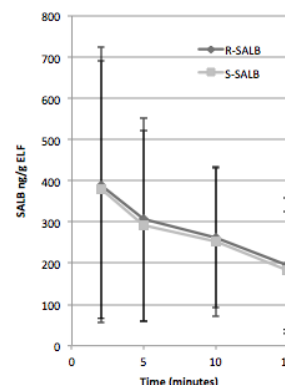
326

A physiologically based pharmacokinetic model for long-circulating nanoparticles

Xiaowen Liang[1], Haolu Wang[1], Jeffrey E. Grice[1], Xin Liu[1], Zhi Ping Xu[2], Michael S. Roberts[1]Therapeutics Research Centre, School of Medicine, The University of Queensland, Woolloongabba, QLD 4102, Australia[1]; Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, QLD 4067, Australia[2].

Introduction. Long-circulating nanoparticles (NPs) have great potential for application in long-term bio-imaging and sustained drug release within vasculature. In order to describe the biodistribution and assess the potential toxicity of long-circulating NP, it is crucial to understand and predict the *in vivo* behavior of these particles in different species.

Aims. To develop a novel physiologically based pharmacokinetic model (PBPK) model to better describe and predict long-circulating NP biodistribution *in vivo* based on physiological studies.

Methods. We developed water-dispersible cadmium telluride/ cadmium sulfide (CdTe/CdS) quantum dots (QDs), to represent long-circulating NPs for investigation their specific *in vivo* behaviour. Inductively coupled plasma-mass spectrometry was used to determine cadmium (representing of QDs) level in each organ after intravenous injection of QDs in mice. The QD location in major organs was real-time monitored using multiphoton microscopy (MPM) and confirmed by transmission electron microscope. A PBPK model was developed to characterize the physiological processes of administrated long-circulating NPs based on the imaging details and quantitative data.

Results. This model accurately characterized and predicted *in vivo* behaviour of QDs and validated by multiple datasets, including experimental inter-route and external inter-species predictive capability. Our results suggest the biodistribution of long-circulating NP is determined by uptake and release of NP by cells in target organs.

Discussion. This PBPK model can be extended to other types of long-circulating NPs by adapting the property-specific parameters. It provides a general framework for predicting the *in vivo* fate of long-circulating NPs and assessing their potential toxicity.
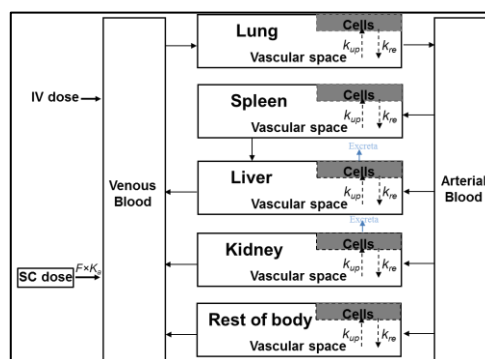
327

A physiologically based kinetic model for elucidating the *in vivo* fate of mesenchymal stem cells

Haolu Wang, Xiaowen Liang, Xin Liu, Michael Roberts. Therapeutics Research Centre, School of Medicine, The University of Queensland, Brisbane, QLD.

Introduction. Mesenchymal stem cells (MSCs) present a promising tool in cell therapy for the treatment of various diseases, such as liver cirrhosis. However, their *in vivo* fate in the body after administration, which is crucial in the prediction and evaluation of the therapeutic efficacy, has still been poorly understood so far.

Aims. To quantitatively analyse *in vivo* fate of MSCs with modelling to better identify the barriers to MSCs delivery, and to propose designs of new formulations and dosing regimens.

Methods. We introduced stable GFP-expressing mouse MSCs into BALB/c nude mice intravenously to mimic the stem cell-based therapy for patients. The *in vivo* fate of MSCs in major organs was real-time monitored using multiphoton microscopy (MPM). Animals were sacrificed at designated times, and MSCs in the blood and major organs were counted using flow cytometry. A physiologically based kinetic (PBK) model was developed to characterize the physiological processes of administrated MSCs based on the MPM details.

Results. Our PBK model successfully described the concentration-time profiles of MSCs in blood and various organs in mice. This model was validated with multiple external datasets, indicating robust inter-route and inter-species predictive capability. The clinical utility of this model was tested with data obtained from stem cell-based therapies to patients with liver cirrhosis. Our results suggest that the targeting efficiency of MSCs is determined by the redistribution from the lung and their arrest, depletion and release rate in target organs.

Discussion. We present the first model for characterizing and predicting the *in vivo* fate of MSCs precisely. This novel method provides a general framework for the study of *in vivo* fate of therapeutic cells to design treatment protocols and to guide future experiments.
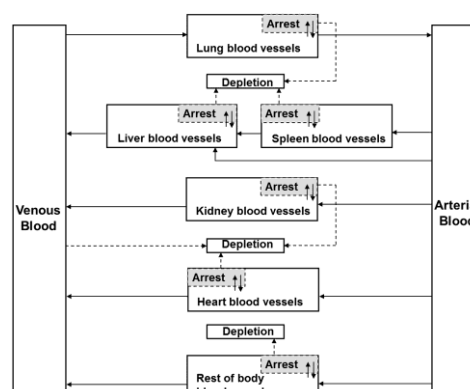
328

Drug and chemical glucosidation by untransfected Supersomes[TM] (u-SUP) and microsomes from *Spodoptera frugiperda* 9 (Sf9) cells

Nuy Chau, Peter I. Mackenzie and John O. Miners. Depart of Clin Pharm, Flinders University, Adelaide, SA.

Introduction. The human UDP-glucuronosyltransferases (UGTs) comprise a superfamily of enzymes that primarily utilize UDP-glucuronic acid (UDP-GlcUA) as the cofactor in the clearance of lipophilic drugs and endogenous compounds. Data from this laboratory has shown that UGT2B7 catalyzes both the glucuronidation and glucosidation of morphine (Chau et al, 2014). However, the relative contribution of each pathway was difficult to determine with recombinant human UGTs expressed in insect cells due to the unexpected involvement of an endogenous glucosidating enzyme(s).

Aim. To characterise the endogenous glucosidation activity of untransfected Sf9 and u-SUP insect cells in order to understand the implications for the measurement of human UGT activity in insect cell expression systems.

Methods. The glucosidation of 15 compounds containing either an aliphatic or phenolic hydroxyl, amine- or carboxyl-acid group by incubations of microsomes from Sf9 and u-SUP cells supplemented with UDP-glucose (UDP-Glc) was quantified by HPLC and verified by high resolution mass spectrometry.

Results. Glucosidation of 10 compounds (morphine, mycophenolic acid, S-naproxen, benzocaine, 21-hydroxyprogesterone, 4-methylumbelliferone, 1-hydroxypyrene (1-OHP), 4-nitrophenol, 1-naphthol, phenethyl alcohol) was observed by both cell systems to varying degrees, with zidovudine and 20-hydroxyecdysone glucosidated only by u-SUP, lamotrigine and trifluoroperazine only by Sf9 and codeine by neither cell systems. Notably, 1-OHP was a high affinity, high turnover substrate. 1-OHP glucosidation by Sf9 and u-SUP microsomes displayed substrate inhibition (K_m=1.24 μmol/L, V_{max}= 2,587 pmol/min/mg) and sigmoidal kinetics (S_{50}=7.95 μmol/L, V_{max}= 11,211 pmol/min/mg), respectively.

Discussion. Insect cells have the capacity to glucosidate a structurally diverse range of xenobiotics metabolized primarily by glucuronidation by human UGTs. Investigation of the role of human recombinant UGTs in drug glucosidation should utilize non-insect cell expression systems.

Chau N et al (2014) J Pharmacol Exp Ther 349:126-137

329

Exemestane and its active metabolite 17-hydroexemestane up-regulate UDP-glucuronosyltransferase (UGT) 2B17 in breast cancer cells

Apichaya Chanawong, Dong Gui Hu, Robyn Meech, Ross A McKinnon, Peter I Mackenzie. Dept. of Clinical Pharmacology and Flinders Centre for Innovation in Cancer, Flinders University, Adelaide, SA.

Introduction. Exemestane, an aromatase inhibitor indicated for endocrine therapy of breast cancer, is primarily converted into its most potent active metabolite 17-hydroexemestane. This metabolite is mainly eliminated via glucuronidation by UGT2B17. Therefore, the expression and activity of UGT2B17 in breast cancer cells is likely to be a major determinant of the clinical efficacy of exemestane.

Aim. To study the potential regulation of UGT2B17 by exemestane and 17-hydroexemestane in breast cancer cells.

Results. We showed that both exemestane and 17-hydroexemestane elevated UGT2B17 mRNA levels in breast cancer MCF-7 and MDA-MB-453 cells, and increased the glucuronidation of UGT2B17 substrates, including androsterone or 17-hydroexemestane in MCF7 cells. We further showed that treatment of cells with the estrogen receptor (ER) antagonist fulvestrant, the androgen receptor (AR) antagonist bicalutamide, or siRNA targeting the ER, AR, or Forkhead box protein A1 (FOXA1) transcription factors, significantly reduced exemestane/17hydroexmestane-induced UGT2B17 expression. Finally, we demonstrated that both exemestane and 17-hydroexemestane stimulated the UGT2B17 promoter activity and this stimulation was significantly reduced by mutation of the FOXA1 binding site in the UGT2B17 promoter. Further investigation is underway to determine the potential role of the previously reported ERE sites at the UGT2B17 promoter.

Discussion. Our results demonstrated that exemestane and its active metabolite 17-hydroexemestane increase the expression and activity of UGT2B17 in breast cancer cells through the promoter via the ER-, AR- and FOXA1-signalling pathways. This induction facilitates removal of 17-hydroexemestane, the most active metabolite of exemestane, from breast cancer cells, and thus may reduce exemestane efficacy or even contribute to the development of exemestane resistance.

330

Inhibition of ocular neovascularization by gene delivery of calreticulin-derived peptide, vasostatin

Guei-Sheung Liu[1,2], Youn-Shen Bee[3], Gregory J Dusting[1,2]. [1]Centre for Eye Research Australia, East Melbourne, VIC; [2]Department of Ophthalmology, University of Melbourne, East Melbourne, VIC. [3]Department of Ophthalmology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

Introduction. The most common and baffling forms of vision loss, particularly those associated with ageing and diabetes, result from damage to the retina. This loss of visual function is exacerbated in these diseases by the growth of new, immature blood vessels that can leak fluid and bleed. Thus, development of cost effective and less-invasive treatments for targeting angiogenesis in the back of the eyes are a priority in ophthalmology. Vasostatin, the N-terminal domain (amino acids 1-180) of calreticulin, is a potent inhibitor of angiogenesis isolated from culture supernatants of an EBV-immortalized B cell line. Vasostatin that could be superior to current therapeutic approaches for it specifically targets endothelial cells and it also has anti-inflammatory properties.

Aims. This study aims to investigate the efficacy of vasostatin gene delivery on experimental choroidal neovascularization (CNV).

Methods. The anti-angiogenic effects of vasostatin were validated by migration and tube formation assays performed on cultured endothelial cells, and by mouse aortic ring assays. CNV lesions were induced in the Brown Norway rats by fundus argon laser photocoagulation. The extent of CNV was examined by fundus fluorescein angiography (FAG) and histological analysis.

Results. In this study, we first showed that vasostatin inhibits angiogenic activity in several assays of angiogenesis including human endothelial cell migration and tube formation, and vessel sprouting from mouse aortic ring explants. We then investigated the therapeutic potential of vasostatin in laser-induced CNV models through subconjunctival gene delivery by an adenoviral vector that releases peptide slowly over 4 weeks. Serial FAG analysis indicated that subconjunctival vasostatin gene delivery significantly reduced CNV lesions on all subsequent days. Histological analysis revealed attenuated CNV lesions and choroidal vascularity in the vasostatin-treated eyes.

Conclusion. The present study provides evidence supporting that sustained delivery of a vasostatin using a minimally invasive procedure promises to revolutionize the management of ocular neovascularization.

331

Novel naphthoquinones against mitochondrial dysfunction-induced seizures

Monila Nadikudi[1]*, Krystel Woolley[2]*, Ayman L.A. Hemasa[1], Jason A Smith[2], Nuri Guven[1]. Pharmacy, School of Medicine, Univ of Tasmania[1], Hobart TAS; Chemistry, School of Physical Sciences; Univ of Tasmania[2], Hobart, TAS.

Introduction. Seizures in nearly 30% of patients are hard to control. Recent studies suggest that seizures can be treated by targeting mitochondrial dysfunction.

Aims. To generate compounds that directly target mitochondrial function and that can be developed into effective anti-seizure drugs using a rational medicinal chemistry approach.

Methods. Novel compounds were synthesized and characterized *in vitro* as a selection process before progressing the most promising compounds to an *in vivo* zebrafish model of drug induced seizures as a second stage.

Results. We synthesised and characterized close to 100 novel compounds. We have improved compounds based on their ability to restore cellular viability at micromolar concentrations in the presence of a mitochondrial inhibitor. We already identified several new compounds that show significantly better cytoprotection and lower toxicity compared to a closely-related, clinically used short-chain quinone.

Discussion. We have identified first structure activity relationships. Our data indicate that a specific level of solubility (logP between 1.5-4.5) as well as a defined balance between polarity and fattiness of the side chain is essential for the compound's cytoprotective effect. Upon testing enantiomer pairs of some compounds, we believe that cytoprotection is based on solubility but could also involve receptor level interactions.
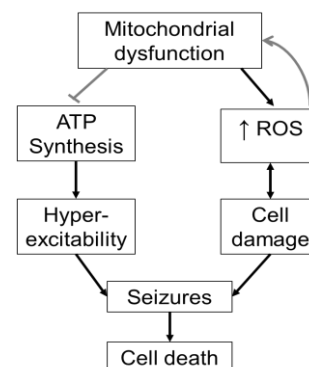
Ahmed M. (2014) Neurochem Res 39(2):394-402

Rahn J J, Bestman J E, Josey B J et al. (2014) Neuroscience 259:142-154

332

Preclinical pharmacological evaluation of CDKI-73 for treatment of acute myeloid leukaemia

Longjin Zhong, Frankie Lam, Julian Adams, Muhammed Hamidur Rahaman, Robert Milne, Shudong Wang. Centre for Drug Discovery and Development, Sansom Institute for Health Research, and School of Pharmacy and Medical Sciences, University of South Australia, Australia.

Introduction: CDKI-73 is one of the most potent cyclin-dependent kinase 9 (CDK9) inhibitors identified to date. It has exhibited marked anti-cancer activity against chronic lymphocytic leukaemia patient-derived samples with minimal toxicity against normal B-cells. It has also demonstrated significant anti-tumour activity against several tumour xenografts.

Aims: To investigate the mechanism of the anti-proliferative effect of CDKI-73 against a human acute myeloid leukaemia MV4-11 cell line and its anti-cancer efficacy *in vivo*.

Methods: MV4-11 cells were incubated with 0.25 µM CDKI-73 for 1, 12 and 24 h. Protein expression in cell lysates were determined by western blot. The effect of CDKI-73 on the cell cycle was determined by flow cytometry; on apoptosis was determined by the Annexin V assay. The anti-cancer efficacy of CDKI-73 was determined in the MV4-11 xenograft model in nude mice. CDKI-73 was administered orally (50 or 100 mg/kg once every three days). Tumour volume and body weight were measured every other day and compared with two-way ANOVA followed by Tukey's multiple comparisons test.

Results: After incubation of MV4-11 with CDKI-73 (0.25 µM) for 1 h, the phosphorylation of RNA polymerase II at Ser 2 and Ser 5 was inhibited, indicating cellular CDK9 and 7 inhibition, respectively. By 12 h, the reduced level of Mcl-1 and caspase-3, companied by an increased level of the cleaved PARP were observed. CDKI-73 induced apoptosis of MV4-11 in a dose- and time-dependent manner without affecting the cell cycle. In the tumour xenograft model, CDKI-73 demonstrated a marked anti-tumour efficacy (P < 0.001) with a minimal toxicity.

Conclusion: In MV4-11 cells, CDKI-73 caused cell death *in vitro* through inhibition of CDK7 and 9 without effect on cell cycle. CDKI-73 possessed striking anti-cancer effect against acute myeloid leukaemia xenograft model with safety *in vivo.*

333

The Involvement of Fatty Acid-Binding Protein 5 in the Blood-Brain Barrier Transport of Docosahexaenoic Acid and Cognitive Function

Yijun Pan, Christopher KH Choy, Jennifer L Short, Christopher JH Porter, Martin J Scanlon, Joseph A Nicolazzo. Monash Institute of Pharmaceutical Sciences, Monash University, VIC.

Introduction. Docosahexaenoic acid (DHA) is an essential fatty acid which has to be transported across the blood-brain barrier (BBB) to maintain normal cognitive function. In order to cross the aqueous cytoplasm of the brain endothelial cell, however, an intracellular carrier protein may be required given the poor aqueous solubility of this fatty acid. We have demonstrated that fatty acid binding protein 5 (FABP5) is expressed at the BBB, however, whether FABP5 facilitates DHA transport across the BBB remains unknown.

Aims. To reveal if FABP5 mediates BBB DHA transport and if FABP5 deficiency leads to cognitive impairment.

Methods. The uptake of ^{14}C-DHA was measured in human brain microvascular endothelial cells (hCMEC/D3) with and without FABP5 genetic silencing and BBB transport of ^{14}C-DHA assessed in wild-type (FABP5$^{+/+}$) and FABP5 deficient (FABP5$^{-/-}$) mice using an *in situ* transcardiac perfusion technique. Endogenous brain concentrations of DHA in each genotype were measured using gas chromatography with flame ionization detection, and cognitive function was assessed using water maze, novel object recognition (NOR), Y-maze, and T-maze memory paradigms.

Results. FABP5 siRNA transfection decreased FABP5 mRNA in hCMEC/D3 cells by 53.2 ± 5.5% (n=4), and this was associated with a 21.7 ± 4.1% (n=3) reduction in FABP5 protein expression and 17.1 ± 2.7% (n=12) reduction in ^{14}C-DHA cellular uptake. BBB transport of ^{14}C-DHA decreased by 40.0 ± 10.7% in FABP5$^{-/-}$ mice (n=5-6), and this was associated with a 27.4 ± 10.3% reduction in endogenous brain DHA levels (n=3). FABP5$^{-/-}$ mice showed impaired spatial learning in the water maze and increased latency to escape (10.5 ± 4.4 sec) in the probe test (n=16-18). In addition, female FABP5$^{-/-}$ mice failed to show exploration differences between novel and familiar arms in the Y-maze (37.1 ± 1.9 % novel arm vs 30.2 ± 1.7% familiar arm). In the NOR paradigm, FABP5$^{-/-}$ mice failed to discriminate the novel object from the familiar object (discriminative index = 0.05). FABP5$^{-/-}$ mice also showed a decrease (9.4 ± 2.4%) in spontaneous alternations in the T-maze paradigm (n=18-19).

Discussion. This study has demonstrated that FABP5 mediates the BBB transport of DHA *in vitro* and *in vivo.* A reduction in DHA brain access is associated with impaired cognitive function, suggesting a crucial role of FABP5 at the BBB in maintaining DHA brain concentrations and consequently normal cognitive function.

334

Development and evaluation of oxaliplatin and irinotecan co-loaded liposomes for enhanced colorectal cancer therapy

Bo Zhang[1,2], Tianqi Wang[1], Shaomei Yang[1], Ya'nan Xiao[1], Yunmei Song[2], Na Zhang[1], Sanjay Garg[2] School of Pharmaceutical Science, Shandong University[1], Jinan, Shandong, China; School of Pharmacy and Medical Sciences, University of South Australia[2], Adelaide, SA, Australia.

Introduction. Drug combinations are widely employed in chemotherapy for colorectal cancer treatment, such as oxaliplatin and irinotecan. Traditional cocktail combinations lead to the uncertainty of treatment, owing to varying pharmacokinetics of different drugs. Therefore, it is critical to co-deliver two drugs into the tumor cells synchronously.

Aims. To design oxaliplatin and irinotecan hydrochloride co-loaded liposomes with synchronised delivery and release, hence improve the colorectal cancer therapy.

Methods. Oxaliplatin and irinotecan hydrochloride were co-loaded into the liposomes using ethanol injection method followed with ammonium sulphate gradient method. The liposomes were characterized for particle sizes, encapsulation efficacy and morphology. *In vitro* release, *in vitro* cellular uptake, *in vitro* cytotoxicity, *in vivo* anti-tumor activity and histopathological analysis were carried out.

Results. The particle sizes of the liposomes were less than 200 nm with uniform size distribution. *In vitro* release study showed that both drugs could be synchronously released from the liposomes. *In vitro* cellular uptake revealed that co-loaded liposomes could efficiently deliver different drugs into the cells. *In vitro* cytotoxicity evaluation demonstrated that co-loaded liposomes exhibited higher cytotoxicity in both CT-26 and HCT-116 cells. Furthermore, Co-loaded liposomes also presented superior anti-tumor activity in CT-26 bearing BALB/c mice. *In vivo* safety assessment demonstrated that liposomes had lower toxicities than their solution formulations.

Discussion. The results indicated that oxaliplatin and irinotecan hydrochloride co-loaded liposomes can be a potential formulation for improving colorectal cancer therapy.

Pathak RK, et al. J Am Chem Soc, 2015, 137: 8324.
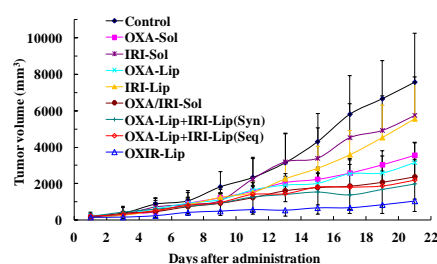
335

Amber teething necklaces: is there any basis to their use in teething infants?

Michael Nissen[1], Esther TL Lau[2], Lisa M Nissen[2] Kathryn J Steadman[1]. School of Pharmacy, The University of Queensland[1], Brisbane, QLD; School of Clinical Sciences, Queensland University of Technology[2], Brisbane, QLD.

Introduction. Baltic amber-bead necklaces or bracelets are commonly used for managing teething symptoms in infants. The effectiveness of these beads is claimed to be from succinic acid release (a compound said to have analgesic and anti-inflammatory properties), which is then absorbed through the skin.

Aims. To investigate whether succinic acid is contained in Baltic amber teething necklaces purchased in Australia, whether it can be released from the beads, and whether it has anti-inflammatory activity.

Methods. Infrared spectroscopy was used to confirm that the teething necklaces were made of Baltic amber. The amount of succinic acid contained within the beads was quantified, and succinic acid release from intact beads was measured in phosphate buffered saline (PBS) pH 5.5 or octanol. Anti-inflammatory activity of succinic acid was compared with ibuprofen, paracetamol and hydrocortisone *in vitro* using THP-1 human macrophages stimulated with LPS. Secretion of the cytokines IL-1a, IL-1b, IL-8 and TNF-α were determined by ELISA.

Results. Each necklace (33 beads in length) contained 19.17 ± 4.89 mg of succinic acid (mean ± se). Over a 6-month period, no succinic acid was detected in PBS. While 0.13 ± 0.09 mg of succinic acid per necklace was released in octanol, this was due to only one replicate of amber beads which had fragmented into shards free-floating in the solvent. Succinic acid had no effect on cytokine secretion unless extremely high concentrations were used and changes were likely to be associated with cell apoptosis and death.

Discussion. No evidence for anti-inflammatory activity was found in the cytokines studied. It is possible that succinic acid could exert an effect via some other mechanism, but while the teething necklaces do contain small quantities of succinic acid, it is highly unlikely to be released from intact beads.

336

A flipped-classroom blended approach to the teaching of pharmacology

Emilio Badoer[1], Laurine Hurley[1,2]. Marian Dobos[1] School of Medical Sciences RMIT University[1], Melbourne, Vic; School of Science, Australian Catholic University[2], Melbourne, Vic.

Introduction. Current and future cohorts of university students are demanding greater flexibility in their learning environments. The dilemma for educators is how to address this flexibility and yet maintain student engagement and satisfaction. We describe the development of a 'flipped-classroom' (FC)-blended learning design implemented in Year 3 pharmacology teaching.

Aims. To design, implement and evaluate a FC-blended learning approach and compare this to a traditional mode.

Methods. Weeks 1-5 of a 12 week semester were taught in the FC-blended mode. This consisted of lectures being replaced by a series of videos complemented by face to face lectorials. Prior to attending the lectorials, the students watched the videos and completed a Student Video Concept Form (SVCF) based on their understanding of the key concepts. The SVCF provided feedback on difficult concepts and was used to direct the learning in the lectorials. The remaining 7 weeks of the semester consisted of traditional, face-to-face lectures and workshop/tutorials. Evaluation of the project included student perception questionnaires, focus groups and lecturers' reflections.

Results. Key findings from a class of 40 students include: >90% of the students found the new teaching approach interesting and helped their learning. Most importantly, 82.5% of the students indicated the new approach addressed their conceptual difficulties vs 35.9% for the traditional mode. Student attendance in the lectorials (71.1%) was markedly higher than in the conventional mode (27.5%). Students reported that the short videos helped them focus on key concepts, and that the FC-model increased flexibility, particularly their ability to view and review videos using mobile devices of their choice; Most importantly, our students reported increased retention of pharmacology concepts as a result of the changed teaching approaches.

Discussion. We conclude that the FC-blended learning model significantly increased student engagement, assisted student learning and increased student satisfaction with the course. Most importantly, the students were able to revisit the learning material and consider their difficulties before the lectorials and reinforce their understanding during the lectorials through student discussion. The SVCF was a valuable tool for both students and staff and critical to the success of this initiative.

337

Increasing pharmacists' capabilities for collaboration in health care teams through successful interprofessional communication

Karen Luetsch[1], Debra Rowett[2]. School of Pharmacy, UQ[1], Brisbane, QLD; DATIS[2], Adelaide, SA

Introduction: Pharmacists are continuously advancing their scope of practice, increasing the need for collaboration with other health professionals involved in team-based healthcare.

Aims: Develop and test a postgraduate learning program which aims at building clinical pharmacists' capabilities and confidence to collaborate with other health professions by learning and applying interprofessional communication skills.

Method: A training and practice module was designed around a framework for successful interprofessional communication for a postgraduate clinical pharmacy program. After completion of the module pharmacists enrolled in the program applied the framework during an educational encounter with a health professional in their workplace. Modelled on educational outreach visits their discussions aimed at the establishment of interprofessional relationships, while focusing on contemporary clinical content. Pharmacists' written reflections (55) and health professionals' feedback (32) on their respective experiences of the encounter were evaluated using qualitative, thematic analysis.

Results: A number of themes relating to interprofessional collaboration and learning were identified. After initial apprehension pharmacists were able to engage clinicians in an interactive exchange of information, learning about, with and from each other. Using the framework to structure their conversations built rapport and their credibility and provided insight into the clinicians' need for information, their current practice as well as barriers to best practice. Pharmacists were starting to move from a transactional approach to improving medication use for individual patients to a role of educator and change agent. They perceived their relationship with the clinician increased in social capital and professional standing.

Health professionals valued the educational aspect of the interaction, how information was tailored to meet to their personal needs and recognised the pharmacists' unique expertise.

Conclusion: Skilled, pro-active interprofessional communication learnt and practiced within a postgraduate program creates potential to demonstrate the value of pharmacists as members of health care teams.

338

Development of a practical class for third year neuropharmacology students using zebrafish to demonstrate the actions of antiseizure drugs

Nicole M Jones[1], Angela M Finch[1]. Dept of Pharmacology, School of Medical Sciences, [1]UNSW Australia, Sydney, NSW.

Introduction. Historically, rodent models (including: pentylenetetrazole (PTZ)) have been widely used to demonstrate antiseizure properties of drugs. These models are not easily performed by experimenters with limited laboratory experience (such as undergraduate students). More recently, newer non-mammalian seizure models have been developed. When zebrafish embryos (4 days post-fertilization) are exposed to convulsants (such as PTZ), they exhibit a well-defined seizure profile which can be reduced by clinically used and novel anticonvulsant drugs.

Aims. We have employed a model of PTZ-induced seizures in zebrafish embryos to develop a new practical class in order to enhance student learning and understanding of the mechanisms of action of antiseizure drugs.

Methods. Students from a 3rd year Neuropharmacology course (64 in 2013, 88 in 2014) performed the experiment. Zebrafish embryos were pre-treated with multiple doses of vehicle solution, carbamazepine or sodium valproate and subsequently exposed to PTZ (15mM). Students observed the time where different behavioural characteristics occurred which define distinct seizure stages and the number of events. Data was collected, analysed and a quiz was administered to students to assess understanding.

Results. In course feedback (2013-2014), students agreed with the statement: "I have developed my lab skills in this course (96-97%) and identified some of the "best features of the course" as relevance of the practical classes to the lectures: "the pracs were challenging and very helpful to learn the mechanism of drugs". Experimental results obtained in the class show a dose-dependent action of antiseizure drugs, and students demonstrated a good understanding of the mechanism of action of the antiseizure drugs according to their quiz responses.

Discussion. This activity integrated well with the lecture material. We have developed an innovative new prac class allowing students to advance their lab skills using a novel screening tool to examine the actions of antiseizure drugs in zebrafish embryos. This practical class allows examination of numerous treatment conditions (drugs and doses) in a single practical class, which would prove very difficult using rodent seizure models in a 3hr time frame. This approach could be readily adaptable to showcase behavioural responses to many different classes of CNS drugs.

339

Pharmacy Simulator: A 3D computer-based virtual patient simulator for training community pharmacists

Ivan K Bindoff[1], Tristan R Ling[1], Luke R Bereznicki[1], Leanne Chalmers[1], Juanita L Westbury[1], Peter R Gee[1], Tristan Garland[1], School of Medicine (Pharmacy), University of Tasmania[1], Hobart, TAS.

Introduction. Computer-based simulated learning experiences allow students to experience realistic, engaging workplace scenarios and apply classroom theory in a safe environment (Lyons, 2012; Susi et al., 2007).

Aims. To provide a computer-based pharmacy practice learning method that is as effective as paper-based scenarios, but more engaging and less labour-intensive.

Methods. We developed a virtual community pharmacy in which students can take on the role of pharmacist, and are given freedom to complete interactive patient scenarios from start to finish. Educators can write scenarios that cover almost any community pharmacy practice issue using the scenario builder tool that we also developed. The simulator was evaluated using a randomized controlled trial with third and fourth year Bachelor of Pharmacy students. We compared the traditional paper-based teaching method against the computer-based approach using equivalent scenarios. The paper-based group had two tutors while the computer group had none. Both groups were given a pre- and post-scenario clinical knowledge quiz and survey.

Results. A total of 33 students participated. Students in the computer-based group showed a trend towards greater improvements in their clinical knowledge score (mean change $+0.63\pm1.31$ vs. -0.24 ± 1.30, $p=0.059$). Third year computer-based students also showed better improvements in history taking ($n=18$, $p=0.029$) and counselling competencies ($n=18$, $p=0.008$). Third year students found the simulation fun and engaging.

Discussion. Overall the pharmacy simulator provided learning outcomes that were generally equivalent to and in some cases better than those provided by a paper-based scenario equivalent, without the need for tutors, and was generally felt to be more fun. The simulator has the potential to augment our existing teaching activities, complementing and better preparing students for experiential learning.

Lyons, J. (2012). Learning with technology: theoretical foundations underpinning simulations in higher education. Proceedings ascilite. M. Brown, M. Hartnett and T. Stewart. Wellington**:** 582-586.

Susi, T., M. Johannesson, et al. (2007). Serious games: An overview. Skövde, University of Skövde.

340

A collaborative approach to embedding cultural competence into the Bachelor of Pharmacy curriculum

Sandra Holmes[1], Mackenzie Williams[1], Virginia Ford[1]. Pharmacy, School of Medicine, Uni of Tas, Hobart, TAS[1]

Introduction. It is important that Bachelor of Pharmacy graduates are prepared for living and working in a global, culturally diverse society. This reflects the University of Tasmania's generic graduate attributes and the Bachelor of Pharmacy accreditation standards and is highlighted by our increasingly diverse student cohort.

Aims. To work collaboratively with staff in other courses within the Faculty of Health to design, develop and embed a cultural competence program in the undergraduate curriculum that would be suitable for diverse student cohorts yet flexible enough to suit discipline specific needs.

Methods. Staff in the Divisions of Pharmacy, Medicine, Nursing and Students and Education developed a *Global Perspectives Program* (*GPP*) based on four key elements of cultural competence: 1 *Awareness* of one's own worldview, 2 *Attitude* towards cultural differences, 3 *Knowledge* of different cultural practices and worldviews, 4 *Skills*, including communication skills. Key features that underpinned program design were: suitable for all students, domestic and international; core module in the first year of the course; core module embedded and assessed within an existing unit; a continuum of learning activities throughout the course; all learning and assessment activities constructively aligned to intended learning outcomes; ongoing program development and improvement informed by student and staff evaluation.

Results. The core module of the *GPP* has been embedded into the first year of the Bachelor of Pharmacy and into seven other undergraduate courses in the Faculty of Health. An interdisciplinary teaching team has delivered the module to diverse student cohorts on Hobart, Launceston and Sydney campuses, using face to face, online and blended delivery methods. The dominant theme in feedback from students was the benefits of getting to know and learn from students outside of their normal peer group. An ideal model for module delivery has emerged in which an interdisciplinary teaching team delivers core content in a series of plenary sessions followed by tutorials or workshops based on discipline-specific examples and scenarios.

Discussion. Our experience has highlighted the benefits of collaborating with staff in other disciplines to facilitate the development of cultural competence in students. The core module of the *GPP* will continue within the Bachelor of Pharmacy as we continue to expand learning opportunities into other years of the course.

341

Creating effective multimedia learning resources for the teaching of pharmacology

Chau N Khuong, Elizabeth A Davis, Wayne C Hodgson. Dept of Pharmacol, Monash Univ, Clayton, VIC.

Introduction. Multimedia resources provide an invaluable method to demonstrate complex biological processes. However, many discipline areas of medical and biomedical sciences, including pharmacology, lack accessible high-quality multimedia resources.

Aim. To develop multimedia resources for the teaching of neurotransmission and neuromuscular pharmacology, and to evaluate their effectiveness as self-directed learning materials.

Methods. The cognitive load theory, cognitive theory of multimedia learning, and Gestalt's theory of visual screen design (Graham *et al.*, 2008; Mayer, 2010) were used to develop a series of diagrams, animations and

simulations to illustrate neurotransmission and neuromuscular pharmacology. These multimedia activities were organized into comprehensive modules and made accessible to first year Monash University medical students via their online Learning Management System. Pre- and post-module tests were used to compare their learning with previous cohorts delivered the same content via the traditional lecture format.

Results. Students who completed the multimedia modules performed better on the post-test than those in the same cohort who did not complete the modules. In addition, delivery of concepts via the self-directed learning modules was associated with higher neurotransmission post-test scores (see figure) and similar neuromuscular pharmacology post-test scores in comparison with presentation of the same content via lectures.

Discussion. The multimedia resources developed were effective learning instructions and can be used as self-directed tools to present concepts, freeing up class time for interactive activities that can model their application and relevance.
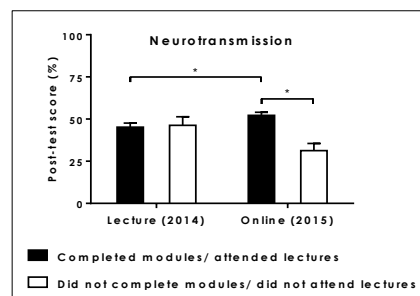
Graham L et al (2008). IJHSS 2: 1-12
Mayer RE (2010). Med Educ 44: 543-549

342

Academic life in the spotlight: Maximizing productivity and fulfillment

Shane P. Desselle, Touro University California College of Pharmacy, Vallejo, CA, USA

Life in the professoriate continues to grow more complex and demanding, with increasing role stress and lack of time inducing turnover, burnout, and shortages of highly qualified faculty. This presentation will examine the current state of academic life in pharmacy, including a unique tool to measure work satisfaction. Results of research on faculty self-efficacies and turnover intention will be shared. These results point to a number of challenges and potential solutions, including guidance on mentorship programs, with an appropriate basis in theoretical learning paradigms also resulting in practical applications. A significant yet often unaccounted for phenomenon is collegiality, the express manifestation of which can be measured as organizational citizenship behaviours. These behaviours, along with perceived psychological contract violations, will complete this presentation on comporting oneself and reacting to the behaviours of colleagues to produce the highest worklife outcomes possible that are mutually beneficial to you and your academic institution.

Authors Index

H

Hall, J	150		Hintermann, S	320
Halls, M	148		Ho, J	206
Halls, M	166		Ho, S	225
Hancock, N	152		Hoban, C	104
Hanrahan, J	225		Hodgson, W	341
Harris, B	233		Hollingworth, S	131
Harris, P	243		Holmes, S	340
Harris, T	167		Hook, S	208
Harrison, I	134		Hopkins, A	246
Haushalter, K	140		Hopper, I	219
Hauta-aho, M	162		Hoyer, D	320
Haynes, J	317		Hu, D	329
Headrick, J	140		Hu, D	303
Held, F	176		Hughes, J	156
Helsby, N	214		Hui, K	237
Hermansyah, A	119		Huizer-Pajkos, A	159, 180, 222
Hibbs, D	225		Hurley, L	336
Hilmer, S	128, 130, 159, 160, 180, 218, 222		Hutchinson, D	143, 146, 314

I

Ilango, S	177		Irving, H	318
Ilomäki, J	175, 312		Isaac, S	231

J

Jacobson, G	179, 325		Johnston, C	212
Jaffar, J	167		Jokanovic, N	126
Jalilzadeh, A	158		Jomaa, I	229
Jap, E	136		Jones, B	180, 222
Jean-Parry, L	136		Jones, N	338
Jensen, D	173		Jordan, M	150, 181
Jeon, Y	226		Joyce, D	232
Johnell, K	129		Jun Du, X	138
Johns, S	162			
Johnston, C	302			

K

Kahlaee, R	155		Keshwani, M	140
Kahlberg, N	136		Khuong, C	341
Kaminskas, L	205		Kichenadasse, G	234
Kanai, Y	300		Kiriazis, H	138, 141
Kane, A	159, 180, 222		Kirkpatrick, C	126, 175, 237, 239
Kannangara, D	238		Klein Herenbrink, C	149
Kanwar, J	242		Kong, D	237
Kassiou, M	322		Kouladjian, L	218
Kathawala, K	245		Krass, I	119, 156
Kaub, P	234		Kreijkamp-Kaspers, S	121
Kelly, D	106		Kroon, M	170
Kemp, R	240		Krum, H	219
Kemp-Harper, B	136, 139		Ku, J	135
Kennan, C	168		Kumar, S	239
Kerr, F	232			

O

Odisho, M	229		Olsen, J	314
O'Doherty, C	246		Ong, G	163
Ofner, S	320		O'Reilly, C	230
O'Halloran, S	232		Orlando, L	211
Oliver, A	323		Ozberk, V	140

P

Page, C	177		Peterson, G	156
Pan, Y	333		Phan, V	233
Parat, M	301		Phillips, L	180
Parayath, N	240		Pijpers, E	121
Parikh, A	245		Pillans, P	177
Parkington, H	217		Pithavala, Y	112
Parry, L	138		Polasek, T	234
Patel, H	140		Porter, C	169, 333
Peart, J	140		Pouton, C	206
Pedagogos, E	237		Prakoso, D	138
Pentony, P	238		Prasad, R	152
Perera, V	110, 233		Proudman, S	246
Perrot, L	320			
Peterson, G	153, 154			

Q

Qian, H	138		Qin, C	313
Qin, C	136, 138, 141		Quach, T	169
			Quirke, T	309

R

Rahaman, M	332		Roberts, J	202
Raidal, S	325		Roberts, M	207, 326, 327
Ramesh, S	132		Roberts-Thomson, S	220
Rawling, T	304		Robson, K	325
Raynor, D	118		Rosli, S	138, 141, 313
Reading, P	166		Roth, D	140
Rees, V	323		Rowett, D	337
Reeve, E	128, 160		Rowland, A	234
Ricardo, S	317		Royce, S	171
Ritchie, R	136, 138, 141, 313			
Roberts, D	201			

S

Saini, B	225, 229, 231		Sianati, S	322
Saini, S	317		Simoons, M	172
Sainsbury, E	119		Simpson, J	223
Salman, S	232		Skiba, M	219
Samuel, C	108, 171		Smith, B	112
Sandow, S	319		Smith, J	331
Sandstrom, A	314		Sobey, C	139
Sapkota, S	157		Somogyi, A	215
Sato, M	143, 314		Song, Y	242, 334
Sawan, M	226		Soon, P	220
Scanlon, M	333		Sorich, M	234
Schilling, J	140		Spark, J	122
Schneider, C	123, 155		Spencer, S	135
Schuliga, M	167		Spizzo, I	217
Scott, B	151		Srewart, A	168
Scott, H	234		Srimongkon, P	224
See Hoe, L	140		Staatz, C	131
Selemidis, S	166, 169		Stanners, M	127
Sexton, P	147, 313		Steadman, K	244, 335