Oral abstracts

100

REMAP-CAP: An adaptive platform trial evaluating multiple candidate treatments for severe COVID-19
Prof Steve Webb, Royal Perth Hospital

REMAP-CAP (Randomised, Embedded, Multifactorial, Adaptive Platform Trial for Community Acquired Pneumonia) is a Bayesian adaptive platform trial. This represents a novel and innovative trial design that provides much greater flexibility and efficiency than conventional clinical trial designs. These advantages include testing multiple candidate treatments simultaneously, evaluating treatment-treatment interactions, evaluating treatment effect in pre-specified sub-groups, and the use of frequent interim analyses to answer questions as soon as there is sufficient statistical confidence, not after a pre-specified sample size has been recruited.

This design has been applied in REMAP-CAP to report the treatment effect of 10 candidate treatments, including identification of tocilizumab/sarilumab, corticosteroids, and anticoagulation (in ward patients) as treatments that improve outcome. Other candidate treatments including antivirals, convalescent plasma, antiplatelet agents, and other immune modulators have been identified as either ineffective or harmful.

101

The cardiometabolic phenotype of orphan GPR37L1: not all mice are created equal
Nicola J Smith[1] and Margaret A Mouat[1,2]. School of Medical Sciences, UNSW Sydney[1], Sydney, NSW, Australia; Molecular Cardiology Division, Victor Chang Cardiac Research Institute, Sydney, NSW, Australia.

Introduction. In the absence of identified ligands, transgenic mice lacking an orphan G protein-coupled receptor (GPCR) of interest are often the only way to discern the potential physiological or pathophysiological impact of a particular receptor. For the brain-specific orphan GPR37L1, the first reported knockout mouse displayed a marked elevation in blood pressure and concomitant cardiac hypertrophy, suggesting it could be a master regulator of blood pressure homeostasis. More recent studies, however, have reported roles in neuroprotection and neurodevelopment, while our own studies have found the cardiovascular and an associated cardiometabolic phenotype of GPR37L1 knockout mice to be far more subtle.
Aim. In this presentation, I will describe our efforts to understand the contribution of GPR37L1 to cardiovascular homeostasis and highlight some of the challenges we faced without available pharmacological tools.
Methods. A series of cardiovascular and cardiometabolic measurements were made in adult and aged cohorts of wildtype or *Gpr37l1*$^{-/-}$ mice, including radiotelemetry, micromanometry and crude tissue measurements, or metabolic profiling in high fat diet-fed cohorts (glucose and insulin tolerance tests, body composition measurement by magnetic resonance spectroscopy, and assessment of energy expenditure by indirect calorimetry).
Results. Each of the studies we performed showed evidence for a cardiometabolic role for GPR37L1 in mice, although the phenotypes themselves were subtle and varied between cohorts. We also noted sex-dependent differences in the studies that included both male and female mice.
Discussion. GPR37L1 has been shown to play a role in cardiovascular tone by several laboratories including our own. However, the individual measures that signal this effect vary between studies and can be contradictory even between cohorts within our own group. Although GPCRs are the most successful drug targets on the market, developing therapeutics at orphan GPCRs lacking even synthetic ligands is incredibly challenging. Given the inconsistent and subtle phenotype evident in *Gpr37l1*$^{-/-}$ mice, we recommend that GPR37L1 is not pursued as a therapeutic target for the treatment of cardiometabolic disease.

102

Understanding the physiological consequences of biased agonism at the GLP-1R

Peishen (Elva) Zhao[1], Tin Truong[1], John Azietaku[1], Jie Gao[1], Dana Hutchinson[1], Shelby Cree[1], Patrick Sexton[1,2], Denise Wootten[1,2.] [1]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences[1], Parkville, VIC, Australia.
[2]ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Monash University, Parkville, VIC, Australia

The Glucgon-like-peptide-1 receptor (GLP-1R) respond to peptide hormones involved in the physiology of glucose regulation, gut motility and satiety, and is a validated therapeutic target for the treatment of diabetes and obesity. Approved GLP-1 peptide mimetics have exhibited diverse spectrum of clinical efficacies and all showed (to different degrees) unfavourable side effect profiles. The GLP-1R can pleiotropically couple to multiple intracellular signalling transducers to generate integrated cellular responses. When targeting this receptor, different drugs can selectively promote distinct signalling profiles, a phenomenon named biased agonism. The current project aims to investigate the physiological consequence of GLP-1R biased agonism. We hypotheses that GLP-1R biased agonism may in part explain the differing clinical efficacies of GLP-1R ligands. We characterised the ligand binding properties, transducer coupling, signalling activation and regulation profile of multiple GLP-1R ligands in recombinant cells overexpress GLP-1R. Ligands with distinct *in vitro* bias profile were then assessed with their ability to promote insulin secretion in pancreatic beta cell lines and reduce glucose tolerance in a diet induced obesity model.

Our result indicated that distinct receptor engagement properties correlated well with ligand's ability to promote GLP-1R coupling to cognate Gs proteins and subsequent cAMP pathways, however this correlation is less obvious with other pathways. In vivo glucose tolerance test revealed differential contribution of G protein mediated pathways versus regulatory pathway to GLP-1R mediated physiological response. Our project starting to provide pivotal link and mechanistic insight into how in vitro biased profile can translate into physiological outcomes.

103

Regulation of GPCR Signalling at Adrenergic Receptor

Walter J. Koch. Department of Cardiovascular Sciences and Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA.

Introduction. Adrenergic signalling is critical in the heart as it supports sympathetic input and the "fight or flight" response to the catecholamine neurotransmitters, norepinephrine and epinephrine. In heart failure, the adrenergic system is dysregulated and this includes up-regulation of kinases that turn off adrenergic receptors. These enzymes known as G protein-coupled receptor kinases (GRKs) are the focus of our lab's research and one particular GRK, GRK2, we believe is a culprit behind dysfunctional adrenergic signalling in the failing heart and a target for heart failure therapy.
Aims. For the last 2+ decades our lab has sought to develop therapeutic approaches to target GRK2 for the development of GRK2 inhibition for heart failure.
Methods. We have used small and large animal models to utilize gene therapy approaches and more recently small molecule pharmacological approaches to inhibit GRK2 in the failing heart. Some of these models will be presented.
Results. Published and unpublished results will be presented showing that a novel small molecule inhibitors of GRK2 can be effective in small and large animal models of heart failure and part of the mechanism appears to be reversal of adrenergic dysfunction and improvement of the fight or flight response.
Discussion. We have identified GRK2 as critical regulator of cardiovascular signaling, metabolism, survival and function, which have wide implications for future research in order to elucidate novel roles for GRKs in physiology and pathology. Overall, we have found GRK2 up-regulation in the stressed and injured heart to be pathological and its inhibition for the treatment of heart failure is nearing translation

1. Sato PY, Chuprun JK, Schwartz M, **Koch WJ**: The evolving impact of G protein-coupled receptor kinases in cardiac health and disease. Physiol Rev 95:377-404, 2015.
2. Pfleger J, Gresham K, **Koch WJ**: G protein-coupled receptor kinases as therapeutic targets in the heart. Nat Rev Cardiol 16:612-622, 2019.

104

Resolving inflammation as an emerging approach to treat vascular complications
Chengxue Helen Qin. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC Australia 3052.

Persistent unresolved inflammation has long been recognized to play a fundamental role in the development of vascular complications in cardiometabolic disease, such as hypertension and diabetes. Formyl peptide receptors (FPR) belong to class A G protein coupled receptor family, a master switch to promote the resolution of inflammation, thus represent an attractive “druggable” target for the cardiometabolic disease. This presentation explores the therapeutic potential and molecular mechanism of targeting this group of receptors to resolve inflammation, improves vascular function, and reduce end-organ damage in preclinical rodent models of vascular complications. Our data suggest that “fine-tuning FPR”, such as by biased agonism, improves resolution of inflammation, delay the progression to the end-organ damage, thus has significant therapeutic potential to treat vascular complications.

105

Threshold vs mastery: A new way to teach and assess
Dr Shannan Maisey, UNSW Sydney, NSW

Chemistry is a central science that equips students with core knowledge that underpins many STEM disciplines. This means that ensuring students leave foundational chemistry courses with the minimum knowledge required for their future programs can be difficult. By acknowledging the differences in learners’ abilities, speed, and motivations, Bloom’s mastery for learning model aims to construct a learning environment where learning is broken down to smaller steps where students can learn at their own pace with sufficient practice and corrective instruction before moving on to the next topic.

In this seminar I will describe how we used this mindset to split the syllabus of a first-year chemistry course into threshold (essential, pass grade competencies) and mastery (higher order interwoven competencies of a merit grade) knowledge. This became the foundation for the assessment design and ultimately whole course design, with student being given multiple opportunities to demonstrate a minimum level of threshold competency, before being challenged by more integrated, authentic mastery concepts. I will reflect on the past 3 years of using this approach, applying a lens looking forward at what the future of course and program design in higher education could look like.

106

Skills priorities for a future ready workforce

Kevin DG Pfleger[1,2,3,4]. Biomedical Innovation, The University of Western Australia[1], Nedlands, WA, Australia; Molecular Endocrinology and Pharmacology, Harry Perkins Institute of Medical Research[2], Nedlands, WA, Australia; ARC Centre for Personalised Therapeutics Technologies[3], Australia; Dimerix Limited[4], Nedlands, WA, Australia

The future employment landscape for pharmacologists and toxicologists in Australasia is changing, creating new opportunities for those open to them. An ecosystem of biomedical innovation and entrepreneurship is developing, supported by organisations such as the medtech and pharma industry growth centre, MTPConnect (Pfleger, 2018). The biotech sector was growing even before COVID-19 (Chiroiu et al, 2019), and the events of the last 18 months have only accelerated this expansion. This is creating opportunities beyond the traditional postdoc-to-lab head pathway in academia. Furthermore, as funding success rates diminish for nationally-competitive grant schemes supporting fundamental research, even those remaining in the university and medical research institute environment will need to consider diversifying their income sources in order to sustain their research activity into the future.

So what skills will be required? MTPConnect has commissioned a number of skills gap analysis reports (www.mtpconnect.org.au/reports/redi-skills-gap) to identify the skills priorities for a future ready workforce as part of the Researcher Exchange and Development within Industry initiative. Such skills include understanding of: quality management systems and protocols; manufacturing processes; project management; clinical trials design and monitoring; cybersecurity; data science, bioinformatics and artificial intelligence; regulatory requirements; health economics; end-to-end translation and commercialisation; unmet needs and the clinical context; how to secure investment and industry collaboration; and reimbursement pathways.

Furthermore, and probably most relevant to ASCEPT members, is the shortage of pharmacologists and toxicologists with drug development expertise, a skills priority for which ASCEPT has an important role to play in addressing.

Pfleger K (2018) Australasian Biotechnology 28:72-73
Chiroiu L et al (2019) Australia's Life Sciences Sector Snapshot 2019, AusBiotech

107

Decision-making by reflection: An interprofessional experience.

Aynsley Peterson, He Rau Kawakawa/ The School of Pharmacy, Te Whare Wānanga o Otāgo/ University of Otago, Aotearoa, New Zealand

Interprofessional (IP) learning opportunities are widely recognised as essential components of well-rounded tertiary curricula worldwide. Delivery of large-scale IP education programmes remains an ongoing challenge in our healthcare programmes at Otago. Examples of these include dentistry, medicine, nursing, occupational therapy, oral health, pharmacy and physiotherapy. Logistical challenges such as aligning timetables and availability of room space and facilitators cause many headaches. We recognise that eliminating such barriers would potentially ease IP programme issues and smooth the way to embedding IP programmes more widely throughout our healthcare courses. To this end, we have explored the use of virtual patients to engage students with therapeutic decision-making while working within an IP team. The virtual patients were generated by SimPHARM and operate autonomously via a cloud-based environment. Students may make observations, order lab tests, question the patient and the healthcare team and make therapeutic interventions to treat their virtual patient in order to attain their therapeutic goals. Students can both liaise at mutually agreed times, and leave asynchronous notes for each other in order to ensure team communication is a focus. Following the virtual learning experience, teams then attend a debrief in small groups with a facilitator. Reflecting upon whether their team decisions met both their therapeutic target goals and the patient's needs forms the basis of their learning process. Facilitators are encouraged to use a good judgment approach to guide the student teams during debriefs.

Experience with using virtual patients in IP sessions has shown that some of the previously mentioned logistical barriers can be eliminated (e.g. avoiding the need for scheduled class time and rooms plus no need for facilitators during the simulation session). The reflective debrief remains a vital tool in closing the circle of learning.

108

Targeting GPCRs for the development of new heart failure therapeutics

Lauren T May[1]. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University[1], Melbourne, VIC, Australia.

Introduction. Myocardial infarction (MI) is the leading cause of morbidity and mortality worldwide. There is an unmet need for the development for novel cardioprotective therapeutics that retain efficacy in high-risk populations, particularly those with diabetes and/or of advanced age. Activation of the adenosine A1 receptor (A1R) is recognised as a powerful mechanism to limit myocardial injury and minimise maladaptive remodelling post-MI. However, prototypical A1R agonists have failed to reached the clinic, likely due to dose-limiting A1R-mediated bradycardia, hypotension and atrioventricular block. Biased agonism, which describes the ability of an agonist to promote a distinct signalling fingerprint as compared to a prototypical A1R agonist, offers the opportunity to selectively design new therapeutics that stimulate cardioprotection whilst avoiding unwanted effects.
Aims. To elucidate the mechanism underpinning A1R biased agonism and assess the influence of advanced age on *in vivo* cardioprotection and cardiovascular adverse effects stimulated by prototypical and biased A1R agonists.
Methods. This study employed a multidiscipline approach, including *in vitro* biosensor signalling assays and an *in vivo* model of myocardial ischaemia-reperfusion in aged (70-72 weeks old) rats.
Results. A1R biased agonists stimulated slower kinetics of G protein activation and subsequent Gβγ-effector interactions, relative to non-biased agonists. As such, a kinetic mechanism appears to underpin A1R biased agonism. In an aged rat model of MI, in contrast to the prototypical agonist, the biased A1R agonist significantly reduced infarct size. Moreover, while the prototypical A1R agonist significantly reduced blood pressure and heart rate upon infusion, the biased agonist had minimal effect.
Discussion. Our A1R biased agonist retains cardioprotective efficacy with minimal effect on heart rate or blood pressure in an *in vivo* model of myocardial ischaemia-reperfusion in aged (70-72 weeks old) rats. The kinetic mechanism that appears to underpin the biased profile offers the opportunity to design clinically relevant A1R agonists for cardiac disease and may offer a framework to design more effective therapeutics for other GPCRs.

109

Bromodomain-containing protein inhibition as a therapeutic strategy in heart failure

Simon R Foster[1,2], Richard J Mills[1], Mary Lor[1], Jessamine E Hazlewood[1], Rebecca L Fitzsimmons[1], James E Hudson[1]. QIMR Berghofer Medical Research Institute[1], Brisbane, QLD, Australia. Dept of Pharmacol[2], Biomedicine Discovery Institute, Monash University, Clayton, VIC.

Introduction. Inflammation is a hallmark feature of chronic heart failure, yet mechanisms and therapeutics for inflammation driven cardiac pathology are lacking. Our team has recently published our discovery of the critical pathways driving inflammation-induced heart dysfunction (Mills RJ et al, 2021), including a central role for the epigenetic regulator bromodomain-containing protein 4 (BRD4). Indeed, pharmacological inhibition of BRD4 recovers dysfunction in human cardiac organoids (hCO) and prevents cytokine-storm mediated cardiac dysfunction in mice. The downstream effectors of BRD-induced dysfunction require further investigation.
Aims. To characterise determine the effectors that control inflammation-BRD-induced cardiac dysfunction in human cardiac organoids.
Methods. Multi-cellular human pluripotent stem cell (hPSC)-derived cardiac organoids were exposed to a cytokine storm cocktail in the absence or presence of Bromodomain and extraterminal domain family inhibitors (BETi) and assessed for cardiac function, transcriptional changes and profiled for cytokine and chemokine release using multiplexed Luminex assays.
Results. 24 hr exposure to cytokine storm induced an inflammatory gene program and led to pronounced dysfunction in hCO, including a 16% increase in contraction duration that was completely blocked by treatment with three different BETi (One-way ANOVA, $p < 0.0001$). Similarly, cytokine storm elicited marked increases in inflammatory cytokine secretion (e.g. IL-6 and CCL5) that were significantly attenuated with BETi treatment ($p < 0.001$).
Discussion. These findings shape our mechanistic understanding of the therapeutic efficacy of BETi in the context of inflammation-induced cardiac dysfunction. Our current work is focussed on delineating the signalling networks that cause BET-mediated dysfunction in the heart.

Mills RJ et al (2021) BET inhibition blocks inflammation-induced cardiac dysfunction and SARS-CoV-2 infection. Cell, 184: 2167–2182.

110

Pharmacological Development of new medicines for heart failure

Peter Molenaar[1,2], Melanie Spratt[1,2], Alex Dashwood[1], Elizabeth Cheesman[1], Nicole Beard[3], Derek R Laver[4]. Northside Clinical School of Medicine, University of Queensland[1], The Prince Charles Hospital, Brisbane, QLD; School of Biomedical Sciences, QUT[2], Brisbane, QLD; Centre for Research in Therapeutic Solutions and the Faculty of Science and Technology, University of Canberra[3], Bruce, ACT; School of Biomedical Sciences and Pharmacy, University of Newcastle and Hunter Medical Research Institute[4], Callaghan, NSW.

Introduction. There is an urgent need for new therapies to reduce mortality of patients with heart failure (HF). Despite advances in medications, mortality and hospital readmission rates have remained largely unchanged with survival rates of ≤ 40% at 5 years from diagnosis. Heart failure patients are at significant risk of sudden cardiac death from ventricular arrhythmia (VA). Sudden cardiac death (SCD) from VA is the leading (45-50%) mode of death. Elucidation of signaling pathways responsible for SCD is fundamental for the development of new medicines.
Aims. The aim of our studies was to elucidate signaling pathways responsible for SCD in heart failure patients in order to identify new targets for drug treatment for the development of new medicines.
Methods. Human hearts from patients with heart failure undergoing heart transplantation were used for investigation. Ventricular trabeculae were used for contractility and arrhythmia experiments. Ryanodine Receptors (RyR) were used to investigate Ca^{2+} channel release and RyR post-translational protein properties.
Results. Activation of both β_1- and β_2-adrenoceptors (AR) caused arrhythmic contractions which were increased by inhibition of PDE3 and PDE4, phosphorylation and increased probability of RyR channel opening. Phenytoin reduced the occurrence of arrhythmic contractions. Phenytoin selectively reduced diastolic Ca^{2+} leak from RyR.
Discussion. PDE activators and RyR inhibitors provide promising new classes of medicines to treat heart failure. The use of non-selective βAR blockers (carvedilol) could offer protection against β_2AR mediated arrhythmias.

Ashna A et al (2020) Mol Pharmacol 97:250-258

111

Novel approaches to heart preservation during transplantation - Hypothermic Ex Vivo Perfusion

Louise E See Hoe[1,2], Karin Wildi[1,2], Mahe Bouquet[1,2], Kei Sato[1,2], Kris Skeggs[3], Margaret Passmore[1,2], Nicole Bartnikowski[1,4], Nchafatso Obonyo[1,2], Sebastiano M Colombo[1], Kieran Hyslop[1,2], Matthew Wells[1,5], Carmen Ainola[1,2], Emily Wilson[1,2], Charles McDonald[1], Silver Heinsar[1,2], Sanne Engkilde-Pedersen[1], Sara Diab[1,2], Gabriella Abbate[1,2], Hollier O'Neill[1,2], Janice Reid[1,2], Tristan Shuker[1,2], Meredith Redd[1], Jae-Sueng Jung[1], Andrew Haymet[1,2], Samantha Livingstone[1,2], Noriko Sato[1,2], Jonathan Millar[1,2], Maximillian Malfertheiner[1], Sacha Rozencwajg[1], Xiomeng Wang[1,2], Yevgeniy Shek[1], Lynnette James[3], Ting He[3], David Mullin[1], David Platts[1], Ai Chi Boon[1,2], Debra Black[1,2], Olivia Zekovic[1,2], Leticia Helms[1], Lucy Bradbury[1,2], Haris Haqqani[1,2], Jason Peart[1,5], Peter Molenaar[2,6], Gianluigi Li Bassi[1,2], Jacky Suen[1,2], David McGiffin[1,7,8], John Fraser[1,2]. Critical Care Research Group, the Prince Charles Hospital[1], Brisbane, QLD, Australia; Faculty of Medicine, University of Queensland[2], Brisbane, QLD, Australia; Princess Alexandra Hospital[3], Brisbane, QLD, Australia; School of Mechanical, Medical and Process Engineering[4], Queensland University of Technology, Brisbane, QLD, Australia; School of Pharmaceutical and Medical Sciences, Griffith University[5], Southport, QLD, Australia; School of Biomedical Sciences, Queensland University of Technology[6], Brisbane, QLD, Australia; Cardiothoracic Surgery and Transplantation, The Alfred Hospital[7], Melbourne, VIC, Australia; Monash University[8], Melbourne, VIC, Australia.

Introduction. Cold static storage (CSS) is the standard method for heart preservation during transplantation (HTx). However, CSS beyond 4 hours increases the risk of primary graft dysfunction (PGD). Hypothermic ex vivo perfusion (HEVP) of donor hearts allows oxygen delivery during preservation, and may facilitate extended donor preservation without increasing PGD risk.
Aims. We sought to compare post-HTx survival, haemodynamic function, systemic inflammation and cardiac function following donor heart preservation by CSS (2 hrs) versus HEVP (2 and 8 hrs).
Methods. Brain death was induced in donor sheep for 24 hrs. Donor hearts were preserved by a) CSS for 2 hrs (n=8), b) HEVP for 2 hrs (n=6), or c) HEVP for 8 hrs (n=7). Orthotopic HTx was performed in matched recipients. Recipients were weaned from cardiopulmonary bypass and monitored for 6 hrs. Recipient blood was collected and assayed for inflammatory cytokines and cardiac markers. Cardiac function was assessed by echocardiography.
Results. Six-hour survival was 75% following CSS, and 100% following 2 and 8hrs HEVP, respectively. Recipients systemic interleukin-6, 10 and 8 levels were reduced using HEVP vs CSS. Post-HTx haemodynamic function was no different between groups, but HEVP recipients required less vasoactive support to maintain adequate haemodynamic function compared to CSS. HEVP was associated with reduced post-HTx lactate, more stable base excess and physiological pH in blood. Post-HTx cardiac function and cardiac troponin I levels were comparable between groups.
Discussion. Preliminary data on donor heart preservation by HEVP shows promising outcomes in comparison to CSS. Heart preservation by HEVP can be extended up to 8 hours, without compromising post-HTx recipient survival. HEVP may assist in overcoming limitations in preservation time associated with HTx, without increasing PGD risk.

112

Development of a novel practical class using crickets to demonstrate the pharmacological actions of drugs for third year neuropharmacology students

Nicole M Jones[1], Jane E Carland[1], Balu Daniel[1], Angela M Finch[1], Ghada Hanna[1], Natasha N Kumar[1]. Department of Pharmacology, School of Medical Sciences, UNSW Sydney[1], Kensington, NSW, Australia.

Introduction. The domestic cricket (*acheta domesticus*) is an invertebrate organism that can be used to investigate neurobiology, physiology and behavior (Stevenson et al. 2000). Crickets use many of the same neurotransmitter systems as mammals, allowing their use as a model organism to teach pharmacological principles of drug action in an *in vivo* system. Locomotor activity in crickets is easily observable in a lab environment by naïve experimenters. We developed a practical class to provide students with hands-on experience at determining the actions of three central nervous system (CNS) active drugs in crickets (nicotine, caffeine and ethanol).
Aims. To employ an invertebrate model (crickets) to demonstrate pharmacological action of CNS active drugs in an *in vivo* system to enhance student learning and engagement.
Methods. Adult crickets of both sexes were used. Each student group (4-5 students, ~21 groups) was provided with 4 crickets for the experiment. Baseline locomotor activity (time moving in the horizontal plane) of each cricket was measured for 15 minutes before intra-abdominal injection (20µl) of drug (water (control), nicotine (0.1 mg/ml), caffeine (50µM) or alcohol (30% w/v solution)). Locomotor activity of each cricket was then measured for a further 15 minutes. Student groups then graphed their data and answered questions about the data and drug mechanisms. A second class in which students identified an unknown drug using pharmacological tools was also developed.
Results. In the course feedback (2021), many students identified some of the "best features of the course" as the hands-on aspects of these cricket practical classes. Some of the experimental results were unexpected, which prompted interesting class discussions and helped to enhance student understanding of drug mechanisms in relation to the experimental data. This was evident in student responses to an exam question on this material.
Discussion. This innovative new practical class allowed students to advance their lab skills using an invertebrate model to examine the *in vivo* effects of various CNS active drugs in a 3 hour practical class. This approach is readily adaptable to showcase behavioral responses to many different classes of CNS drugs.
Stevenson AP, et al (2000) Journal of Neurobiology 43, 107-120.

113

Attendance but not delivery mode correlates with academic performance in a newly developed pharmacology subject

Elvan Djouma, Antony Vinh and Ross D. O'Shea. Dept of Physiol, Anatomy and Micro, School of Life Sci, La Trobe Uni, Bundoora, VIC, Australia

Introduction. Given the continued challenges of teaching impacted by COVID-19 lockdowns and the desire to increase flexibility for students, we delivered a new pharmacology subject where students could choose their mode of attendance for workshops (face-to-face [F2F] or online) using La Trobe's 'StudyFlex' model.
Aims. To investigate whether workshop teaching modality and attendance are associated with differential academic outcomes for students undertaking this pharmacology subject.
Methods. 138 students were enrolled in the subject 'General Principles of Pharmacology' in Semester 1, 2021: 52 students selected F2F workshops, while 86 students selected the online option. Workshops consisted of a 2-hour weekly class where all students had access to the workshop notes in advance on the Learning Management System (LMS). For F2F workshops, students worked in teams in class while the online group worked in breakout rooms via Zoom. The same academics conducted all workshops for both groups, regardless of mode. All other learning materials were delivered online via the LMS. We compared academic performance for both F2F and online students as measured by results on the individual assessments (two exams worth 60% of the total) and the overall subject mark, which included additional individual and team assessments. Workshop attendance for both groups was taken 15 minutes after the start of workshops, and the correlation between attendance and marks was analysed.
Results. Overall workshop attendance was not different between the two modes of delivery (66% F2F vs 70% online, $P > 0.05$) and there was no difference between test marks or overall marks across cohorts (unpaired t-tests, both $P > 0.05$). Non-parametric Spearman correlations revealed a significant correlation between the percentage of workshops attended and both test marks and overall marks for students in both modes of attendance (all $P < 0.05$).
Discussion. The mode of workshop attendance had no significant effect on academic performance, but greater workshop attendance, independent of mode, was correlated with improved academic performance. This analysis highlights the utility of online workshops, which were equally effective as F2F classes in pharmacology teaching in this subject.

114

Using a design thinking approach to develop a large-scale, inter-professional medication safety micro-curriculum

Kellie A. Charles[1,2], Nicholas Buckley[1], Sarah Hilmer[1,3], Lisa Koulajian O'Donnell[3], Rebekah Moles[4], Stephen Carter[4], Astrid Frotjord[5], Carl Schneider[4]. 1. Discipline of Pharmacology, School of Medical Sciences, 2. Medical Education Unit, Sydney Medical School, 3. Northern Clinical School, Sydney Medical School 4. Sydney Pharmacy School, 5. Sydney Nursing School, University of Sydney[1,2,3,4,5], Sydney, NSW, Australia.

Introduction. Medication safety is a core principle for all professionals involved in safe and effective management of drugs. Medication management is taught traditionally in silos within health professional degree programs. Inter-professional education enables students from multiple professions to learn core clinical and teamwork skills within authentic clinical teams. Medication management provides an exemplar activity for interprofessional education.
Aims. To develop an innovative medication safety module for medical, pharmacist and nursing students to better understand the impact of team-based approaches to quality use of medicines and safe medicines prescribing practice.
Methods. We used design thinking principles (empathise, ideate, prototype and evaluate) to iteratively implement and test individual components prior to delivering a fully integrated, multi-week, inter-professional medication safety module within the MD, B.Pharmacy and B.Nursing degrees, (n=650 students) in Sem 1, 2021. Students follow the medication management cycle (medical students prescribe, then medication review by pharmacy and nursing students) as well as coming together in person to review as an inter-professional team. Student experience, knowledge, skills and attitudes to patient safety, interprofessional teamwork and clinical pharmacology were investigated before and after the module. All medication chart and progress notes were analysed for session debriefing of quality use of medicines and feedback on creating highly effective healthcare teams.
Results and Discussion. There was variable confidence in core pharmacology knowledge and application of this knowledge within each of the degree (44% - 55%) prior to the module. Improvements in medication charts were observed following team review. Teaching in the Medication Safety Module was rated by students has highly effective (85% agree) and increased the confidence in core pharmacology knowledge and application by 11% and 15% across the whole cohort. Further evaluation with a realist model is ongoing to investigate the relationships between contexts and mechanisms explaining how inter-professional teamwork generates improvements in the education of the quality use of medicines.

115

Upskilling Teaching Associates: a bespoke Virtual Teaching Associate Training program

Betty Exintaris, Nilushi Karunaratne, Stefan Huth, Thao Vu, Steven Walker, Zoe Ord, Lorenna Reynolds, Harry Al-Wassiti. Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia.

Introduction. Teaching Associates (TAs) play a critical central role in student learning, student engagement and therefore, the overall student experience, yet university faculties have limited time and resources to adequately mentor TAs, many of whom have little to no teaching experience [1]. At the Faculty of Pharmacy and Pharmaceutical Sciences (FPPS) at Monash University, a need for goal-oriented training focusing on orientation, induction, TA teaching responsibilities and fostering the development of the individual TA was recognised.
Aims. To design, develop and implement a flexible, evidence-based, self-regulated Virtual Teaching Associate Training Program with a focus on learner engagement to train and support the development of TAs at the FPPS.
Methods. The program was designed with learner engagement and preparedness in mind, whilst incorporating a variety of content formats, knowledge checks, reflective practice and peer-to-peer feedback. The resource developed contained 5 distinct training modules each focusing on a specific aspect of TA: 1) General training; 2) Understanding Students and the Learning Environment; 3) Degree-specific Learning and Teaching; 4) Facilitating Learning; 5) e-Portfolio (fostering the development of the individual TA).
Results. The program, across 6 semesters over 3 years, has directly influenced student learning and engagement by helping TAs to be more prepared to teach better. Anonymous feedback from TA participants (response rate: >80%) show that the majority of TAs felt better prepared for teaching and learnt new skills to teach better. TAs' ability to apply strategies to ask questions and respond to student questions was clearly demonstrated from student feedback in SETU and faculty-based student feedback surveys. Feedback from Unit Coordinators has shown the positive influence of TAs on student engagement. This program has been used to train nearly 200 individual TAs.
Discussion. This training program has provided TAs an opportunity to gain the confidence and skills to give them a successful start into teaching practice, regardless of their background and prior experience. Furthermore, improving training for TAs not only benefits the TAs, who are largely PhD students, but also results in benefits to various stakeholders impacted by the quality training of TAs.
1. Komarraju.2008. A Social-Cognitive Approach to Training TAs. Teaching of Psychology; 35(4): p.327-334.

116

Nimble pharma®: Pandemic pivoting to a synchronous proxy practical class examining new treatments for Covid-19

Graham A. Mackay[1], Makhala M. Khammy[1], Mark D. Habgood[1], Alastair G. Stewart[1]. [1]Dept. of Biochemistry and Pharmacology, The University of Melbourne, Parkville, VIC 3010.

Introduction. For many years, we have run a practical class where students develop an assay suitable for running a high-throughput screen (HTS) to identify inhibitors of the serine protease thrombin. The pitch of this task is pharma/biotech-related, with a focus on developing new anti-thrombotic drugs. With the Covid-19 pandemic, this task was pivoted to reflect another important facet of the pharma industry: seizing new opportunities.
Methods. We used two 4-hour practical sessions, separated by discussion-based Zoom workshops. Given restricted practical laboratory access, students participated via Zoom breakout rooms each with a designated demonstrator who conducted the experiments on their behalf (by proxy). Demonstrators were instructed to follow guidance from student teams, and whilst offering advice, were encouraged to complete as instructed even when knowing a poor outcome was likely. Thrombin activity was quantified using two substrates with platereader-based data output.
In the first session, students varied experimental conditions to optimise the assay. Results were returned to student teams via Lab Archives electronic notebooks in real time, with students discussing, plotting data and calculating Z' factors to identify assay conditions optimal for HTS. In usual years, the second session would see progression to screen a series of 10 unknown compounds in a mock-HTS. However, in 2021 this session was pivoted to an industry-focused scenario where students were presented with a series of serine protease inhibitors that had been identified to inhibit the COVID-19 target, transmembrane serine protease 2 (TMPRSS2). Their task was to screen these compounds using their assay, to determine if off-target thrombin inhibition might be a significant adverse effect of these compounds.
Results. Whilst not formally assessed, student teams engaged strongly in the proxy class, interacting effectively with demonstrators and academic leads, analysing data promptly and displaying clear connection to 'their' data sets.
Discussion. Whilst not a replacement for the hands-on skills of the practical class, the proxy-based approach provided an authentic opportunity for students to engage with a highly relevant and much publicised therapeutic scenario. Importantly, the by-proxy nature of the class also allowed the students the opportunity to 'fail' in their experimental design and then identify and correct mistakes with all the long-lived learning that this experience provides.

117

Characterisation of chemokine GPCRs signalling in monocytes using state-of-the-art phosphoproteomics approach

Rina Pokhrel[1], Simon R Foster[1], Cheng Huang[1,2], Anup D Shah[1,2,3], Ralf B Schittenhelm[1,2], and Martin J Stone[1]. Biomedicine Discovery Institute & Dept of Molecular Biology and Biochemistry,[1] Monash Biomedical Proteomics Facility[2], Monash Bioinformatics Platform[3], Monash University, Clayton, VIC, Australia

Introduction. Chemokine receptors are G protein-coupled receptors (GPCRs) expressed on the surface of leukocytes that bind to chemoattractant cytokines called chemokines. They are the key player in inflammation. Chemokine-receptors interaction is a crucial step in recruiting monocytes at the inflammation site for regulation of inflammatory responses. Monocytes abundantly express CCR1 and CCR2 receptors, which are potential therapeutic targets in inflammatory diseases including atherosclerosis and rheumatoid arthritis. Lack of effective anti-inflammatory drugs and failure of many clinical trials till date could be due to complexity in the chemokine-receptor signalling network.
Aim. To characterise the signalling network of robustly activated chemokine receptors in monocytes using state-of-the-art phosphoproteomics and computational modelling approaches.
Methods. We carried out well-established cell-signalling assays, including chemotaxis (96- well MultiScreen plates, Merck) and ERK phosphorylation (AlphaLISA Surefire Ultra, Perkin Elmer) in monocyte-like THP-1 cells expressing CCR1. To characterise downstream CCR1-dependent signalling events, we performed a phosphoproteomics study using data-dependent acquisition (DDA) mass spectrometry to quantify changes in phosphopeptides between untreated and CCL5-stimulated THP-1 cells.
Results. We have previously demonstrated that the chemokines, CCL5 and CCL7 elicited a concentration-dependent increase in chemotaxis of THP-1 cells and phosphorylation of ERK. A CCL5 stimulation time course monitored by phosphoproteomics, revealed regulation of 931 phosphosites and 534 unique proteins (1-way ANOVA with FDR <0.05), including phosphorylation of CXCR2. A similar study showed that treatment with CCL2, acting via the receptor CCR2, resulted in regulation of 460 phosphosites and 329 unique proteins, including phosphorylation of CCR1.
Discussion. These findings provide critical insights into complexity of chemokine receptor signalling cascade. The extensive signalling network identified from protein phosphorylation data includes expected chemokine receptor signalling pathways as well as previously unrecognised signalling mechanisms therefore, result to potential novel target related to CCR1 and CCR2 signalling.

118

The cardiometabolic expression profile of the sweet taste receptor

Mariah R Stavrou[1], Angela M Finch[1], Nicola J Smith[1]. Dept of Pharmacol, School of Medical Sciences, UNSW Sydney[1], Sydney, NSW.

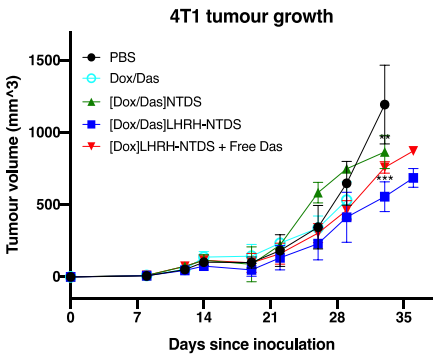
Introduction. The sweet taste receptor (STR) is a family C G protein-coupled receptor responsible for cellular responses to sweet stimuli. The functional heterodimer, consisting of the TAS1R2 and TAS1R3 subunits, localises with the G protein α-gustducin in taste-sensory epithelium. It is commonly reported that elements of the STR signaling system are present in cardiometabolic tissues, including pancreatic β-cells, enteroendocrine cells and adipocytes, suggesting a role in nutrient sensing and metabolic regulation. However, these studies often rely on poorly validated antibodies or RNA-sequencing in rodent models.
Aims. To use publicly available datasets to compare STR mRNA expression in human pancreatic β-cells, enteroendocrine cells and adipocytes, and to determine whether this expression is likely physiologically significant.
Methods. Human gene expression data was mined from large-scale and tissue-specific RNA-sequencing studies. Sequencing counts were extracted as transcripts per million RNA reads for TAS1R2, TAS1R3, GNAT3 and control genes including: (1) marker genes ubiquitously expressed in the specific tissues tested, (2) other GPCRs with known physiological roles in the specific tissues tested, (3) other GPCRs with known expression exclusively outside the tissues of interest. Housekeeping genes with minimal variation between tissues were chosen for normalisation.
Results. Gene expression of STR signalling elements in human cardiometabolic tissue rarely exceeded 0.5 transcripts per million RNA reads, which was negligible when compared to physiologically active genes.
Discussion. We found scant evidence for physiologically-relevant STR gene expression levels in human cardiometabolic tissue, particularly when measured against GPCRs ubiquitously expressed in these tissues, suggesting that the STR is unlikely to be a promising target for the treatment of cardiovascular and metabolic diseases. Our results may reflect a discrepancy between human and rodent models for analysis of STR expression and function, as rodent models appear to be the default choice for studies that claim extra-oral expression is substantial.

119

Novel targeted nanotheranostic delivery systems for precision therapy of triple negative breast cancer

Pegah Varamini[1,2]. Behdad Delavari[1], Bahareh Bigdeli[1]
Sydney Pharmacy School, University of Sydney, Sydney, NSW, Australia [1]
The University of Sydney Nano Institute, University of Sydney, Sydney, NSW, Australia[2]

Introduction. Triple-negative breast cancer (TNBC) represents 15–20% of all breast cancers and tend to behave more aggressively with the worst survival rate compared to other breast cancer subtypes (Garrido-Castro et al., 2019). Chemotherapy is the mainstay of treatment to which TNBC cells exhibit a high level of intrinsic/acquired resistance (Vagia et al., 2020). The presence of breast cancer stem-like cells (BCSCs), which are intrinsically resistant to chemotherapy (Park et al., 2019), and the expression of P- glycoprotein (P-gp) play a key role in conferring resistance to cytotoxic and targeted chemotherapy in TNBC (Abd El-Aziz et al., 2021).
Aims. We aimed to develop a novel therapeutic/diagnostic technology (nanotheranostic) that specifically delivers a chemotherapy agent combined with an anti-BCSC drug. The second agent has also been shown to inhibit P-gp overexpression in TNBC cells, potentially overcoming therapy resistance in TNBC patients and improving their survival. Thus, our novel targeting approach will improve TNBC patients' quality of life.
Methods. This nanotheranostic technology was fabricated and examined in various cell-based and animal pharmacological models of TNBC.
Results and discussion. The new targeted nanotheranostic technology showed a synergistic anticancer activity of the two combined agents with a significant increase in the efficacy of chemotherapy in preclinical models of TNBC. The successful development of this nanotheranostic technology will have a far-reaching impact on the survival and quality of life of TNBC patients.



Abd El-Aziz, Y.S., et al. (2021) Biosci Rep 41.
Garrido-Castro, A.C., Lin, N.U., Polyak, K. (2019) Cancer Discov 9, 176-198.
Park, S.-Y., Choi, J.-H., Nam, J.-S. (2019) Cancers 11, 965.
Vagia, E., Mahalingam, D., Cristofanilli, M. (2020) Cancers 12, 916.

120

Afatinib is a novel and effective drug candidate for the treatment of human uveal melanoma

Fanfan Zhou[1], Wenying Shu[1,2], Xue Zhu[3], Ke Wang[3], Svetlana Cherepanoff[4], R. Max Conway[5], Michele C Madigan[6,7], Hong Zhu[8], Ling Zhu[6], Michael Murray[1]. Sydney Pharmacy School, Univ of Sydney[1], NSW; Dept of Pharmacy, Guangzhou Medical Univ[2], Guangzhou, China; Jiangsu Institute of Nuclear Medicine[3], Wuxi, China; SydPath, St Vincent's Hospital[4], Sydney, NSW; Sydney Eye Hospital[5], Sydney, NSW; Save Sight Institute[6], Sydney, NSW; School of Optometry and Vision Sciences, Univ. of NSW[7], NSW; College of Pharmacy, Zhejiang Univ.[8], Hangzhou, China.

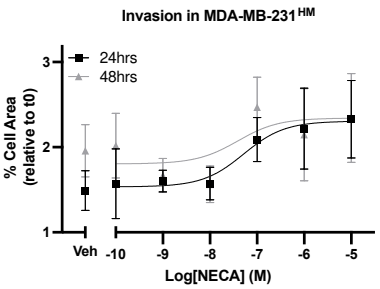
Introduction. Uveal melanoma (UM) is the most common intraocular malignancy in adults and has both a poor prognosis and high rate of recurrence. At present there are no effective treatments for primary or metastatic UM. Multi-kinase inhibitors that target dysregulated protumorigenic signaling cascades have revolutionized the treatment of a range of cancers, but their efficacy in UM has not been established.
Aims. A screen of multi-kinase inhibitors identified afatinib as a highly effective agent in decreasing UM cell viability. Accordingly, the anti-UM efficacy of afatinib was assessed in greater detail using in vitro, ex vivo and in vivo models.
Methods. Cell viability, cell death and cell cycle assays were used to evaluate the anti-cancer actions of afatinib, while cell migration and reproductive death assays were performed to assess its anti-metastatic activity. The altered expression of signaling intermediates was assessed to characterise the cell killing mechanisms and molecular targets of afatinib in UM. The anti-cancer actions of afatinib were also evaluated in a UM xenograft mouse model.
Results. Afatinib markedly activated apoptosis and cell cycle arrest in UM cell lines and in patient tumor-derived primary cell lines. Afatinib also impaired cell migration and enhanced reproductive death in UM cells. At the molecular level afatinib-induced cell death was accompanied by activation of STAT1 and downstream inhibition of the anti-apoptotic Bcl-xL and proliferative cyclin D1 signaling factors that control cell survival and cell cycle progression. Afatinib attenuated the activation of HER2-AKT/ERK/PI3K signaling in UM cell lines, which may be an early event underlying its anti-cancer actions. Consistent with these findings, afatinib also suppressed tumor growth in vivo in mice carrying UM xenografts.
Discussion. Afatinib has emerged as a novel and effective candidate drug for the treatment of UM and the prevention of metastasis. Afatinib activates UM cell death and targets the HER2-AKT/ERK/PI3K cascade, which modulates STAT1-Bcl-xL/cyclin D1 signaling. From these findings, targeting HER2 may be a novel therapeutic strategy in UM.

121

The adenosine A_{2B} receptor accelerates invasion in aggressive triple negative breast cancer tumour cells

Sandra Sursock, Terrance Lam, Bonan Liu, Chantel Mastos, Alastair C Keen, Michelle L Halls. Drug Discover Biology, Monash University, Melbourne, VIC, Australia.

Introduction. High levels of adenosine are present in the tumour microenvironment. Adenosine signals via a family of four G protein-coupled receptors, the A_1, A_{2A}, A_{2B}, and A_3 receptors, to activate a range of signalling pathways. These receptors are therefore emerging cancer targets. However, it remains unknown whether the effect of adenosine receptor activation changes during the progression of breast cancer.
Aims. To determine whether adenosine receptors differentially affect invasion and whether adenosine receptor stimulation results in distinct signaling profiles between normal MDA-MB-231 tumour cells (parental) compared to a highly metastatic variant of the same cells (MDA-MB-231^{HM}).



Methods. Adenosine receptor expression in both cell types was determined using qRT-PCR. Signalling downstream of adenosine receptor activation was determined by measuring cAMP accumulation and calcium mobilisation. Finally, we measured the effect of adenosine on cell invasion and proliferation.
Results. MDA-MB-231 parental cells express A_{2A} and A_{2B} receptors, whereas the HM cells only express A_{2B} receptors at the RNA level. A stable adenosine analogue, NECA, increases cAMP and calcium in both cell types. However, NECA accelerates invasion only in the HM cells; there was no effect of adenosine receptor activation on invasion of the parental cells (p=0.0005 and p=0.6112, respectively; maximum NECA invasion vs vehicle control, two-way ANOVA with Sidak's post-test, n=4-6). There was no effect of adenosine receptor activation on proliferation in either cell type.
Discussion. While adenosine receptors couple to the same signalling pathways in the two cell types, there is an increase in invasion in response to NECA only in the HM cells. This suggests that either the A_{2B} receptor can also activate other signalling in the HM cells, or that the signals occur in a different location inside the cell that links to invasion.

122

Combined Antiplatelet/Anticoagulant Drug For Cardiac Ischemia/Reperfusion Injury

Laura A Bienvenu[1,2,3], Ana Maluenda[1,2], James D McFadyen[1,3,4], Amy K Searle[1,2,4], Eefang Yu[1,2,4], Carolyn Haller[5,6], Elliot L Chaikof[5,6], Karlheinz Peter[1,3,4,#,^], Xiaowei Wang[2,3,4,#,^]. [1]Atherothrombosis and Vascular Biology Laboratory; [2]Molecular Imaging and Theranostics Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia; [3]Cardiometabolic Health, University of Melbourne, VIC; [4]Medicine, Monash University, Melbourne, VIC; [5]Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston; [6]Wyss Institute of Biologically Inspired Engineering of Harvard University, Boston; [#, ^]equally contributing senior authors.

Introduction: Myocardial infarction (MI) is typically caused by rupture of atherosclerotic plaques leading to thrombotic occlusion of coronary arteries. Reperfusion of the artery results in further tissue damage, known as ischemia/reperfusion (I/R) injury, by provoking major inflammatory and microthrombotic responses, leading to loss of cardiac function. Current antiplatelet/anticoagulant drugs, particularly in combination, are associated with bleeding complications representing a major cause of mortality/morbidity. Aims: To target anticoagulant activity at the site of platelet accumulation in the I/R heart to improve cardiac function post-MI. Methods: We developed a single-chain antibody (scFv), which specifically binds to activated glycoprotein GPIIb/IIIa, which mediates platelet crosslinking/aggregation and thrombus formation, and genetically fused it to tick-anticoagulant-peptide (TAP), a FXa inhibitor, generating a unique dual-function antiplatelet/anticoagulant drug (Targ-TAP). Results: Using a cardiac I/R mouse model (occlusion of the left anterior descending artery of mice for 60 min) we showed that Targ-TAP improved cardiac function 4wks post-MI (increased ejection fraction and fractional shortening, less cardiac strain) compared to non-targeted control (Mut-TAP) and PBS. This protection of cardiac function by Targ-TAP correlated with a significantly reduced infarct size in comparison with controls, as assessed by Evans Blue/triphenyltetrazolium chloride staining. Confirming that Targ-TAP does not exhibit systemic effects on hemostasis, mice treated with Targ-TAP did not display prolonged tail bleeding times or increased blood loss. Discussion: We describe Targ-TAP as a highly effective anti-thrombotic drug uniquely combining localized antiplatelet and targeted anticoagulant effects while preserving hemostasis for the treatment of cardiac I/R injury.

123

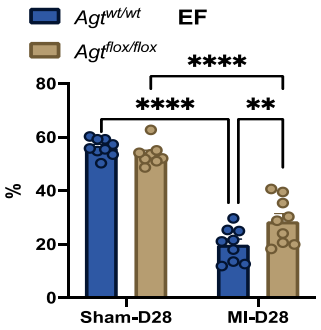
Inactivation of the local cardiac renin angiotensin system improves cardiac performance after myocardial infarction

Zunhan Lu, Yusuke Yoshikawa, Monique Lowes, Melisa Reichelt, Walter Thomas. School of Biomedical Science, University of Queensland, Brisbane, QLD, Australia.

Introduction. The renin-angiotensin system (RAS) regulates blood pressure via angiotensin II (Ang II), which is generated in blood from angiotensinogen (AGT). In addition to the blood borne RAS, a local cardiac RAS has been identified that is activated following heart injury. Based on RNAseq data from isolated cardiac cells (1), AGT is primarily expressed from cardiomyocytes.

Aims. We aimed to examine the role of the local cardiac RAS in the remodelling process post-myocardial infarction (MI) by specifically deleting AGT from adult cardiomyocytes to prevent local activation of the RAS.

Methods. Four weeks prior to myocardial infarction $AGT^{wt/wt}$ and $AGT^{fl/fl}$ mice were injected with adeno-associated virus (AAV9), which drives the expression of Cre enzyme specifically in cardiomyocytes. The expression of transgenes and AGT deletion were confirmed by RT-qPCR; fibrosis was analysed by histological staining and cardiac function post MI was assessed by echocardiography.



Results. The AAV-Cre approach successfully deleted AGT in cardiomyocytes. This AGT knockdown reduced MI-induced inflammatory, hypertrophic, and fibrotic responses at 7 days after MI. At 28 days after MI, control mice ($AGT^{wt/wt}$) showed profound impairment of cardiac output, stroke volume and ejection fraction (see figure), whereas AGT deleted $AGT^{fl/fl}$ mice showed significantly improved systolic cardiac function.

Discussion. These results indicate a functional, local cardiac RAS, which is active following myocardial infarction and contributes to the fibrosis and functional impairment associated with cardiac damage/repair. The findings of this study provide fundamental insights into the contribution of the local RAS in the setting of cardiac pathology and may have clinical relevance when considering local versus systemic RAS inhibition.

1.Quaife-Ryan Gregory A, Sim Choon B, Ziemann M, Kaspi A, Rafehi H, Ramialison M, et al. Multicellular Transcriptional Analysis of Mammalian Heart Regeneration. Circulation. 2017;136(12):1123-39.

124

Activation of the Angiotensin II Type 2 Receptor Prevents Progression of Cardiac Fibrosis associated with Hypertension and Concomitant Diabetes

Mandy Li[1], Levi Nguyen[1], Dorota Ferens[1], Iresha Spizzo[1], Chengxue Helena Qin[2], Tracey Gaspari[1], Robert Widdop[1], Dept. of Pharmacol and Biomedicine Discovery Institute[1] and Drug Discovery Biology[2], Monash University, Australia

Introduction. The AT_2R has demonstrated anti-fibrotic and concurrent anti-inflammatory and antioxidative properties in type 2 diabetes mellitus (T2DM) and hypertension. These effects are yet to be studied in a comorbidity model of type 1 diabetes mellitus (T1DM) with hypertension.
Aims. The aim of this study was to investigate the effects of AT_2R stimulation, with or without AT_1R blockade in a model of hypertension with concomitant T1DM.
Methods. 6-week-old spontaneously hypertensive rats (SHRs) were given either citrate or single dose of streptozotocin (STZ) (55mg/kg, i.p.) to induce diabetes. After 4 weeks of diabetes, animals were administered vehicle (saline), β-pro^7-AngIII (75pmol/kg/min via osmotic mini-pump), candesartan (2mg/kg/day via drinking water) or a combination for a further 8 weeks.
Results: After STZ injection, diabetic animals maintained a fasting blood glucose >16mM and blood pressure of >160mmHg in all groups. Candesartan had no impact on elevated cardiac fibrosis (picrosirius red staining), inflammation and oxidative stress but lowered blood pressure. While β-pro^7-AngIII had no effect on blood pressure, there was a significant increase in cardiac interstitial collagen associated with diabetes (6.06±0.54% vs 8.16±0.36% $P<0.05$) that did not occur with β-pro^7-AngIII (6.31±0.5%). Furthermore, β-pro^7-AngIII alone, but not candesartan, significantly reduced protein expression of the fibrotic and inflammatory markers, TGF-β1 (3.04±0.4% vs 4.32±0.27%, $P=0.05$), α-SMA (1.11±0.13% vs 1.81±0.1%, $P=0.02$), pIkB (0.43±0.05% vs 0.73±0.05%, $P=0.005$), and a similar trend for reduced cardiac superoxide levels (2.65±0.27% vs 3.5±0.18%, $P=0.07$), compared to the hypertensive-diabetic vehicle. The effects of β-pro^7-AngIII in combination with candesartan were similar to those of β-pro^7-AngIII alone.
Discussion: These findings demonstrated that AT_2R stimulation with β-pro^7-AngIII alone, or in combination with AT_1R blockade, may improve the cardiac structural changes implicated in hypertension with concomitant diabetes that are heavily associated with inflammation and oxidative stress, independent of blood pressure regulation

125

Targeting formyl peptide receptors as a novel approach to treat hypertension-induced end-organ damage.

Jaideep Singh[1,2], Kristy L Jackson[2], Audrey Gumanti[1,2], Rebecca H Ritchie[3], Owen L Woodman[1], Geoffrey A Head[2], Chengxue Qin[1]. Cardiovascular Pharmacology, Monash University[1], Parkville, Melbourne, VIC, Australia; Neuropharmacology, Baker Heart and Diabetes Institute[2], Melbourne, VIC, Australia; Heart Failure Pharmacology, Monash University[3], Parkville, Melbourne, VIC, Australia;

Introduction: Formyl peptide receptors (FPR), a family of G-protein-coupled-receptors, play a critical role in the regulation of resolution of inflammation, an important mediator of hypertension-induced end-organ damage. The FPR agonist Cmpd17b has cardioprotective effects against inflammatory ischaemic insults, but its impact on a chronic inflammatory insult, such as in hypertension, has not been explored. Aim: To investigate the effect of the FPR-prototype Cmpd17b on mean arterial pressure (MAP) and end-organ damage in a model of angiotensin-II (AngII)-induced hypertension. Methods: Male C57BL/6 mice (12 weeks old) were implanted with a radiotelemetry probe to measure MAP. Hypertensive mice (AngII infused at 0.7 mg/kg/day, s.c.) and normotensive mice (saline infused, s.c.) underwent 24 hour blood pressure recordings via telemetry at baseline and during 4 weeks daily treatment with Cmpd17b (50 mg/kg/day i.p.) or its vehicle. At the end of the study, tissue was collected and cardiac and kidney fibrosis were assessed by Picro Sirius red staining. Results (see Table): AngII infusion significantly increased blood pressure, cardiac hypertrophy and fibrosis and kidney fibrosis. Interestingly, Cmpd17b significantly lowered MAP (~5 mmHg), cardiac (LV) hypertrophy and fibrosis and kidney fibrosis. Discussion: Our study demonstrated that the FPR agonist reduced hypertension-induced end-organ damage, supporting the development of FPR-based therapy to treat complications related to hypertension.

Results as mean ± SEM (n)	Saline infused + Vehicle	Saline infused + Cmpd17b	AngII infused + Vehicle	AngII infused + Cmpd17b
Delta MAP (mmHg)	-1.4 ± 1.7 (8)	-1 ± 0.8 (6)	27.1 ± 3.3* (6)	21.9 ± 3.5$^\#$ (6)
LV weight (mg/BW)	3.1 ± 0.1 (11)	3.1 ± 0.1 (12)	4.0 ± 0.1* (12)	3.6 ± 0.1$^\#$ (12)
LV collagen (%)	2.8 ± 0.4 (7)	2.5 ± 0.4 (8)	4.6 ± 0.7* (7)	3.1 ± 0.3$^\#$ (9)
Kidney collagen (%)	1.5 ± 0.2 (9)	1.3 ± 0.1 (10)	3.3 ± 0.6* (10)	1.3 ± 0.1$^\#$ (11)

*$P<0.05$, compared to saline infused vehicle; $^\#P<0.05$, compared to AngII infused vehicle (Mixed and Two-way ANOVA with Bonferroni's and Fisher's post-hoc for multiple comparisons)

126

Human amnion epithelial cells reduce blood pressure and prevent aortic inflammation and collagen expression during experimental hypertension

Quynh Nhu Dinh[1], Cecilia De Silva[1], Henry Diep[1], Shenpeng R Zhang[1], Liz J Barreto Arce[1], Rebecca Lim[2], Grant R Drummond[1], Christopher G Sobey[1], T. Michael De Silva[1]. Centre for Cardiovascular Biology and Disease Research, La Trobe University [1], Bundoora, VIC, Australia; The Ritchie Centre, Hudson Institute of Medical Research [2], Clayton, VIC, Australia.

Introduction. Vascular inflammation and fibrosis are hallmarks of hypertension and contribute to the development of cardiovascular disease. Current treatments for hypertension may reduce blood pressure but do not target the associated pathological changes and end-organ damage. Cell therapy has great therapeutic potential as unlike single pharmacological agents, cells can deliver multiple mediators which could more effectively target complex disease mechanisms. Human amnion epithelial cells (hAECs) have many properties (eg. anti-inflammatory, anti-fibrotic, and immunologically inert) that make them attractive candidates for a cell-based therapy for vascular pathology.
Aims. To test the potential of hAECs to treat vascular pathology in angiotensin II-induced hypertension.
Methods. Male C57Bl6 mice (8-12 weeks) were administered vehicle (saline; n=35) or angiotensin II (0.7 mg/kg/d, n=37) for 14 d via an osmotic minipump. After minipump implantation, a subset of mice were injected with 10^6 of hAECs intravenously. Systolic blood pressure was measured using tail-cuff; inflammation was assessed using flow cytometry and markers of fibrosis using quantitative PCR.
Results. Angiotensin II infusion increased BP and promoted accumulation of aortic leukocytes, specifically macrophages and monocytes, which were elevated by ~3-fold compared to vehicle-infused mice (n=9-11, $P<0.05$). Angiotensin II also increased aortic mRNA expression of collagen type 1 alpha 1 (*Col1a1*) by ~4-fold and collagen type 5 alpha 1 (*Col5a1*) by ~7-fold compared to vehicle (n=6-8, $P<0.05$). Co-administration of hAECs limited the development of hypertension by angiotensin II (185±5 mmHg vs 165±4 mmHg; n=9-11, $P<0.05$), as well as the aortic infiltration of macrophages and monocytes (n=9-11, $P<0.05$) and expression of *Col1a1* and *Col5a1* (n=6-8, $P<0.05$).
Discussion. Intravenous administration of hAECs blunted angiotensin II-induced hypertension, aortic inflammation and collagen expression in male mice. This study suggests that hAECs or their cellular products could be explored as treatments for vascular pathology during hypertension.

127

Antidepressant Deprescribing Recommendations in Clinical Practice Guidelines for Depression: A Systematic Review

Patricia TY Yee[1], Aili V Langford[1], Carl R Schneider[1], Ivan D Florez[2,3], Danijela Gnjidic[1]. School of Pharmacy, Faculty of Medicine and Health, The University of Sydney[1], Sydney, NSW, Australia; Department of Health Research Methods Evidence and Impact, McMaster University[2], Juravinski Site, G Wing, 2nd Floor, 711 Concession Street, Hamilton, ON L8V 1 C3, Canada; Department of Paediatrics, Univeresidad de Antioquia[3], Calle 57 #53-08, 050001, Medellin, Colombia

Introduction. Clinical practice guidelines (CPGs) for the management of depression often contain prescribing recommendations for antidepressants. However, there are gaps regarding guidance on medication withdrawal or deprescribing recommendations.
Aims. To identify and evaluate the presence, nature and clarity of deprescribing recommendations for antidepressants in depression CPGs.
Methods. A comprehensive systematic search from January 2016 to April 2021 was conducted of both guideline developer websites and citation databases including MEDLINE, EMBASE, CINAHL, PsycINFO, Scopus, and Web of Science, as well as supplementary citation tracking. Presence and nature of deprescribing recommendations were extracted from CPGs for depression, and consideration of AGREE-II tool and GRADE approach components were also documented. Nature of guidelines were characterised by mapping deprescribing recommendations to the deprescribing framework which included the following criteria: initiation, patient-centred approach, taper rate and duration, pharmacokinetic and pharmacodynamic considerations, and review period.
Results. Preliminary analysis have identified 34 guidelines, with 24 guidelines classified as depression-specific and 10 related to depression in the context of other medical conditions. Twenty nine guidelines contained at least one deprescribing recommendation as specified by the deprescribing framework; 10 applying AGREE-II or GRADE for guiding their development, and 27 included the need for deprescribing with tapering being the most frequent recommendation. None-of the CPGs fulfilled all the criteria for deprescribing recommendations.
Discussion. Our preliminary results provide some insights into the poor clarity of deprescribing recommendations in current depression CPGs. There is insufficient guidance to clinicians on how to deprescribe antidepressants in the available CPGs, which can contribute to overuse of antidepressants in the community.

128

A stewardship program to facilitate anticholinergic and sedative medication deprescribing using the Drug Burden Index integrated in Electronic Medical Records

Nashwa Masnoon[1,2], Sarita Lo[1], Sarah N Hilmer[1,3]. Kolling Institute, Faculty of Medicine and Health, University of Sydney[1], Sydney, NSW, Australia; Department of Pharmacy, Royal North Shore Hospital[2], Sydney, NSW, Australia, Departments of Clinical Pharmacology and Aged Care, Royal North Shore Hospital[3], Sydney, NSW, Australia.

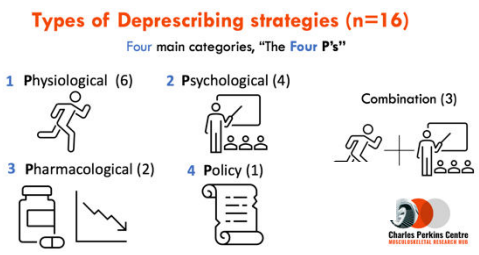
Introduction. The Drug Burden Index (DBI) provides a score measuring a person's total exposure to anticholinergic and sedative medications. These medications are commonly associated with adverse outcomes such confusion and falls.
Aims. Through incorporating the DBI in routinely used Electronic Medical Records and implementing a DBI stewardship program, we sought to explore i) potential opportunities for deprescribing sedative and anticholinergic medications in older inpatients and ii) uptake of the Steward's deprescribing recommendations by the medical team.
Methods. Electronic medical records of patients aged 75 years or older, admitted under General Medicine or Aged Care at the Royal North Shore Hospital (Sydney, Australia) with a DBI score greater than zero were reviewed by the DBI Stewardship Pharmacist remotely due to COVID-19 pandemic restrictions. The Steward identified potential deprescribing opportunities and discussed these over telephone with the Medical Registrar looking after each patient.
Results. Preliminary findings from the first 2.5 weeks of stewardship demonstrated that out of 51 patients reviewed, the Steward made 30 recommendations for 26 patients. The Registrars agreed with 24 recommendations (80% of all recommendations). The most common drug classes involved were antipsychotics, opioids, antiepileptics and antidepressants. Of the 24 agreed recommendations, 18 (75%) were actioned. Nine recommendations (50% of all recommendations actioned) were implemented in hospital, whilst the remaining nine were included as suggestions in the discharge summary for follow-up by the General Practitioner.
Discussion. Preliminary findings demonstrate existing opportunities for deprescribing sedative and anticholinergic medications in older inpatients. Whilst a remote stewardship model was necessitated by pandemic restrictions and may have limited uptake of recommendations, it has the advantage of efficient use of a pharmacist's time and resulted in recommendations which were mostly agreed upon by Registrars. Evaluation of factors enabling implementation of agreed changes may increase impact of the stewardship program. Longer term follow-up of patients may elucidate sustainability of deprescribing changes and subsequent clinical outcomes.

129

Deprescribing paracetamol in pain conditions: A scoping review

Thomas G Patterson[1], Paula Beckenkamp[1], Manuela Ferreira[1], Justin Turner[2], Danijela Gnjidic[1], Ana Reis[1], Yanyu Chen[1], Carlos Castrillion[1], Paulo Ferreira[1], Faculty of Medicine and Health, The University of Sydney[1], Sydney, NSW, Australia; Faculty of Pharmacy, University of Montreal[2], Quebec, H3T 1J4, Canada.

Introduction. New research has not only questioned the efficacy of paracetamol use for musculoskeletal conditions, such as low back pain and osteoarthritis, but also its safety. No previous review has summarised the evidence on deprescribing strategies targeting individuals using paracetamol to manage pain, and the effectiveness of these strategies.
Aims. To examine evidence on deprescribing paracetamol in pain conditions and inform future strategies for paracetamol deprescription.
Methods. A scoping review was conducted searching MEDLINE, Embase, PsycINFO, CENTRAL and JBI Ovid databases via Ovid and CINAHL via EBSCO from inception to 31 July 2020.
Results. 16 original articles were included. Deprescribing strategies were grouped into 4 main categories: (1) Pharmacological, (2) Psychological, (3) Physiological, (4) Policy. We found strategies were predominately consumer-focused, conducted in community settings and involved individuals experiencing musculoskeletal pain. All 16 studies were on some level effective at deprescribing paracetamol although the effectiveness of deprescribing strategies were highly variable, ranging from the majority of participants discontinuing their paracetamol use, to less than 10% reducing their paracetamol use upon the latest follow-up.



Discussion. Only one study directly targeted deprescription as a primary outcome. Many of the studies were published in the last 5 years. There are clear opportunities for prospective trials to be designed more purposely and primarily focused to influence reduction and cessation of paracetamol for specific pain conditions where deprescription is appropriate.

130

OPTIMIZE: A pragmatic cluster randomized deprescribing trial in primary care

Emily Reeve[1], Elizabeth Bayliss[2], Susan Shetterly[2], Melanie Drace[2], Jonathan Norton[3], Ariel Green[3], Linda Weffald[4], Matthew Maciejewski[5], Orla Sheehan[3], Jennifer Wolff[3], Kathy Gleason[2], Jennifer Sawyer[2], Courtney Kraus[2], Mahesh Maiyani[2], Marilyn Du Vall[2] & Cynthia Boyd[3]. Clin and Health Sci, Univ of South Australia[1], Adelaide, SA, Australia; Kaiser Permanente Colorado[2], Denver, CO, USA; Johns Hopkins Univ[3], Baltimore, MD, USA; Veterans Affairs Med Center[4], Durham NC, USA.

Introduction. People with living with dementia and multiple chronic conditions are at increased risk for polypharmacy, potentially inappropriate medication (PIM) use and adverse drug events. Deprescribing may improve outcomes for these individuals. However, optimal approaches to deprescribing in primary care are unknown.
Aims. To assess the outcomes of a patient, family and provider deprescribing education and activation intervention.
Methods. Pragmatic, cluster randomised trial at 9 intervention and 9 control primary care clinics in a not-for-profit integrated delivery system in the United States. Participants were ≥65 with dementia or mild cognitive impairment plus ≥1 other chronic condition, taking ≥5 medications. The intervention consisted of a brochure and short survey sent to patients and carers before a primary care visit to prepare them for conversations about deprescribing; and monthly tip sheets about deprescribing for clinicians plus alerts in the electronic health record that their patient had received the brochure.
Results. 1,433 intervention patients received, and 1,579 control clinic patients would have been eligible to receive, the full intervention (N=3,012). Both groups at baseline had a mean age of 80, 7 chronic medications, and approximately 30% took ≥1 PIM. At 6 months, adjusted estimated number of medications and proportions of PIMs did not differ significantly between intervention (6.4 and 17.8% respectively) and control groups (6.5, 21.0%) in the overall cohort but were significantly lower in the intervention group in the pre-planned subgroup with ≥7 medications at baseline (7.9 vs 8.1, p=0.03; 25.1% vs. 31.7%, p=0.04, adjusted for baseline counts, age, gender and race).
Discussion. Combining patient and carer education and activation with longitudinal clinician education about deprescribing reduces chronic medications and PIMs in people with dementia or mild cognitive impairment taking 7 or more chronic medications. Taken to scale, this approach could improve clinical outcomes and could provide a foundation for additional medication optimisation strategies.

131

Effect of self-monitoring urate on allopurinol adherence

Jian S Chan[1,2,3], Matthew J Coleshill[1,2,4], Dyfrig Hughes[5], Toni Michael[3], Jane Spragg[3], Parisa Aslani[3], Richard O Day[1,2], Sophie L Stocker[1,2,3]. St Vincent's Clinical School, Univ of New South Wales[1], Sydney, NSW, Australia; Clin Pharmacol & Toxicology, St Vincent's Hosp[2], Sydney, NSW, Australia; School of Pharmacy, Univ of Sydney[3], Sydney, NSW, Australia; Black Dog Institute, The Univ of New South Wales[4], Sydney, NSW, Australia; Health Economics & Medicines Evaluation, Bangor Univ[5], Bangor, GWYNEDD, Wales.

Introduction. Gout is a prevalent and debilitating disease with safe and effective therapies. Yet, it is still sub optimally managed. A significant barrier to optimal gout management is poor adherence to urate lowering therapies.
Aims. To determine the effect of self-monitoring urate on allopurinol adherence.
Methods. Participants over 18 years, diagnosed with gout, prescribed allopurinol, and not using a weekly medication planner, have been recruited. Participants were asked to self-monitor their urate concentrations, at least once a month, using a point-of-care device (*Humasens2.0plus).* Adherence to allopurinol is being measured as a proportion of days with correct dosing using electronic monitoring (*MEMS)*. Participants will be observed for 12-months. Feedback on their urate concentrations is being provided at monthly follow-ups. Adherence information is unavailable to participants during data collection.
Results. Participants (n=31) are predominantly male (94%), with a mean (SD) age of 58.6 (12.5) years, and a median (interquartile range) baseline allopurinol dose of 300 (150-300) mg daily. Preliminary data from 11 participants indicates a mean adherence to allopurinol of 87.8% (95% CI 79.3-96.4%) with a mean (SD) follow-up period of 52.3 (16.0) days. The mean baseline urate was 0.34 mmol/L (95% CI 0.30-0.37 mmol/L). The majority (67%) of participants have reported optimal urate concentrations (<0.36 mmol/L). Two gout flares have been reported.
Discussion. Self-monitoring of gout offers a patient-led approach to gout management and is anticipated to promote allopurinol adherence. The observed allopurinol adherence rates, at this early stage, are promising. The majority of gout patients are achieving target urate concentrations. The full observation period will allow us to assess the long-term efficacy of this novel intervention.

132

The development and application of BRET-based assays to characterise molecular pharmacology.

Elizabeth KM Johnstone[1,2,3,4], Kevin DG Pfleger[1,2,3,5]. Mol Endocrinol and Pharmacol, Harry Perkins Inst of Med Res[1], Nedlands, WA, Australia; Centre for Med Res, Univ of Western Australia[2], Crawley, WA, Australia; Aust Res Council Centre for Personalised Therapeutics Technologies[3], Australia; School of Biomed Sci, Univ of Western Australia[4], Crawley, WA, Australia; Dimerix Limited[5], Nedlands, WA, Australia.

Introduction. Uncovering the complexities of receptor molecular pharmacology requires the use of novel tools and techniques. Bioluminescence resonance energy transfer (BRET) is one such platform technology that has emerged as a leading tool for investigating all aspects of receptor pharmacology allowing pharmacology to be monitored in live cells in real time. Our laboratory has been at the forefront of developing BRET-based assays to investigate receptor pharmacology, and has also expanded its application beyond receptors.
Aims. To develop and utilise BRET-based assays to investigate molecular pharmacology.
Methods. Proteins of interest are genetically labelled with BRET donor (luciferase) and acceptor (fluorophore) proteins. Coexpression of these proteins in cells (e.g. HEK293s) enables monitoring of the proximity of the two proteins, and investigation of modulation of this proximity through ligand treatment.
Results. I have been involved in the development and application of novel BRET-based assays such as the NanoBRET ligand binding assay, the Receptor-Heteromer Investigation Technology and a BRET trafficking assay with a broad suite of trafficking markers. My work in this area has led to characterisation of the angiotensin II and bradykinin heteromers, the AT_2-B_2 heteromer and the AT_1-B_2 heteromer, as well as other angiotensin AT_1 receptor complexes: with the epidermal growth factor receptor, the receptor for advanced glycation end products (RAGE), and the CCR2 chemokine receptor, the latter research forming the basis for a therapeutic that is currently in three Phase III clinical trials. I have also expanded the application of BRET assays into cancer biology, looking at the cellular effects of a novel therapeutic and a newly discovered oncogene.
Discussion. The use of novel BRET-based assays has enabled characterisation of a multitude of different aspects of molecular pharmacology, including receptor signalling, trafficking and heteromerisation.

133

Tackling dementia: From structure to clinic targeting muscarinic receptors

Prof Andrew Tobin, The University of Glasgow, UK

Symptomatic treatment for, and slowing the progression of, dementia is currently one of the worlds most intractable global heath challenges. Despite a huge research focus the molecular nature of the cause of many forms of dementia and the mechanism of the spread of disease throughout the brain remain areas of uncertainty. It is with this paucity of information regarding the fundamental elements of disease that we have been investigating the possibility that targeting the muscarinic receptor family might offer the prospect of next generation medicines to treat Alzheimer's disease (AD), one of the most prevalent forms of dementia. I will present in my talk the challenges of understanding the most appropriate pharmacological properties to be designed into an effective muscarinic receptor agonist that will rescue defective memory loss in AD. In particular, I will focus on our recent collaboration with Sosei-Heptares that has framed our understanding of how to apply structural based drug design to the generation of the optimal properties of an orthosteric agonist targeting the M1/M4-muscarinic receptors for the treatment of memory loss in AD. I will also focus on the limitations of such an agent to deliver disease modification and how our collaboration with the teams at Vanderbilt University and Monash University (MIPs) has highlighted the potential of allosteric modulators at the M1-muscarinic receptor as medicines that might not only restore memory loss in AD but that might also slow the progression of neurodegenerative disease.

200

Skills coaching: an innovative initiative to support professional skill development in pharmacology students

Nilushi Karunaratne
Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia.

Introduction. As the pharmacology discipline evolves with new paths requiring specialised technical skills, it is critical that educators simultaneously maintain focus on developing core professional skills (e.g. communication, teamwork and scientific inquiry etc.) in students. The Faculty of Pharmacy and Pharmaceutical Sciences (FPPS) at Monash University has embedded such core professional skills into the undergraduate curriculum.
Aims. To develop a learner-centred program to support the development of relevant professional skills that allows pharmacology students to robustly demonstrate their skill attainment to others.
Methods. A Skills Coaching Program, where academics performed the role as coach, was developed to support the development of core professional skills in undergraduate students. The program adopted a learner-centred approach where students met with their skills coach every 2-4 weeks completing 3-4 cycles of skills coaching each semester. Students used Borton's model (1) of reflection and upload Personalised Learning Plans (PLPs) consisting of reflections, associated evidence, and actions for improvement, into an e-portfolio. Coaches provided feedback using a standardised format (Keep, Start, Stop) (2). Following this, students met as a group with their skills coach.
Results. The Skills Coaching Program consisted of the following components: students were taught, had an opportunity to practice, and were regularly assessed on professional skills using a custom designed e-portfolio. The e-portfolio facilitated student awareness of their skill development, and demonstrated attainment of skill-based competencies. The learner-centred approach has benefited students in terms of developing their ability to be in control of their skills development and at the same time, feel supported by academic skills coaches.
Discussion. Skills development is a learning process that needs to be deliberate, explicit and embedded from commencement, to graduation, and into the workplace. The Skills Coaching Program has instilled reflective practice in pharmacology students allowing them to articulate, focus and develop core professional skills essential for the workforce.
1. Borton, T. Reach, touch, and teach: student concerns and process education. New York: McGraw Hill; 1970.
2. DeLong T. Three Questions for Effective Feedback. Harvard Business Review. 2011 [cited 19 Aug 2019].

201

Unbiased and hypothesis-free approaches to studying the brain and its disorders using whole-brain imaging technique

Hitoshi Hashimoto, Laboratory of Molecular Neuropharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University, and University of Fukui, Division of Bioscience, Institute for Datability Science, Osaka University, Open and Transdisciplinary Research Initiatives, Osaka University, Department of Molecular Pharmaceutical Sciences, Graduate School of Medicine, Osaka University

As the brain has a high degree of functional specialization and integration, imaging of the entire brain at a subcellular resolution and subsequent processing of the resulting images are prerequisites to investigate anatomical and functional brain networks to understand their function and dysfunction. To address this issue, we have recently developed highly scalable and high-speed imaging system (block-FAce Serial microscopy Tomography, FAST). By using this system, we have successfully imaged whole brains in mice and marmosets and part of a human postmortem brain, and conducted whole-brain anterograde and retrograde neuronal circuit tracing and activation mapping in the transgenic immediate early gene reporter mice. These image data can be used for quantitative comparisons of whole-brain structures and neuronal activities at the cellular level using the spatial coordinated numeric data of brain cells and pattern recognition methods. The FAST system thus paves the way for imaging analyses of the brain and provides new opportunities for unbiased and hypothesis-free approaches that contributes to investigate molecular mechanisms and therapeutic drug targets for brain disorders. In this presentation, I will introduce our recent progress and discuss the directions of future research that would contribute to a better understanding of the brain systems and brain disorders.

202

Gamma-hydroxybutyrate Overdose in Inner-Sydney Emergency Departments: a retrospective review

Oliver Harris[1], Krista Siefried[2], Angela Chiew[3], Gonzalo Aguirrebarrena[4], Darren Roberts[5], Anna Sordo[6], Jonathan Brett[5]. School of Medicine, Univ of New South Wales[1], Sydney, NSW; Alcohol and Drug Service, St. Vincent's Hospital[2], Sydney, NSW; Emergency Dept, Prince of Wales Hospital[3], Sydney, NSW; Emergency Dept, St. Vincent's Hospital[4], Sydney, NSW; Clinical Pharmacology and Toxicology Dept, St. Vincent's Hospital[5], Sydney, NSW; Univ of Sydney, Sydney[6], NSW

Introduction. GHB overdose can result in severe toxicity and mortality, yet there remains a lack of consensus regarding acute management.
Aims. This study aims to describe the spectrum of GHB toxicity and management in two urban EDs and identify factors associated with intubation.
Methods. We conducted a retrospective study of GHB-related overdoses presenting in August each year to St Vincent's Hospital Sydney's (SVHS) ED between 2001-2021 and Prince of Wales' ED in August between 2010-2021. We collected data on demographics, triage category, changes in vital signs, incidence of intubation, disposition, on presentation and length of stay. We performed bivariate then multivariable logistic regression to determine associations between patient characteristics at presentation and intubation.
Results. We recorded 538 episodes of GHB overdoses across two hospital EDs. The mean age of participants was 32.2 (± 9.3), 68% (n = 366) identified as male; 13.8% (n = 74) were intubated; and 47.1% (n = 222) presented with coingestion of an illicit drug or alcohol in addition to GHB. The average time spent in ED was 154 (± 389) minutes, and mean time to extubation was 4.4 (± 3.2) hours. Hospital location (SVHS [OR 0.13, P<0.001] vs POW [OR 1.51, P= 0.1]), presenting symptoms including agitation (OR 0.25, P=0.002), seizures (OR 7.37, P<0.001), bradycardia (OR 2.49, P=0.002), hypotension [OR 3.0, P=0.01] and presenting GC S [OR = 0.62, P<0.001] were significant associated with intubation in bivariate analysis. Only presenting GCS and seizures remained significant in the multivariable model.
Discussion. Here we describe the demographics, presentation, and management of GHB overdose in two inner-city ED's. We identify seizures and decreased GCS as the factors most significantly associated with intubation. This information is relevant when developing guidelines to manage GHB overdose.

203

Polypharmacy with high Drug Burden Index (DBI): association with microbiome and global function in aged mice

Gizem Gemikonakli[1,2], John Mach[1,2], Fan Zhang[3], Martyn Bullock[2], Trang Tran[1,2], Emad El-Omar[3] & Sarah N Hilmer[1,2]. Lab of Ageing and Pharmacology, Kolling Institute, Royal North Shore Hosp, NSW, Australia [1]. University of Sydney, NSW, Australia [2]. Microbiome Research Centre, St George and Sutherland Clinical School, UNSW, NSW, Australia [3].

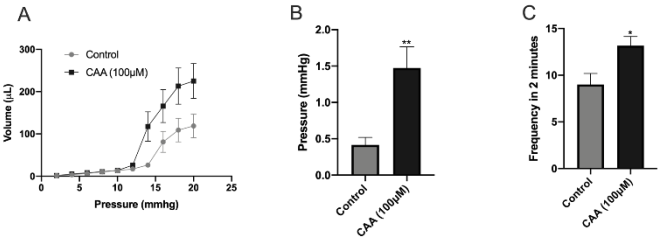
Introduction. Ageing, disease, and medication use are associated with changes in microbiome and global function.
Aims. We investigated the effect of polypharmacy with high Drug Burden Index (DBI, measure of total anticholinergic and sedative medication exposure) and/or gradual cessation (deprescribing) on the microbiome, and whether this was associated with global outcomes.
Methods. 12-month-old male C57BL/6 mice received either control diet or chronic high DBI polypharmacy (metoprolol, simvastatin, citalopram, oxycodone, oxybutynin) at therapeutic doses. At 21 months, high DBI polypharmacy mice were re-randomised to continue treatment or to gradual cessation. Faecal samples were collected, and functional assessments were conducted at 12 months (before starting medication), 15 months, 21 months, and 24 months (n=16-10). Following DNA extraction, 16S sequencing was used.
Results. Microbiome Alpha diversity decreased with age in control (12 vs 21 months p=0.02, 12 vs 24 months p=0.04) and high DBI polypharmacy deprescribed (overall p=0.026, and 15 vs 24 months p=0.011), but no change was seen with age in mice administered high DBI polypharmacy. Beta diversity PCoA plots evaluated by Bray-Curtis distances showed significant difference with increasing age (R2=0.039, p=0.001), polypharmacy compared to control (R2=0.041, p=0.001), and deprescribing compared to polypharmacy (R2=0.089, p=0.022) but not control at 24 months, indicating reversal with deprescribing. Regression analyses for observed and Shannon Alpha diversity of all animals pooled, correlated with age at death and maximum grip strength (p<0.05). In control mice, significant compositional changes included *Clostridiaceae*, *Rikenellaceae* and *Bacteroidales* families, compared to changes in *Bifidobacteriaceae* and *Lactobacillaceae* in polypharmacy, and *Dehalobacteriaceae*, *Desulfovibrionaceae*, *Lachnospiraceae* in deprescribed.
Discussion. Chronic administration of high DBI polypharmacy appeared to attenuate the age-related reduction in alpha diversity, which was not sustained after deprescribing. In contrast beta diversity and bacterial composition varied with age, polypharmacy and deprescribing. Future studies will investigate the role of these changes on physiology and pharmacology.

204

Chloroacetaldehyde, the toxic urinary metabolite of cyclophosphamide, alters urinary bladder function

Eleanor J West[1], Donna Sellers[1], Catherine McDermott[1], Russ Chess-Williams[1]. Centre for Urology Research, Bond University[1], Gold Coast, QLD, Australia.

Introduction. Cyclophosphamide (CPO) is a commonly administered chemotherapeutic originally developed to treat a variety of different cancers. Following treatment with CPO patients experience urotoxic adverse effects that are most commonly attributed to the urinary metabolite acrolein. However, another metabolite of CPO, chloroacetaldehyde (CAA) may also contribute to the adverse effects experienced in patients.



Aims. Investigate the effects of intravesically administered CAA on bladder function, in an ex-vivo whole bladder preparation

Methods. Female C57BL/6J mice (12-13 weeks) were used in this study. Mice were euthanised, bladders were isolated and placed in 37°C Krebs-bicarbonate solution and aerated with carbogen (95% O2 and 5% CO2) gas. Whole bladder preparations were performed as previously described by our group (West, E.G. et al, 2018). Spontaneous activity, bladder compliance and accommodation, detrusor contractility and nerve-evoked contractile responses were measured.

Results. Intravesical administration of CAA, increased bladder compliance at pressures above 12mmHg (Figure 1A); however, accommodation of bladders following filling was not affected. Amplitude and frequency of spontaneous contractions during accommodation were significantly increased in CAA treated bladders compared to controls (Figure 1B, $P<0.01$; Figure 1C, $P<0.05$, one-tailed, unpaired Student's t-test).

Discussion. Bladder compliance and spontaneous activity during accommodation was significantly increased in response to CAA. Intravesical CAA causes pharmacological and physiological changes to the mouse urinary bladder which may explain the contribution of CAA to urotoxic side effects experienced by patients treated with CPO.

References. West, E.G, et al, 2018. J. Pharmacol. Exp. Ther. 366: 282-290.

205

In vitro demonstration of herbal exacerbation of paracetamol-induced hepatotoxicity

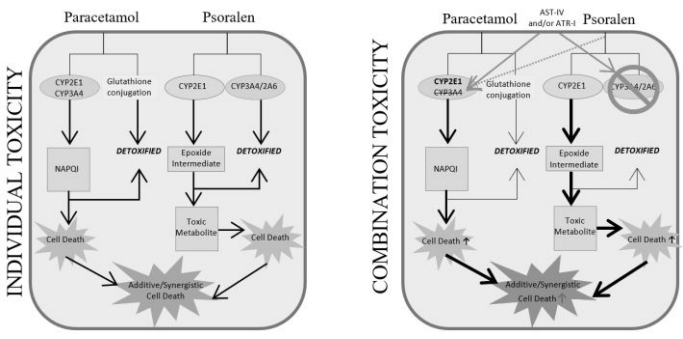
Susan M Britza[1], Rachael Farrington[1], Roger W Byard[1, 2], Ian F Musgrave[1]. Pharmacology, School of Biomedicine, The University of Adelaide[1], Adelaide, SA, Australia; Pathology, Forensic Science SA[2], Adelaide, SA, Australia

Introduction. Paracetamol is one of the most accessible pharmaceutical analgesic and antipyretic agents. Similarly, traditional herbal medicines including *Psoralea corylifolia, Astragalus propinquus* and *Atractylodes macrocephala* have been used for centuries to treat cold and flu-like symptoms. As herbal medicines are considered 'natural and safe', the likelihood of combination with over-the-counter pharmaceuticals is high. Paracetamol and herbal medicines are associated with many adverse effects, hepatotoxicity being a common complication.

Aims. To determine whether concomitant use of paracetamol with phytochemicals commonly found in herbal medicines, including psoralen, astragaloside IV (AST-IV) and atractylenolide I (ATR-I) may produce synergistic toxicity.

Methods. A human liver carcinoma cell line (HepG2) was exposed to paracetamol (0-50 mM), psoralen (0-1000 μM) and AST-IV and ATR-I (0-300 μM). Interactions were determined using fixed concentrations of 200 μM psoralen with paracetamol (0-50 mM), and 10 mM paracetamol with AST-IV or ATR-I (0-300 μM).

Results. Paracetamol and psoralen demonstrated significant concentration-dependent toxicity individually ($P < 0.05$), however AST-IV and ATR-I alone or in combination with each other had little effect ($P > 0.05$). Fixed 200 μM psoralen with 20 – 50 mM paracetamol produced an approximately 20% increase in cell death compared to paracetamol with no psoralen; thus, paracetamol and psoralen demonstrated increased toxicity through synergistic interactions ($P < 0.01$; $CI < 1$).



Discussion. This study highlights the potential risks that herbal medicines can have on paracetamol-induced liver injury and may explain the underlying mechanisms where patients have developed liver failure and necrosis in the presence of low levels of paracetamol.

206

Expression and localisation of mas-related G protein-coupled receptors F and D: a novel study in the human colon

Patrick Hawker[1], Irit Markus[1], Lu Liu[1]. Department of Pharmacology, UNSW Sydney[1], NSW 2052, Australia.

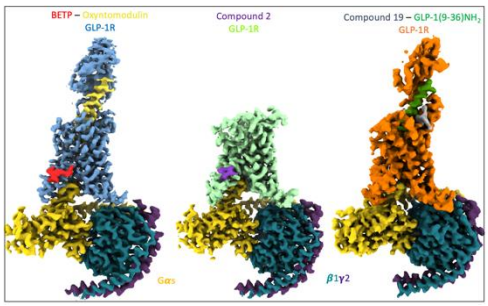
Introduction. Mas-related G protein-coupled receptors have recently gained attention for their role in nociception and neuroimmune interactions (Serhan et al, 2021). Mas-related G protein-coupled receptors F and D (MRGPRF and MRGPRD) may participate in enteric nervous system regulation (Avula et al, 2011; Zhou et al, 2019) but their involvement in inflammatory bowel disease (IBD) has not been studied.
Aims. Determine expression and localisation of MRGPRF and MRGPRD in the healthy human colon and assess for changes in ulcerative colitis or Crohn's disease tissue.
Methods. Immunofluorescence double labelling and immunohistochemical 3, 3'-diaminobenzidine (DAB) staining was performed using MRGPRF (HPA028811) and MRGPRD (HPA031346) antibodies at 1:100 dilution incubated in human colon tissue. Slides were scanned with Aperio Scanner and DAB optical density quantified with QuPath software.
Results. MRGPRF immunoreactivity (IR) was abundant in muscularis mucosae, longitudinal muscle, and circular muscle. Co-expression was observed with smooth muscle marker, alpha-actin. MRGPRF-IR did not alter in Crohn's disease samples but was significantly downregulated in ulcerative colitis tissue (circular muscle P = 0.0093, longitudinal muscle P = 0.0059) compared to controls. MRGPRD-IR was observed in a population of immune cells present in mucosa. However, MRGPRD-IR was not observed in cells positive for CD3 (T cell marker), MUM-1 (lymphoid cell marker), or MCT (mast cell marker). The density of MRGPRD-IR was similar across colon tissue from IBD patients and controls.
Discussion. This is the first report to show the expression and localisation of MRGPRF and MRGPRD in the human colon. The downregulation of MRGPRF-IR in the muscularis propria of human colon suggests MRGPRF may play a role in colonic dysmotility often associated with IBD. MRGPRD was highly expressed in immune cells within the lamina propria, however their identity remains unknown and requires further investigation.

Serhan N et al (2021) Neurosci Lett 749:135724
Avula LR et al (2011) Histochem Cell Biol 139:639-658
Zhou C et al (2019) Cell Tissue Res 377:259–268

207

Structural insights into allosteric modulation of the human glucagon-like peptide-1 receptor

Xin Zhang[1,2], Matthew J. Belousoff[1,2], Fabian Eisenstein[3], Radostin Danev[3], Patrick M. Sexton[1,2], Denise Wootten[1,2]. Drug Discovery Biology[1], and ARC Centre for Cryo-electron Microscopy of Membrane Proteins[2], Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia; Graduate School of Medicine, University of Tokyo[3], Tokyo, Japan.



Introduction. The glucagon-like peptide 1 receptor (GLP-1R) is a well-established clinical target for type II diabetes with several clinically approved GLP-1R agonists. A variety of GLP-1R positive allosteric modulators (PAMs) have also been identified, such as compound 2, BETP and compound 19, however, the molecular details of PAM binding and how they modulate receptor function are largely unknown.
Aims. Determine structures of GLP-1R-Gs complexes bound by different PAMs and endogenous peptide agonist (GLP-1 and its metabolite, and oxyntomodulin), and correlate these with pharmacological profiles.
Methods. Structures of GLP-1R-Gs complexes were determined using cryo-electron microscopy (cryo-EM), and dynamics were assessed using CRYOSPARC 3D variability analysis. Pharmacological profiles were assessed using assays of well-studied downstream signalling (cAMP production and calcium mobilisation) and regulatory (arrestin recruitment, internalisation) events.
Results. Cryo-EM structures reveal compound 19 binds high in the helical bundle at an interface between TM1 and TM2, and forms direct interactions with the metabolite GLP-1(9-36)NH_2. This correlates with the probe dependent properties of the PAM, which modulates metabolite signalling, but has no effect on other endogenous peptides. In contrast, compound 2 and BETP bind at the intracellular end of TM6 and modulate the signalling and regulatory events of all peptide agonists, but to different extents. Different PAMs also induce distinct complex conformations and dynamics.
Discussion. Structural differences can be correlated to functional data revealing molecular insights into modulation of endogenous peptide activity by different PAMs. These findings will facilitate rational structure-based discovery of non-peptidic drugs targeting the GLP-1R and other related class B1 G protein-coupled receptors.

208

Ligand-directed G protein coupling and downstream signalling of the GLP-1R

John Teye Azietaku[1], Peishen (Elva) Zhao[1,2], Madeleine M. Fletcher[1], Patrick M. Sexton[1,2], Denise Wootten[1,2]
Drug Discovery Biology[1], and ARC Centre for Cryo-electron Microscopy of Membrane Proteins[2], Monash University[1], Parkville, VIC

Introduction. The glucagon-like peptide-1 receptor (GLP-1R) is a validated therapeutic target for the treatment of metabolic disease. Current FDA-approved GLP-1R agonists are efficacious in lowering blood glucose levels, but exhibit varying efficacies in other beneficial effects, including cardiovascular protection and lowering body weight. The underlying mechanisms for these varying clinical profiles are unknown, but are proposed, in part, to be due to GLP-1R biased agonism. GLP-1R agonists exhibit varying degrees of biased agonism when assessing downstream signalling pathways, however a comprehensive understanding of how this arises, and in particular the involvement of different G protein subtypes, is lacking.
Aims. (1) Establish G protein coupling profiles mediated by GLP-1 (endogenous agonist), peptide 19, semaglutide (the current "gold standard" clinical GLP-1R agonist), and the non-peptide agonist, PF 06882961 (currently in clinical trials). (2) Determine the contribution of different G protein subtypes to GLP-1R mediated downstream signalling.
Methods. Heterotrimeric G protein dissociation was monitored using TRUPATH G protein BRET sensors in GLP-1R expressing HEK cells. cAMP production and intracellular calcium (Ca^{2+}) mobilisation were measured in wildtype HEK cells and cells lacking endogenous Gs, Gq/11, or G12/13 proteins; all stably expressing the GLP-1R.
Results. Differences in the potency and maximum BRET signal were observed for different G protein sensors in a ligand-dependent manner. Relative to GLP-1, all ligands were full agonists for Gs and Gq activation, with PF 06882961 displaying a 15-20 fold lower potency. PF 06882961 and semaglutide were partial agonists for G12/13 activation relative to GLP-1 and peptide 19. Gs was essential for cAMP signalling for all agonists, and Gq/11 was essential for Ca^{2+} signalling. However, both Gs and G12/13 subtypes also contributed to the Ca^{2+} response. Re-introduction of Gs expression, in HEK cells lacking Gs, enhanced GLP-1R mediated Gq/11 activation and Ca^{2+} signalling mediated by all agonists.
Discussion. The data revealed bias in the G protein activation profiles of semaglutide and PF 06882961, relative to GLP-1. Interestingly, Ca^{2+} signalling, while dependent on Gq/11, is influenced by Gs and G12 proteins, suggesting a role for these proteins in modulating Gq/11 dependent signalling downstream of GLP-1R activation.

209

The importance of a methylene group in the species variability of VU0467154 at the M_4 muscarinic receptor.

Emma T van der Westhuizen[1], Jack K McDonald[1], Elham Khajehali[1], KH Christopher Choy[1], Geoff Thompson[1], Vi Pham[1], David M Thal[1], Celine Valant[1], Patrick M Sexton[1], Andrew B Tobin[3], Arthur Christopoulos[1]. Drug Discovery Biology, Monash University[1], Parkville, VIC, Australia. Centre for Translational Pharmacology, University of Glasgow[3], Glasgow, United Kingdom.

Introduction. Targeting M_4 muscarinic acetylcholine receptors (mAChRs) with positive allosteric modulators (PAMs) is a novel strategy to treat the negative symptoms of schizophrenia. VU0467154 is an M_4–selective PAM that reverses amphetamine and MK-801-induced deficits in various mouse models of learning, memory and psychosis (Bubser et al., 2014). VU0467154 shows substantial species variability, robustly potentiating the effects of acetylcholine (ACh) at the mouse (m) M_4 mAChR *in vitro* but only weakly potentiating ACh at the human (h) M_4 mAChR.
Aims. To identify amino acid residues underpinning the species variability of VU0467154.
Methods. Allosteric parameters for affinity, cooperativity and efficacy were determined from equilibrium binding assays and ERK1/2 phosphorylation assays in FlpInCHO cells stably expressing human, mouse or mutant M_4 mAChRs.
Results. At the hM_4 mAChR, VU0467154 had weaker affinity (pK_B = 5.7±0.1 hM_4; 6.0±0.1 mM_4) and binding cooperativity (logα; 1.6±0.1 hM_4; 2.2±0.1 mM_4) compared to the mM_4. Similarly, at the hM_4 VU0467154 was a weaker allosteric agonist ($log\tau_B$ = -0.3±0.1 hM_4; 2.1±0.1 mM_4) and had weaker functional cooperativity (logαβ = 0.3±0.3 hM_4; 2.1±0.3 mM_4) compared to the mM_4. The functional cooperativity was restored at the hM_4(V91L/D432E/T433R) mutant mAChR (logαβ = 1.9±0.1) composed of the hM_4 mAChR with several allosteric site residues mutated to their mM_4 counterparts. Likewise, the functional cooperativity was restored at the hM_4(D432E) mAChR (logαβ = 1.8±0.2).
Discussion. A single amino acid change (D432E) in the hM_4 mAChR is sufficient to restore the allosteric effects of VU0467154 at this receptor. The shorter R chain of the aspartic acid (D) residue naturally occurring in the hM_4 mAChR may have an electrostatic clash with the electronegative oxygen atoms of the trifluoro(methylsulfonyl)methane pendant of VU0467154. These results provide important insights into the mechanism underlying species variability at the M_4 mAChR and will guide future medicinal chemistry efforts around the VU0467154 scaffold.

Bubser M, et al., (2014) ACS Chem Neurosci **5**: 920-942

210

Orthosteric or allosteric: implications for determining mechanisms of probe dependence

Wessel A. C. Burger[1], Arthur Christopoulos[1], Celine Valant[1], David M. Thal[1]. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University[1], Melbourne, VIC, Australia.

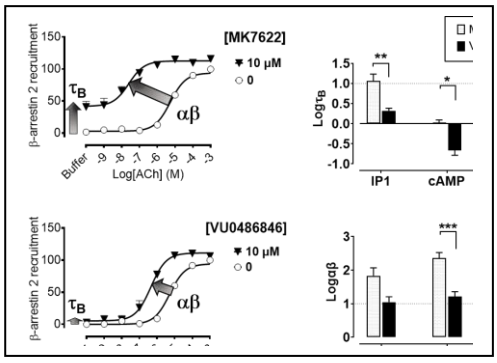
Introduction. LY2033298 is a M_2/M_4 muscarinic acetylcholine receptor (mAChR) selective allosteric modulator that has shown promise in preclinical models predictive of antipsychotic drug behaviour (Chan et al, 2008). Pharmacological characterisation has revealed that the degree and direction that LY2033298 modulates orthosteric ligand activity is dependent on the orthosteric ligand. Specifically, LY2033298 enhances the signalling of the endogenous agonist ACh, but decreases the signalling of another agonist, xanomeline, at the M_2 mAChR (Valant et al, 2012). This makes LY2033298 unique as it selectively stabilizes an ACh active-state receptor conformation whereas it destabilizes the xanomeline active state conformation. Most allosteric modulators stabilize all active state conformations and destabilise inactive-state conformations, or vice versa (Canals et al, 2012). It is uncertain how this occurs and whether this is common to all allosteric modulators at the M_2 mAChR.
Aims. This study characterises the interaction of LY2033298 and a more recent M_2 mAChR selective allosteric modulator, LY2119620, with a range of orthosteric agonists. To determine the minimal functional unit required for probe dependence at the M_2 mAChR we utilise monomeric M_2 mAChR nanodiscs.
Results. We show through radioligand binding studies that LY2119620 and LY2033298 require only a monomeric M_2 mAChR as a functional unit to modulate orthosteric agonist binding. In addition, we show that LY2033298 and LY211298 display different forms of probe dependence with xanomeline in functional assays. However, radioligand dissociation experiments unexpectedly show that xanomeline binds to the allosteric binding site at the M_2 mAChR. Further interrogation using radioligand dissociation experiments reveal that LY2119620 and LY2033298 interact differently with xanomeline in the allosteric binding site explaining the difference observed in functional assays.
Conclusion. This study has important ramifications for understanding mechanisms of allostery and the initial screening of allosteric modulators.

Canals et al. (2012). JBC, 287:650-659
Chan et al., (2008), PNAS. 105:10978–10983
Valant et al., (2012), Mol. Pharmacol. 81:41-52

211

Less is more when it comes to allosteric modulation of the M_1 muscarinic receptor

Huong Thi Mai Nguyen[1], Celine Valant[1], Emma T van der Westhuizen[1], Elham Khajehali[1], Arthur Christopoulos[1]. Analytical Structural Neuropharmacology, Drug Discovery Biology, Monash University[1], Melbourne, VIC, Australia.



Introduction. Targeting the allosteric site of M_1 muscarinic acetylcholine receptors (M_1-mAChRs) is a promising strategy to treat major neurocognitive disorders. Many positive allosteric modulators of the M_1-mAChR (M_1-PAMs), with diverse structures, have been developed. However, in contrast to traditional orthosteric drugs, allosteric modulators are characterized by at least four parameters including binding affinity, binding cooperativity, efficacy, and functional cooperativity. To date, it remains unknown what is an optimal "mix" of pharmacological properties of M_1-PAMs that is sufficient to elicit therapeutic effects (TEs) while limiting potential adverse effects (AEs).
Aims. To characterize the *in vitro* pharmacological profile of 5 structurally distinct M_1-PAMs to explore their role in TEs versus AEs.
Methods. The human M_1 mAChR was stably expressed in HEK293A cell line. Radioligand binding assays were performed to determine the binding affinity (K_B) of the 5 M_1-PAMs and their binding cooperativity (α) with the endogenous ligand, ACh. IP_1 accumulation, β-arrestin 2 recruitment and cAMP production were performed to quantify the efficacy (τ_B) of the M_1 PAMs and their functional cooperativity ($\alpha\beta$) with ACh.
Results. Of all M_1-PAMs tested, VU0486846, which showed no AEs in *in vivo* studies, has a lower binding cooperativity (α) with ACh than other PAMs. It also has weaker allosteric agonist activity (τ_B) and moderate modulatory effect ($\alpha\beta$) across all tested signalling pathways (Fig). M_1-PAMs with cholinergic AEs like MK7622 (Fig), PF06767832 and MIPS1780 have greater allosteric agonism, stronger binding and functional modulation than VU0486846.
Discussion. Our findings support the hypothesis that an M_1-PAM with weak/no agonist activity and moderate modulatory activity at the M_1-mAChR may provide benefits whilst limiting AEs.

212

Human amniotic epithelial cells reduce brain injury after tissue plasminogen activator therapy in ischemic stroke

Liz J Barreto Arce[1], Hyun Ah Kim[1], Shenpeng R Zhang[1], Rebecca Lim[2], Thanh G Phan[3], Christopher G Sobey[1]. Department of Physiology, Anatomy & Microbiology and Centre for Cardiovascular Biology and Disease Research, School of Life Sciences, La Trobe University[1], Melbourne, VIC, Australia; The Ritchie Centre, Hudson Institute of Medical Research[2], Melbourne, VIC, Australia; Department of Medicine, School of Clinical Sciences at Monash Health, Monash University[3], Melbourne, VIC, Australia

Introduction. Currently, tissue plasminogen activator (t-PA) is the only drug treatment available for ischemic stroke. However, the use of t-PA is limited by its narrow therapeutic window with an increased risk of cerebral haemorrhage after 4.5 h of stroke onset. It is known that intravenous administration of human amnion epithelial cells (hAECs) in mice reduces brain injury at 24-72 h following stroke. However, it remains to be tested whether hAECs are protective in the presence of t-PA.
Aim. To assess the neuroprotective efficacy of hAECs in the presence of t-PA.
Methods. Male C57BL/6 mice (aged 8-12 weeks) were subjected to middle cerebral artery occlusion for 1 h followed by reperfusion. Immediately following reperfusion, vehicle (saline, n=18) or t-PA (Alteplase, 10 mg/kg; n=83) was administered via the tail vein. After 30 min of reperfusion, t-PA-treated mice were injected intravenously with either hAECs ($1x10^6$ cells; n=39) or vehicle (2% human serum albumin; n=44). Mice were randomly assigned to one of the three treatment groups and euthanised at 3, 6 or 24 h post-stroke (n= 21, 35, and 45, respectively), at which time brains were collected for blinded assessment of infarct volume progression via analysis of thionin-stained sections.
Results. While there was no mortality within 6 h of stroke, there was a high mortality at 6-24 h in t-PA-treated mice also receiving vehicle compared with mice given t-PA plus hAECs (60% vs 27%), a skewing that prevented an unbiased comparison of infarct volume of survivors at 24 h. Infarct development at 3 h was not different between groups (data not shown). However, at 6 h, infarcts in the subcortical region (corresponding to the expanding infarct core) were ~50% larger in t-PA- vs vehicle-treated mice (22 ± 3 mm^3 vs 15 ± 2 mm^3) but were ~40% smaller in mice receiving hAECs (13 ± 2 mm^3, P=0.03 vs t-PA + vehicle).
Discussion. When administered in combination with t-PA, hAECs attenuated infarct growth and mortality following stroke.

213

Inhibition of oxidative stress prevents the onset of glucose intolerance associated with cigarette smoking.

Stanley MH Chan[1], Kevin Mou[1], Simone N De Luca[1], Kurt Brassington[1], Huei Jiunn Seow[1], Aleksandar Dobric[1], Rana Alateeq[1], Alina Akhtar[1], Stavros Selemidis[1], Steven Bozinovski[1], Ross Vlahos[1]
[1]School of Health and Biomedical Sciences, RMIT University, BUNDOORA, VIC 3083, Australia

Introduction. Smokers are 30 to 40 percent more likely to develop type 2 diabetes than non-smokers and the effectiveness of insulin is reduced in smokers. Glucose intolerance is a form of metabolic disturbance where the body's ability to handle glucose becomes impaired, marking the condition of pre-diabetes. It is understood that smoking increases oxidative stress which plays a major role in the onset and development these diseases. We propose that inhibition of oxidative stress may alleviate metabolic disturbances associated with cigarette smoking (CS).
Aim. To examine the effect of oxidative stress inhibition on CS-induced metabolic disturbances.
Methods. Male BALB/c mice were exposed to either room air (sham) or CS generated from 9 cigarettes per day, 5 days per week for up to 6 months with or without the NADPH oxidase inhibitor and hydrogen peroxide scavenger − apocynin ($5mg{\cdot}kg^{-1}{\cdot}day^{-1}$, i.p. injection) administration.
Results. CS exposure increased tissue oxidative stress and resulted in glucose intolerance ($p<0.05$, n=6). The glucose intolerance was detected as early as 2 months of CS and became more pronounced by 6 months ($p<0.01$, n=6). Indirect calorimetry analysis revealed that smoking increased oxygen consumption (VO_2), while diminishing substrate switching ability (respiratory exchange ratio, RER) without alterations in physical activity. Inhibition of oxidative stress by apocynin effectively lowered the CS-induced oxidative burden in tissues and the onset of glucose intolerance. However, apocynin treatment had no significant effect on the various metabolic parameters, including VO_2, RER and physical activity. Molecular analysis found that smoking blunted insulin-stimulated Akt phosphorylation (serine 473, n=6) in skeletal muscle, which determines glucose uptake and utilisation in muscle. The blunted insulin-stimulated Akt phosphorylation was partially ameliorated by apocynin treatment.
Discussion. Oxidative stress is a major underlying mechanism linking CS to metabolic disturbances. Targeting the excessive oxidants associated with smoking appears to be a viable pharmacological strategy in minimising the onset of more debilitating systemic comorbidities in patients with COPD.

214

Metabolic profiling of mice with deletion of orphan GPCR, GPR37L1

Margaret A Mouat[1,2], Brendan P Wilkins[1], James L J Coleman[2], Eileen Ding[1], Hemna Govindaraju[1], Robert M Graham[2], Nigel Turner[1], Nicola J Smith[1]. School of Med Sci, UNSW Sydney[1], Sydney, NSW; Mol Cardiol Div., Victor Chang Cardiac Research Institute[2], Sydney, NSW.

Introduction. The physiological role for GPR37L1, an orphan G protein-coupled receptor, is not well understood. GPR37L1 has a known role in cardiovascular homeostasis (Coleman et al, 2018; Mouat et al, 2021), and pilot data from our laboratory indicated that *Gpr37l1* knockout mice had elevated body weight between 6-12 months of age.
Aims. This study aimed to thoroughly characterise the metabolic phenotype of mice with genetic deletion of *Gpr37l1* in order to determine how this receptor may be affecting whole body energy homeostasis.
Methods. 8-week-old male *Gpr37l1*$^{-/-}$ mice and wildtype littermate controls (C57BL/6J) were fed high fat diet (HFD) or standard chow for 12 weeks and phenotyped metabolically (glucose/insulin tolerance tests, body composition measurement by magnetic resonance spectroscopy, energy expenditure assessment by indirect calorimetry). A tandem cohort of littermate wildtype and *Gpr37l1*$^{-/-}$ mice were aged for 1 year on a standard chow diet with regular body composition measurements. Results analysed by two-way ANOVA or ANCOVA, with Holm-Sidak post-hoc test.
Results. HFD robustly induced obesity and impaired glucose tolerance in wildtype and *Gpr37l1*$^{-/-}$ mice to a similar degree. *Gpr37l1*$^{-/-}$ mice had an elevated respiratory exchange ratio during their inactive period (7am-7pm; genotype effect P=0.027), indicating preference for glycolytic metabolism. Further, there was elevated energy expenditure by *Gpr37l1*$^{-/-}$ mice during the inactive period (genotype effect P=0.052). This is consistent with the aged cohort; lower body weight and fat mass were observed in the *Gpr37l1*$^{-/-}$ mice at 52 weeks of age when compared to wildtype mice (time X genotype interaction effect P<0.0001 for fat mass accumulation).
Discussion. Minor changes in energy metabolism seen in young *Gpr37l1*$^{-/-}$ mice are likely to contribute to the reduced accumulation of fat mass seen in the aged cohort. This study provides evidence for a modest role of GPR37L1 in whole body energy homeostasis.

Coleman JLJ et al (2018) Biol Sex Differ 9: 14
Mouat MA et al (2021) Front Pharmacol 11: 2384-2404

215

Influenza A virus induces peri-vascular adipose tissue inflammation and vascular dysfunction in pregnant mice

Authors: Osezua Oseghale[1], Stella Liong[1*], Madison Coward-Smith[1], Eunice E. To[1], Jonathan R. Erlich[1], Raymond Luong[1], Felicia Liong[1], Mark Miles[1], Shaghayegh Norouzi[1], Cara Martin[4], Sharon O'Toole[4], Robert D. Brooks[4], Steven Bozinovski[1], Ross Vlahos[1], John J. O'Leary[4], Doug A. Brooks[3] and Stavros Selemidis[1,2*] [1]School of Health and Biomedical Sciences, RMIT Uni, [2]Department of Pharmacology, BDI, Monash University, School of Pharmacy and Medical Sciences, [3]Division of Health Sciences, Uni SA, [4]Emer Casey Research Lab, Molecular Pathology Laboratory, Dublin.

Introduction. Influenza A virus (IAV) infection during pregnancy promotes maternal vascular dysfunction by inflammation and T cell activation, but the details of the mechanism are yet to be clearly defined.
Aim. To examine the IAV and immune profile in the Peri Vascular Adipose Tissue (PVAT) and its contribution to maternal vascular dysfunction
Methods. Eight-to-twelve-week old time-mated pregnant (E12 gestation) C57BL/6 mice were infected intranasally with IAV (HKx31; 10^4 PFU) or with PBS (n=6-8 per group). Mice were euthanized 6 h, 1, 3 and 6 days post-infection for analysis of the viral burden in the vessel wall and PVAT, and maternal vascular immune profile by qPCR, immunofluorescence and flow cytometry. Maternal thoracic aorta vasodilation to Ach and SNP were assessed via wire myography.
Results. Here we demonstrate that IAV disseminates into the PVAT of the aorta, as early as 6 h post infection (p.i), which preceded the significant impairment in endothelium-dependent relaxation to acetylcholine. IAV mRNA levels in the PVAT continued to increase through Days 1-3 post infection with the mRNA levels being ~2 orders of magnitude greater compared with the vessel wall. IAV infection also increased Ly6C^{low} patrolling monocytes and Ly6C^{high} pro-inflammatory monocytes in the vessel wall at 3 days p.i. which was then followed by a greater homing of these monocytes in the PVAT at Day 6 p.i. This vascular immune phenotype was characteristic of a "vascular storm"- like response, with increases in neutrophils, pro-inflammatory cytokines, and oxidative stress markers in the PVAT and arterial wall. IAV also triggered a PVAT compartmentalised elevation in $CD4^+$ and $CD8^+$ activated T cells.
Discussion. In conclusion, the PVAT can act as a vascular niche that supports IAV dissemination and a site for initiating a profound innate inflammatory and adaptive T cell response. This specific role of the PVAT in IAV infection is likely to be central to the genesis of cardiovascular complications caused by respiratory viral infections during pregnancy.

216

Single Cell Transcriptomic Profiling of the Hypertensive Mouse Aorta

Tayla A Hughes[1], Malathi Imiyage Dona[2], Ashleigh Sherriff[1], Henry Diep[1], Gabriella Farrugia[2], Taylor Gaynor[2], Alex Pinto[1, 2], Grant Drummond[1], Maria Jelinic[1], Antony Vinh[1], Centre for Cardiovascular Biology and Disease Research, La Trobe University[1], Bundoora, VIC, Australia; Baker Heart and Diabetes Institute[2], VIC, Australia.

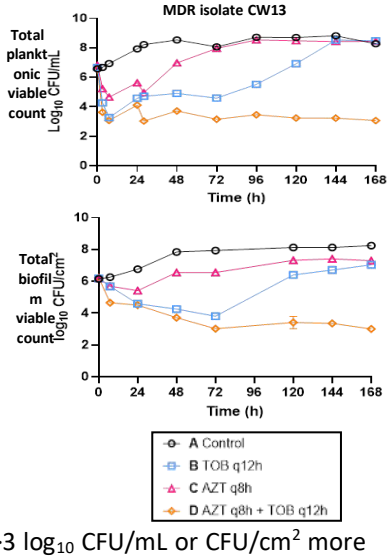
Introduction. Hypertension is the leading cause of global death and morbidity, affecting one third of adults worldwide. Aortic stiffening is a hallmark of hypertension that manifests from changes to both functional (vasoconstriction) and structural (vascular fibrosis and hypertrophy) properties of the vessel wall. While current antihypertensive medications may address the former, there are no therapies that directly target structural changes. There is an urgent need to define the cellular processes that drive pathological aortic remodelling and fibrosis during hypertension.
Aim. To characterise the cellular landscape of the hypertensive mouse aorta and identify novel fibrogenic cells that drive aortic stiffening.
Methods. 12-week-old male C57BL/6 mice were randomly assigned to receive angiotensin (Ang) II- (0.7 mg/kg/day) or vehicle (saline)-infusion via osmotic minipump (*s.c.*). After 28 days, mice were killed, and aortae were harvested and enzymatically dissociated into single cell suspensions. Metabolically active live cells were collected using FACS and prepared for single-cell RNA sequencing using Chromium 10x and NovaSeq genomics platforms.
Results. Single cell transcriptomic analysis of 22,207 cells identified 17 distinct cell types including 14 distinct fibroblast subclusters. Importantly, a novel fibroblast subcluster that uniquely expressed the profibrotic gene *Cthrc1* was identified in Ang II treated aortae (>70-fold increase), which was almost non-existent in vehicle-treated mice. This *Cthrc1*$^{+}$ fibroblast cluster also exhibited upregulation of genes relating to ECM remodeling (*Thbs2*, *Cdh11* and *Postn*) and collagen (*Col1a1*, *Col3a1* and *Col5a1*) at the highest rate in comparison to any other fibroblast subcluster. Thus, gene ontology revealed upregulation of profibrotic signaling pathways in the *Cthrc1*$^{+}$ fibroblast subcluster (i.e., cell adhesion, ECM organisation and collagen fibril organisation). Immunohistochemistry revealed that *Cthrc1*$^{+}$ was localized in the adventitia of hypertensive mouse aortae but absent in that of vehicle-control mice.
Discussion. We report the first ever comprehensive analysis of the aortic cellulome in the setting of hypertension. The identification of a novel profibrotic fibroblast that was only present in aorta from hypertensive mice, raises the exciting possibility that they may be a key driver (and potential future therapeutic target) of aortic stiffening.

217

Targeting hypermutable cystic fibrosis *Pseudomonas aeruginosa* clinical isolates with an inhaled aztreonam and tobramycin dosing regimen in a dynamic *in vitro* biofilm model

Siobhonne Breen[1], Kate E. Rogers[1], Wee Leng Lee[1], Jessica R. Tait[1], Roger L. Nation[1], Cornelia B. Landersdorfer[1]. Drug Delivery, Disposition and Dynamics, Monash University[1], Parkville, VIC, Australia.

Introduction. *Pseudomonas aeruginosa* (Pa) is a biofilm-forming opportunistic pathogen commonly infecting patients with cystic fibrosis (CF). Antibiotic resistance is one of the greatest threats to humans and ensuring effective use of antibiotics is essential. Suboptimal antibiotic regimens have supported the rise of multidrug-resistance (MDR), particularly with the hypermutable Pa strains that are prevalent in CF.
Aims. To evaluate aztreonam (AZT) plus tobramycin (TOB) regimens against clinical hypermutable Pa strains in a dynamic *in vitro* biofilm model (BF).
Methods. MDR isolates, CW5 and CW13 (MIC_{AZT} ≥32mg/L, MIC_{TOB} 8mg/L), from patients with CF were investigated in static concentration time-kill studies before being studied in a 168h BF (inoculum $10^{5.5}$ CFU/mL). Inhaled AZT (75mg, q8h) and TOB (300mg, q12h) dosing regimens were examined in mono and combination therapies. The BF simulated the PK of AZT and TOB in lung fluids based on published PK in CF patients ($t_{1/2}$=3h). Total viable counts were determined for planktonic and biofilm bacteria. Resistant bacteria were quantified at 5 time points.



Results. For both isolates, TOB monotherapy demonstrated >2 log_{10} CFU/mL initial killing of planktonic bacteria at 7h. All monotherapies led to amplification of resistant populations and regrowth close to the control by 168h. The combination was synergistic (>3 log_{10} CFU/mL or CFU/cm^2 more bacterial killing compared to the best monotherapy) against planktonic and biofilm bacteria of both isolates at 168h. Minimal resistant subpopulations were detected in the combination samples.
Discussion. The combination of AZT and TOB was required to suppress regrowth and resistance of the planktonic and biofilm bacteria of hypermutable MDR Pa isolates from patients with CF. As this inhaled combination regimen demonstrated promising synergistic activity, further investigation is warranted.

218

Polypharmacy with high Drug Burden Index affects physical performance differently in mice of varying age and sex, over 23 hours

Trang Tran[1,2], John Mach[1,2], Gizem Gemikonakli[1,2], Harry Wu[1,2], Heather Allore[3], Susan Howlett[4], Christopher Little[5] & Sarah N Hilmer[1,2]. Lab of Ageing and Pharmacology, Kolling Institute, Faculty of Medicine and Health, Univ of Sydney, Sydney, NSW, Australia[1]; Clinical Pharmacology and Aged Care, Royal North Shore Hosp, Sydney, NSW, Australia[2]; Dept of Biostatistics, Yale Univ, Connecticut, USA[3]; Dalhousie Univ, Halifax, NS, Canada[4]; Raymond Purves Bone and Joint Research Lab, Kolling Institute, Sydney, NSW, Australia[5].

Introduction. Polypharmacy is associated with adverse outcomes, which is a global healthcare challenge for the ageing populations. The Drug Burden Index (DBI) measures cumulative exposure to anticholinergic and sedative medications. DBI exposure is associated with impaired physical function in older adults and in mice. There is limited understanding of the sex-specific impacts of polypharmacy and DBI, particularly on daily physical activities across the diurnal cycle.
Aims. We investigated how polypharmacy with high DBI (HDBI) affects spontaneous physical behaviours over 23 hours in young (4 months) and old (23 months) C57BL/6 male and female mice.
Methods. Mice (n=6-8) were randomised by age and sex strata to receive control feed or HDBI polypharmacy feed (simvastatin, metoprolol, oxybutynin, oxycodone, and citalopram) for 4 weeks. An additional cohort of young males and females (n=6) received monotherapy of metoprolol or simvastatin. Mice were singly caged in the LABORAS device, which automatically records different behaviours continuously over 23 hours.
Results. After 4 weeks of treatment, compared to control, HDBI polypharmacy significantly decreased active behaviours, including distance travelled, mean gait speed, durations of locomotion and rearing, during the light period but increased them during the dark period in mice of both ages and sexes *($p<0.05$).* The declines observed were more marked in females than males *($p<0.05$)*. In young mice, metoprolol and simvastatin monotherapies increased some exploratory activities, compared to administration of these medications within the HDBI polypharmacy *($p<0.05$)*.
Discussion. Polypharmacy with HDBI impaired physical function variably across the diurnal cycle, and with greater impact in females. This method can be applied to future studies exploring the effects of a range of polypharmacy and monotherapy regimens in mice of different ages and sexes across the diurnal cycle, and investigation of the mechanism.

219

Tobacco cessation medications in tobacco smokers receiving treatment for opioid use disorder

Lobbe C, Bahnisch J, Lin C, Shakoor A, Murnion B. Central Coast Clinical School, Faculty of Medicine and Health, University of Newcastle, NSW

Introduction: Despite effective interventions for tobacco cessation, up to 90% of people in Opioid Agonist Treatment (OAT) smoke, compared to the population average of 12%. This is associated with high levels of tobacco related morbidity and mortality. There is advocacy to make unregulated e-cigarettes available to high risk populations.
Aims: To explore treatment experience with, knowledge of and attitudes to smoking cessation medications (nicotine replacement therapy (NRT), bupropion and varenicline) and attitudes to e-cigarettes in patients receiving OAT for opioid use disorder and clinicians in a regional drug and alcohol service.
Methods: Cross-sectional surveys of patients (PP) and clinicians (HCPP) in two public OAT clinics were conducted during April to May 2021.
Results: 91 PP and 10 HCPP completed the surveys. Most PP had at least one lifetime quit attempt, and many were currently trying to quit. There was high levels of knowledge and experience with NRT, lower levels with varenicline and very limited knowledge or prior experience of bupropion. Most PP reported inadequate knowledge of bupropion and varenicline to rate helpfulness or likelihood of use. Subjects ranked NRT as their preferred tobacco cessation intervention, followed by e-cigarettes. Despite this, subjects scored e-cigarettes higher than NRT for perception of helpfulness with cessation. Few subjects had discussed smoking cessation interventions (SCI) with their clinicians or been offered smoking cessation medications. HCPP acknowledged tobacco harms, thought patients should be supported with SCI but reported poor knowledge and confidence about tobacco cessation medications and SCI.
Discussion: There are high rates of tobacco cessation planning, attempts and experience of NRT in this population but low levels of knowledge and experience with varenicline and bupropion. E-Cigarettes are considered favourably. SCI is infrequent. Improving knowledge about tobacco cessation medications in both patients and clinicians could improve SCI and uptake of approved and available medications.

220

Comparing Effects of Polypharmacy on Inflammatory Profiles in Older Adults and Mice: Implications for Translational Ageing Research

Harry Wu[1,2], John Mach[1,2], Danijela Gnjidic[3,4], Vasi Naganathan[5,6], Fiona M. Blyth[6,7], Louise M. Waite[5,6], David J. Handelsman[8], David G. Le Couteur[4,5,6], Sarah N. Hilmer[1,2.] [1]Laboratory of Ageing and Pharmacology, Kolling Institute, University of Sydney and [2]Royal North Shore Hospital, Sydney, NSW, Australia. [3]Sydney Pharmacy School, [4]Charles Perkins Centre, University of Sydney, NSW, Australia. [5]Department of Aged Care, Concord Hospital, [6]Centre for Education and Research on Ageing (CERA), ANZAC Research Institute, University of Sydney, Concord, NSW, Australia. [7]School of Public Health, University of Sydney, Sydney, NSW, Australia. [8]Department of Andrology, Concord Hospital and ANZAC Research Institute, Sydney, NSW, Australia.

Introduction: Polypharmacy is associated with increased morbidity and mortality in older adults. Inflammation plays important roles modulating drug metabolism and drug effects. No previous studies have compared inflammatory profiles in humans and mice with polypharmacy. Methods: A cross-sectional analysis of data from the five year wave of the Concord Health and Ageing in Men Project, a population-based study of community-dwelling men aged ≥ 70 years. Serum concentrations of 27 cytokines were measured using a multiplex immunoassay. Associations between polypharmacy (≥ 5 medications) and cytokines were evaluated using multivariable linear regression adjusting for age, frailty, comorbidities and individual drug classes. Interaction between polypharmacy and Drug Burden Index (DBI - exposure to drugs with anticholinergic and sedative effects) was analysed. Effects of polypharmacy and DBI on serum levels of 23 cytokines, also measured with a multiplex immunoassay, were determined in ageing male mice treated with chronic polypharmacy or control. Results: Inflammatory profiles differed significantly between CHAMP participants exposed to polypharmacy (n=409) and those who were not (n=495). Participants with polypharmacy had significantly higher concentrations of IL-8, IL-6, CCL3, Eotaxin, IL-1ra, IL-1β, IP-10 and lower concentrations of anti-inflammatory cytokine IL-4. In fully-adjusted multivariable models, polypharmacy was positively associated with concentrations of IL-8 and CCL3. There were no significant differences in inflammatory profiles between control and polypharmacy-treated mice. The relationship was not influenced by DBI. Conclusions: Inflammatory markers associated with polypharmacy in older adults were not seen in healthy aged mouse models, and may be related to underlying diseases. The polypharmacy mouse model provides opportunities for mechanistic investigations in translational research.

221

A systematic review of adverse drug reactions or adverse drug events of heart failure treatment in frail older adults

Mai Duong[1], Danijela Gnjidic[2], Andrew McLachlan[2], Marissa Sakiris[3,] Sarah Hilmer[1]. Northern Clin School, Fac of Med and Health, Univ of Sydney, Kolling Institute, Royal North Shore Hosp[1], Sydney, NSW, Australia; Sydney Pharmacy School, Univ of Sydney[2], Sydney, NSW, Australia; Dept of Pharmacy, Royal North Shore Hosp[3], Sydney, NSW, Australia.

Introduction. Frail older adults with heart failure (HF) have a high risk of mortality and morbidity and may be more vulnerable to adverse outcomes due to multiple co-morbidities and polypharmacy.
Aims. To determine the type, prevalence, causality and severity of adverse drug reactions (ADRs) or adverse drug events (ADEs) reported in frail older adults related to their HF treatment.
Methods. A systematic search of electronic databases (e.g. CENTRAL, MEDLINE, Embase, Ageline, CINAHL, International Pharmaceutical Abstracts, PsychInfo, Scopus), registries and citations was conducted according to PRISMA 2020 checklist. Eligible studies included randomised controlled trials (RCTs) or observational studies of people diagnosed with HF, aged ≥65 years, with frailty defined by an objective and validated measure, and reported ADRs or ADEs of guideline directed HF treatments. Two reviewers screened all studies (Kappa=0.88) and assessed the quality (GRADE) and risk of bias (RoB2, ROBINS-I). A second reviewer validated data extracted from 20% of studies.
Results. On preliminary analysis, two RCTs (n=15,032) and three cohort studies (n=1644) were included. Severe ADEs such as mortality and hospitalizations were higher in frail compared to non-frail patients (HR 1.18-2.19, P<0.001) across all HF treatments reported in the RCTs. One observational study reported ADRs according to the Naranjo and Hallas scales. Fourteen percent (n=13/96) of early readmissions were attributed to ADRs and two were attributed to digoxin use. Falls, tiredness and nausea were classified as 'probably avoidable' ADRs, as alternative options or mitigating treatments existed. Analysis of ADEs in a RCT showed that compared to robust patients, very frail patients (frailty index score≥0.311) taking sacubitril/valsartan, aliskiren or ACEIs/ARBs were twice as likely to fall and more likely to experience hypotension, hyperkalaemia, fractures, angioedema and drug discontinuation (P<0.001).
Discussion. Frail older adults were more at risk of severe ADRs or ADEs. ADRs or ADEs were not routinely reported in well-defined frail populations, demonstrating a need for investigation and inclusion in clinical trials and future research.

222

The orphan GPCR GPR101 contributes to growth hormone secretion via constitutive activation of Gs and Gq/11

Julien Hanson[1].
[1]Laboratory of Molecular Pharmacology, GIGA-Molecular Biology of Diseases, University of Liège, Liège, BELGIUM.

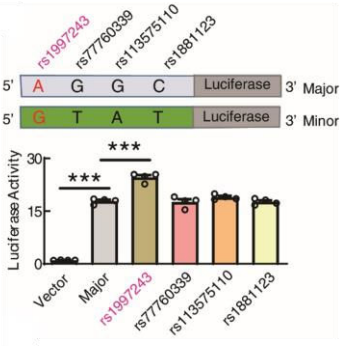
Introduction. G protein coupled receptors are seven transmembrane proteins that are involved in nearly all physiological processes. They represent the most important source of drug targets. However, around 100 receptors remain orphans with unknow ligands and function. Growth hormone (GH) is a key modulator of growth, secreted by specialized cells of the anterior pituitary called somatotropes. GH secretion is under the control of hypothalamic hormones such as GH-releasing hormone (GHRH) and somatostatin that act on their respective receptors on somatotropes. GH over-secretion can lead to gigantism and one form is X-linked acrogigantism (X-LAG), in which infants develop GH-secreting pituitary tumors over-expressing the orphan G-protein coupled receptor, GPR101.
Aims. Although GPR101 is associated with X-LAG, its role in GH secretion remains obscure. Thus, we aimed at investigating its function in the pituitary.
Methods. We studied GPR101 signaling pathways and their effects in HEK293 and rat pituitary GH3 cell lines, human tumors and in transgenic mice with elevated somatotrope Gpr101 expression driven by the rat Ghrh receptor (Ghrhr) promoter ($Ghrhr^{Gpr101}$) in somatotropes.
Results. We observed that Gpr101 causes elevated GH/prolactin secretion in transgenic $Ghrhr^{Gpr101}$ mice but without hyperplasia/tumorigenesis of the pituitary, which is a usually associated with elevated GH. We show that GPR101 constitutively activates not only G_s but also $G_{q/11}$ and $G_{12/13}$, which leads to GH secretion but not proliferation. These signatures of GPR101 signaling, notably PKC activation, are also present in human pituitary tumors with high GPR101 expression.
Discussion. These results point to a role for GPR101 in the regulation of somatotrope axis function. Interestingly, the combined activation of several G proteins-mediated pathways led to increased GH secretion but did not affect proliferation of somatotropes. This opens interesting perspectives as the activation of other receptors involved in GH secretion such as GHRHR, coupled to G_s only, promotes both GH secretion and somatotropes proliferation.

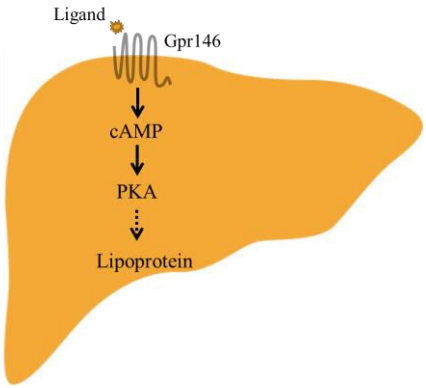
223

Orphan GPR146 regulates blood cholesterol levels in humans and mice

Yan Wang. Department of Biochemistry, College of Life Sciences, Wuhan University, China

Dyslipidemia has strongly heritability. However, currently the known mutations can only explain 10-20% of the heritability. Large scale Genome-wide association studies (GWAS) have been performed extensively on blood lipids levels and thousands of variants have been identified that are strongly associated with blood lipids levels. However, about 95% of those GWAS variants are located in genome noncoding regions with unknown function, which is one of the biggest challenge at post GWAS era.





Combining with bioinformatics analysis and experimental verification, we systematically characterized those noncoding GWAS variants and found that a noncoding SNPs rs1997243 is the disease-causing variant at *7p22* locus. We further found that rs1997243 specifically up regulates the expression level of an orphan G-protein coupled receptor GPR146. And decreasing the expression of gpr146 in livers of mice leads to significantly reduced blood cholesterol levels and protects the mice from high-fat or high-fat high-cholesterol diet induced hypercholesterolemia. Those results provide the molecular mechanistic explanation for the strong association between *7p22* locus and hypercholesterolemia in human. GPR146 is highly expressed in liver of hepatocytes and is specifically localized on the plasma membrane. It responses to heat-inactivated serum and activates cAMP-PKA-Creb pathway. To our knowledge, this is the first report showing a GPCR directly regulates blood cholesterol levels in both human and mouse. Our study suggests that GPR146 is a potential drug target to treat hyperlipidemia and cardiovascular diseases.

224

Do RAMP and MRAP accessory proteins modulate orphan putative cannabinoid receptors GPR18 and GPR55?

Nathaniel A.K. Glenn[1], David B. Finlay[2], Kathy G. Mountjoy[1], Christopher S. Walker[3], Natasha L. Grimsey[1]. School of Medical Science, Faculty of Medical & Health Science, University of Auckland, NZ[1]; School of Biomedical Science, Division of Health Science, University of Otago, NZ[2]; School of Biological Science, Faculty of Science, University of Auckland, NZ[3].

Introduction. Receptor activity-modifying proteins (RAMP1-3) and melanocortin receptor accessory proteins (MRAP1-2) are best known for their roles in modulating the calcitonin receptor-like receptor (CLR) and melanocortin receptor 2 (MC2) (respectively). However, interactions with other GPCRs have been reported. GPR18 and GPR55 are orphan putative cannabinoid receptors, which are also proposed to have other endogenous ligands (N-arachidonoyl Glycine [NAGly] and Lysophosphatidylinositol [LPI] respectively). However, there are considerable discrepancies in ligand efficacy and subcellular localisation between studies suggesting other factors may be involved. We hypothesised that accessory proteins may influence GPR18 and GPR55 function.
Aims. We aimed to investigate the effects of RAMPs 1-3 and MRAPs 1-2 on GPR18 and GPR55 expression, subcellular localisation and signalling. Cannabinoid receptors CB1 and CB2 were investigated concurrently.
Methods. Receptors (HA-tagged) and either accessory proteins (untagged or FLAG-tagged) or benign plasmid control were transiently transfected into HEK-293S cells. CLR and MC2 were utilised as controls. Cell surface and total expression of receptor and accessory proteins were quantified using immunocytochemistry with automated imaging. Signalling was detected via CAMYEL cAMP biosensor, arrestin translocation biosensor, TRUPATH G protein biosensors, and AlphaLISA pERK assay.
Results. CLR and MC2 responded as expected. Several novel effects of accessory proteins on receptor expression, or vice versa, were noted. Differing patterns across the dataset suggested these were not bystander artefacts of over-expression. Notably, GPR55 and both MRAPs exhibited reciprocal reductions in cell surface and total expression. No influence of accessory proteins on CB1 or CB2 signalling was detected. GPR18 did not respond to putative ligands regardless of accessory protein expression. GPR55 signalling is under investigation.
Discussion. Our findings indicate the potential for specific pairings of the accessory proteins and receptors studied to influence each other's expression and/or function. Importantly, where accessory proteins and accessory-interacting receptors are natively co-expressed, sequestration of accessory proteins may be a mechanism for receptor crosstalk.

225

GPR52 and its role in cognition

Gregory D Stewart[1], Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University[1], Parkville, Vic., Australia

Schizophrenia is a chronic and debilitating psychiatric disorder affecting 1% of the population, with over 50 million diagnosed cases in 2020. Patients exhibit a heterogeneous collection of symptoms including acute psychotic episodes (positive symptoms), anhedonia (negative symptoms), and cognitive deficits. Cognitive deficits are the major contributor to poor quality of life of schizophrenia patients and their severity is a predictor of patient outcomes. Targeting G protein-coupled receptors (GPCR) remains a viable and tractable approach to treat schizophrenia and cognitive deficits, however many GPCR-targeting compounds that demonstrate promise in preclinical cognitive testing fail to translate to the clinic; potentially due to the permissive nature of common preclinical tests. GPR52 is an orphan GPCR with a unique expression profile. It is almost exclusively found in the brain in the striatum and the prefrontal cortex – both of these regions are associated with psychosis and cognition, respectively. Preclinical testing of tool GPR52 agonists suggest that activation of GPR52 has antipsychotic and pro-cognitive potential.
To understand the molecular mechanisms of GPR52 signalling, we studied the effects of the GPR52 agonist, 3-BTBZ, on cell signalling (cAMP accumulation and beta-arrestin-2 recruitment), western blotting and immunofluorescence (key neuronal signalling molecules) assays, *in vitro* and *ex vivo*. We also assessed the pro-cognitive capacity of 3-BTBZ we employed the translational mouse touchscreen spatial working memory task, trial-unique, delayed non-matching-to-location (TUNL). Our studies revealed that 3-BTBZ: increased cAMP concentration and engaged with beta-arrestin-2 in recombinant cells and primary neurons, and modulated key neuronal signaling molecules, ex vivo. 3-BTBZ also demonstrated pro-cognitive effects in its own right and in a psychotomimetic-induced deficit model of cognitive deficit.
Together, the delineation of GPR52 signalling mechanisms in native systems and the pro-cognitive effects of 3-BTBZ in a translational touchscreen model of cognition suggest GPR52 may be a viable target for the treatment of cognitive deficits in schizophrenia.

226

The National Prescribing Curriculum–supporting confident and rational prescribing by health professional graduates

Clare E Weston[1], Content and Interventions Manager, Jeff Elliott, Program Support Lead[1] NPS MedicineWise[1], Sydney NSW, Australia.

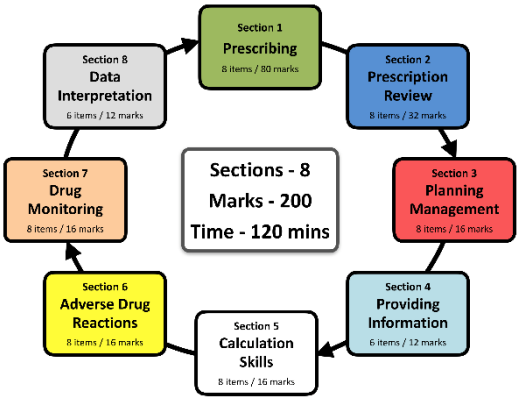
Introduction: The NPS MedicineWise National Prescribing Curriculum (NPC) is a nationally available interactive case-based online course funded by the Australian Government Department of Health. It is designed to develop skills and confidence in prescribing by medical and pharmacy students. Currently comprising 24 general modules and 3 dental modules, the NPC covers elements of the curriculum around diagnosis, treatment and prescribing for major diseases.
Aims: This session will outline the history of the NPC since its introduction in 2002, highlight key characteristics of the modules, and share recent and future developments.
Methods. Each module follows a stepwise approach as per the WHO's Guide to Good Prescribing and content is also mapped to the national Prescribing Competencies Framework. Students work through each module answering the in-built question, create their own drug formulary, and complete a prescribing activity. Expert feedback is provided as to best practice. The NPC modules undergo regular review to ensure they reflect contemporary guidance and practice.
Results:. A 2019 international evaluation of online clinical pharmacology curriculum resources for medical students ranked the NPC the highest out of 8 resources.[1] In 2020 48,028 NPC modules were completed by 4,840 medical students and 443 pharmacy students from most Australian medical and pharmacy schools. Tutor and student feedback supports the NPC's usefulness and relevance as a teaching tool to improve student confidence in prescribing.
Discussion: Developments include ongoing update, adaptation for sharing on university learning platforms, support for the Prescribing Skills Assessment and collaboration with Pharmacy Schools to adapt some modules to further address pharmacy student needs.

1. Zhang XY, Holbrook AM, Nguyen L, et al. Br J Clin Pharmacol. 2019 Nov;85(11):2599-2604.

227

Recent developments with the Prescribing Safety Assessment

Simon Maxwell. Clinical Pharmacology Unit and Medical Education Centre, University of Edinburgh, Edinburgh, UK.



The UK *Prescribing Safety Assessment* (PSA) was the first national online prescribing assessment dedicated to testing the competence of new medical graduates to undertake their responsibilities as prescribers of medicines in a modern healthcare environment. This issue has assumed particular importance for healthcare providers around the world since it has been demonstrated that a significant proportion of the prescriptions written by newly qualified doctors contain errors and that many patients experience avoidable adverse drug reactions. Many providers and national authorities are seeking an assessment that can be a surrogate marker for prescribing competence. Drawing on the experience of the PSA over the last 10 years, this presentation will discuss the development, delivery, quality assurance and governance of the assessment and highlight some of the challenges encountered. It will highlight the major impact that this project has had in raising the profile of UK clinical pharmacology and prescribing education. This approach is currently being piloted in Australia, New Zealand and several other countries

Maxwell SRJ, Coleman JJ, Bollington L, Taylor C, Webb DJ. Prescribing Safety Assessment 2016: Delivery of a national prescribing assessment to 7,343 UK final-year medical students. Br J Clin Pharmacol. 2017;83:2249–2258.
Maxwell S, Cameron IT, Webb DJ. Prescribing Safety Assessment. Lancet. 2015;385:579–581.
Maxwell SRJ, Webb DJ. Improving medication safety: focus on prescribers and systems. Lancet. 2019;394:283–285.

228

The Prescribing Skills Assessment - a journey across Australia and New Zealand

Claire Harrison[1]. Department of General Practice, School of Public Health and Preventive Medicine, Monash University[1], Melbourne, VIC, Australia

Introduction. The Prescribing Skills Assessment (PSA) is an online teaching and assessment tool in medication safety. In partnership with the British Pharmacological Society, it has been used in an increasing number of medical schools across Australia and New Zealand. It provides users with opportunities to practice and obtain feedback on multiple skills required for safe prescribing. These include prescribing for paediatric and adult patients, identification of adverse effects and drug interactions, dose calculations, provision of information, identification of success or failure of medication regimens and alteration of treatments informed by relevant investigations. It encompasses a strong focus on scenarios susceptible to medication harm, from error-prone medications to patient factors such as age, renal or liver disease and polypharmacy. It encourages use of formularies as an intrinsic component of safe practice.
Aims. To describe the implementation of the PSA, its impact and to explore potential future directions.
Methods. A narrative account of the collaborative process of implementation and its impact, with an analysis of quantitative and qualitative data relating to participation, performance and user experience.
Results. From an initial school in 2015, participation has increasingly expanded to its current use in 13 medical schools across Australia and New Zealand. As at 2020, a multidisciplinary team drawing on over 50 senior and junior staff across Universities, countries and clinical settings, has delivered the Prescribing Skills Assessment to 11,157 students. In exam sittings, this equates to the marking of 669,420 medication safety related items, including 89,256 prescriptions. It is perceived as an acceptable and relevant tool. It has raised the visibility of, and driven educational initiatives in clinical pharmacology, therapeutics and prescribing. It has brought to the fore the critical requirement for medication safety as a graduate outcome.
Discussion. The Prescribing Skills Assessment is a key initiative to support safe and rational prescribing and to contribute to the global aspiration to reduce medication errors. Its journey has drawn together a rich collaboration of stakeholders across academia, clinical settings and industry. Future plans may include use by increasing numbers of medical schools, engagement with additional stakeholders and opportunities for interprofessional educational innovations.

229

A programmatic interprofessional approach to prescribing education: medication safety as a team sport

Kellie Charles[1] and Carl R Schneider[1]. Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney[1], Sydney, NSW, Australia.

Introduction. Medication management is a collaborative inter-professional process, with both individual and team responsibilities (Figure 1). Medication-related patient safety has been shown to be compromised when poor teamwork is encountered in critical areas of shared care (e.g. hospital admission, handover, discharge from hospital, community practice). Therefore, it is essential that all health professional students from disciplines managing any aspects of medication management have appropriate team socialisation and communal skill development to prepare them for practice.
In this presentation, we will discuss the implementation of the WHO Framework for Action on Interprofessional Education and Collaborative practice in relation to the medication management cycle and application to curriculum development as a guiding principle for the Therapeutics vertical in the new MD program at The University of Sydney.

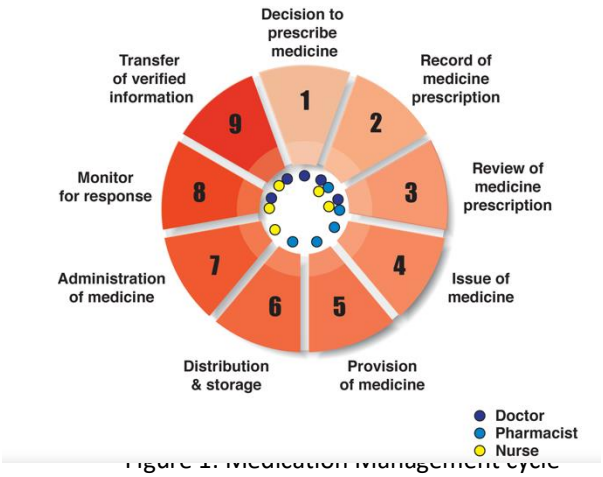


Figure 1. Medication management cycle

References:
World Health Organisation (2010) Framework for Action on Interprofessional Education & Collaborative Practice, WHO Reference Number: WHO/HRH/HPN/10.3, https://www.who.int/publications/i/item/framework-for-action-on-interprofessional-education-collaborative-practice
Yan J, Gilbert JHV, Hoffman SJ (2007) World Health Organization Study Group on Interprofessional Education and Collaborative Practice. Journal of Interprofessional Care, 21:588–589.

230

Impact of adherence on HIV, malaria, and TB treatment and prevention

Rada Savic[1]. Department of Bioengineering and Therapeutic Sciences, University of California San Francisco[1], San Francisco, CA, USA.

Poor adherence to prescribed medication is a common phenomenon across cultures, ages, and disease states that results in treatment failure or disease recurrence. Adequate patient adherence is necessary but challenging to achieve, especially in complex settings. Tuberculosis (TB), malaria, and HIV are manageable diseases that yet kill more than 2 million people per year. Their treatment and prevention strategies consist of long and complex drug combinations that need to be adhered to. However, information is lacking on what level and pattern(s) of poor adherence are putting patients at risk, and on what additional factors (pharmacogenomic diversity, environmental factors, and comorbidities) might further increase risk and significantly impacting disease outcome. The talk will address this topic and showcase the interplay between adherence, comorbities and treatment outcome for major infectious diseases.

231

A decision support tool for the management of adherence in patients with gout

Natalia Smith Diaz[1], Sophie L. Stocker[2], Daniel FB Wright[1]. School of Pharmacy, University of Otago[1], Dunedin, NZ. School of Pharmacy, University of Sydney [2], Sydney, Australia.

Introduction: Adherence to allopurinol therapy is often suboptimal[1]. A tool to identity poor adherence would aid screening in clinical trials and therapy decisions in clinical practice.
Aim: To develop and validate a tool to identify poor-adherence to allopurinol therapy.
Methods: A simulation study using an oxypurinol population pharmacokinetic model to predict trough and peak oxypurinol concentrations assuming perfect adherence was conducted. Two thousand stochastic simulations were performed at doses of 100-800mg daily, under different creatinine clearance bands, and accounting for diuretic use and ethnicity. Patient characteristics were sampled as a group from a population of virtual patients derived from the Gout in Aotearoa study [2]. The 20th percentile of the predicted concentrations defined the threshold below which poor adherence was assumed. The tool was evaluated by simulating a population of gout patients with a 50% reduction in adherence (missed every 2nd dose). The predictive performance of the tool was assessed using sensitivity, specificity (S&S), ROC curves, and negative and positive predictive value (NPV, PPV).
Results: Sensitivity and specificity values for the trough concentrations were 89% - 95% and 76 - 83% respectively with ROC curve AUCs ranged from 0.84–0.88. PPV and NPV were found to be 79-84% and 88-94% respectively.
Discussion: The predictive performance of the tool is suitable for screening and decision-support. Further research to detect imperfect adherence based on urate concentrations would enhance utility.

1. Scheepers L et al (2018). Semin Arthritis Rheum 47(5):689-702.
2. Phillps-Green et al (2016). Ann Rheum Dis 75(1):124-30

232

Adherence monitoring in Medication Assisted Treatment of Opioid Dependence

Bridin Murnion[1,2]. Clinical Pharmacology, St Vincent's Hospital[1] and Discipline of Addiction Medicine, Faculty of Medicine and Health, University of Sydney[2], Sydney, NSW, Australia

There are over 50,000 people receiving Medication Assisted Treatment of Opioid Dependence (MATOD) in Australia. Interventions used to monitor and improve adherence are multi-faceted, reflecting the unique challenges of MATOD. They include varying levels of treatment supervision, drug screening in biological matrices and most recently development of depot formulations of medication. Frequency of drug screening in biological matrices can depend on the clinical or legal scenario and in some instances can be required more than weekly. Choice of biological matrix can similarly depend on the clinical or forensic context. There are multiple assay methods for urine drug screening that have varying sensitivity and specificity. Perceived clinical benefit of point of care urine testing and assay cost can also direct the assay method used but the limitations of the assay chosen may not be appreciated. There is often multi-agency involvement in care with multi-agency interpretation of drug screening in biological matrices. Non-clinical staff may have an even more limited understanding of the limitations of the matrix and assay, with consequences that extend beyond the biomedical domain. This presentation will explore the complexities of drug screening as adherence monitoring in MATOD.

233

Estimating forgiveness using trials of placebo-substituted active drugs

Dyfrig A Hughes[1], Abdullah Al-Bassam[2]. School of Medical and Health Sciences, Bangor University[1], Bangor, Wales; Department of Pharmacy Practice, Kuwait University[2], Kuwait City, Kuwait.

Introduction. The ability of medicines to continue providing therapeutic benefit despite the occasional missed dose depends on their property of 'forgiveness'. This is defined as the maximum duration of dose interruption during which sufficient drug effect is maintained.
Aims. To perform a systematic review of randomised controlled trials (RCTs) that examine the duration of permissible interruption in antihypertensive therapy by substituting active drug doses(s) with placebo.
Methods. Following protocol registration with PROSPERO, RCTs were identified from electronic databases using search terms relating to antihypertensive treatments and missed doses, connected by Boolean operators. Studies were included if they involved randomising hypertensive patients to groups that allow comparison within- or between- different antihypertensive drugs, of one or more consecutively missed dose using placebo substitution. Data were extracted on study population, methods (experimental design, blinding design, interventions, primary outcomes) and study results. Each RCT was assessed using the Cochrane risk of bias tool.
Results. Among the included studies were trials comparing angiotensin II receptor antagonists (mainly losartan and telmisartan), angiotensin-converting enzyme inhibitors (mainly perindopril), calcium channel blockers (mainly amlodipine), aliskiren or indapamide. Methodologies were inconsistent across studies; however, they typically centred around the comparison of the change from baseline in 24-h ambulatory blood pressure following a missed dose at steady state of drug A versus B. Risk of bias was low in the majority of studies.
Discussion. RCTs involving placebo substitutions can provide evidence to support labelling instructions on missed doses, and identify medicines that may diminish the potentially hazardous effects of non-adherence.

234

New perspectives on drug safety and the mitochondria: from toxicological target to personal susceptibility factor

Dr Amy Chadwick, Department of Pharmacology and Therapeutics, The University of Liverpool, UK

Dr Amy Chadwick is a lecturer working in the Department of Pharmacology and Therapeutics at the University of Liverpool where she leads the Bioenergetics Group, with the aim of defining and understanding the role of the mitochondria and dynamic bioenergetic responses in the onset of adverse drug reactions in some individuals.

Individual energy phenotype arises from a complex interaction of mitochondrial and nuclear genetics against a background of environmental factors. It is hypothesised that individuals vary in terms of bioenergetic status, based on activity and efficiency, and that this baseline can influence a patient's susceptibility to toxicity. The aim of this research was to variation in bioenergetic status and susceptibility to mitotoxicants in freshly isolated human hepatocytes (FHH), using real-time respirometry to monitor ATP production rates (OXPHOS and glycolytic).

A marked variation in basal metabolic function FHH and susceptibility to rotenone (IC_{50} 0.1 - 100 M) was defined. Strikingly, we found that glycolytic energy metabolism plays an essential role in protection when OXHOS is compromised, a pathway not well documented in healthy hepatocytes. The robustness of this response varied between FHH donors. Finally, significant association between basal bioenergetic function parameters and susceptibility to rotenone was observed and a mathematical model was constructed, using the measured parameters of bioenergetic function, which was predictive of rotenone IC_{50} (R_2 = 0.782, p = 0.002).
The group's most recent work investigates the influence of mitochondrial genotype on drug toxicity through the creation of transmitochondrial cybrid HepG2 cell lines. These cells are made by incorporating donor mitochondria (platelet-derived), of known haplogroup, in to HepG2 (p0 - devoid of mitochondria) cells, thus creating a cell panel of distinct mtDNA haplogroups with a constant nuclear background. Ten cybrids have been created, and their potential utility as a personalised model of drug-induced mitochondrial dysfunction will be described.

300

Novel targets for the treatment of chronic pelvic pain

Dr Luke Grundy. Department of Clinical Pharmacology, College of Medicine and Public Health, Flinders University, Adelaide, SA, Australia

Introduction. Chronic pelvic pain conditions originating in the bladder affect more than 5% of Australians, causing debilitating illness that severely impacts every aspect of life. Despite this burden, current therapeutics are ineffective, providing very few patients with acceptable relief. There is a clear and urgent need to develop better therapeutics for chronic pelvic pain. However, this is a significant challenge, as we have an insufficient knowledge of the pathological mechanisms that contribute to the development of chronic pain.
Aims. The aim of my research program is to provide vital mechanistic insight into the pathological mechanisms responsible for chronic pelvic pain and identify novel targets for therapeutic interventions.
Methods. Combining electrophysiology, pharmacology, microbiology, and single cell genomics we have begun to systematically unravel the mechanisms that regulate bladder sensation both in health and disease.
Results. We have identified that sensitisation of bladder-innervating sensory neurons is a critical mechanism for the establishment of chronic pelvic pain. We have determined the anatomical location of the sensory nerves innervating the bladder, unravelled the mechanosensory and molecular properties of these bladder-innervating sensory neurons, and identified the innervation patterns of their terminal ends within the spinal cord. We have discovered multiple key ion channels underlying bladder sensation, including voltage gated sodium channels and T-type calcium channels, identified neuro-immune interactions as key regulators of bladder hypersensitivity in chronic pain, and revealed a novel treatment paradigm for chronic pelvic pain that exploits viscero-visceral cross-talk.
Discussion. This research program has generated key insights into the mechanisms underlying the development and maintenance of chronic pelvic pain and identified pathways that can be exploited to develop more specific and effective future therapeutic treatments.

301

Oral Nanotherapeutic Formulations of Insulin

Nicholas J Hunt[1], Glen P Lockwood[1], Zdenka Kuncic[1], Peter McCourt[1], David Le Couteur[1], Victoria Cogger[1]. University of Sydney[1], Sydney NSW, Australia.

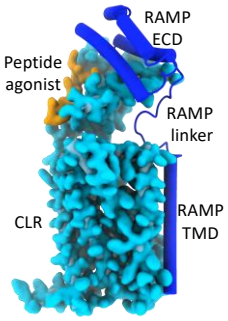
Introduction. Nanotechnology, particularly, quantum dots (QDs), demonstrate rapid intestinal uptake and drug delivery to the liver following oral adminstration[1]. Previously we have shown drastic improvements in drug oral bioavailability following surface attachment to QDs, these changes were facilitated by changes in intestinal and hepatocyte uptake pathways[2].
Aims. To investigate the oral bioavailability of larger peptides such as insulin (that must normally be given via SC injection) and examine if QDs could act as effective oral delivery agents for larger peptides. We also aimed to investigate the use of glycopolymers of chitosan and glucose for functional targeting of hepatocytes as well as pH dependent aggregation techniques and glycosidase dependent enzymatic cleavage to control the release of insulin in vivo.
Methods. Silver sulfide (Ag_2S) QDs were conjugated to insulin and encapsulated within a chitosan/glucose glycopolymer. Bioavailability and biodistribution were investigated in WT C57BL/6J using ^{14}C radiolabelled insulin, with pharmacodynamic data obtained using oral glucose tolerance testing. Finally, we investigated insulin tolerance testing in animal models of type 1 diabetes.
Results. QD-insulin demonstrates a 4% systemic bioavailability compared to SC-insulin. However, biodistribution within the liver was 3-fold higher than blood. Both SC-insulin (2 IU/kg) and QD-insulin (20 IU/kg) produced similar reductions in oGTT AUC in WT C57BL/6J mice. In diabetic NOD mice and STZ treated rats both SC-insulin and QD-insulin demonstrated similar reductions and time frame of action in ITT AUC compared to WT mice.
Discussion. This work demonstrates the effectiveness of QDs in facilitating the delivery of the usually non-orally bioavailable peptide, insulin. Further we have shown that oral QD-insulin is able to reduce blood glucose levels in both diabetic and non-diabetic animals.

[1]Hunt N (2020) ACS Nano 14 (2), 1492-1507
[2]Hunt N (2021) ACS Nano 5 (3), 4710-4727

302

RAMPing up dynamics at G protein coupled receptors

Tracy M Josephs[1,2], Matthew J Belousoff[1,2], Yi-Lynn Liang[1], Sarah J Piper[1,2], Jianjun Cao[1,2], Daniel J Garama[3,4], Grace V Mennen[1], Peishen Zhao[1,2], Radostin Danev[5], Denise Wootten[1,2], Patrick M Sexton[1,2]. Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences[1] & ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences[2], Monash University, Parkville, VIC 3052, Australia; Hudson Institute of Medical Research, Monash University[3] & Department of Molecular and Translational Science, Monash University[4], Clayton 3168, VIC, Australia; Graduate School of Medicine, University of Tokyo[5], Tokyo, Japan.



Introduction. Receptor activity-modifying proteins (RAMPs) are accessory proteins that interact with G protein-coupled receptors (GPCRs) to alter their ligand selectivity and function. Adrenomedullin (AM_1R) and calcitonin gene-related peptide (CGRPR) receptors are two of the best-known examples of RAMP-GPCR modulation. CGRPR and AM_1R are heterodimers of the calcitonin-like receptor (CLR) and RAMP1 or RAMP2, respectively. Cryo-EM structures show limited interaction of the CGRP and AM peptide agonists with RAMPs suggesting that RAMPs mediate their effects through allosteric receptor interactions. However, the mechanistic basis for RAMP modulation is unknown.
Aims. To understand the allosteric contribution of RAMPs to CLR ligand selectivity.
Methods. Our work combines analytical pharmacology with cryo-EM and hydrogen-deuterium exchange (HDX) of an essentially unmodified receptor to probe RAMP modulation and allostery at the CLR.
Results. Analysis of the dynamic data obtained using HDX revealed increased dynamics of the RAMP1 linker region connecting the transmembrane domain (TMD) and the extracellular domain (ECD) upon ligand binding compared to RAMP2. Accordingly, chimeric exchange of the less dynamic linker region of RAMP2 with RAMP1 resulted in a loss in transducer coupling potency in response to CGRP but had little to no effect on AM.
Discussion. CGRPR activation requires the more dynamic RAMP1 linker for high affinity CGRP binding and transducer coupling, whereas AM is tolerant to both the flexible or less dynamic linker region of RAMP2. The differential dynamics of the RAMPs support a role for allosteric modulation of CLR *via* the RAMP linker region in controlling receptor ligand selectivity and function.

303

Therapeutic Drug Monitoring for Cancer Care

Dr Madele van Dyk, Flinders University

Abstract not yet provided

304

GPCR-CoINPocket identifies peptide GPCR ligands with novel off-target activity on the β_2 adrenoceptor.

Sean S So[1], Tony Ngo[2], Brendan P Wilkins[1], Angela M Finch[1], Irina Kufareva[2], Nicola J Smith[1]. Dept of Pharmacol, UNSW Sydney[1], Kensington, NSW; Skaggs School of Pharmacy and Pharmaceutical Sciences, UCSD[2], La Jolla, CA, USA.

Introduction. To assist in the discovery of chemical probes for orphan GPCRs, we developed GPCR Contact-Informed Neighbouring Pocket (GPCR-CP): a structure- and ligand-independent metric of pharmacological similarity between Family A GPCRs. Unexpectedly, the human β_2 adrenoceptor (hβ_2-AR) was predicted to share ligands with peptide GPCRs.
Aims. Here, we aim to experimentally verify the GPCR-CP-predicted pharmacological similarity between hβ_2-AR and various peptide GPCRs including the somatostatin 2 and 4, oxytocin, gonadotropin-releasing hormone, melanin-concentrating hormone 1, orexin 2, bombesin 3, and neuropeptide S receptors.
Methods. Using 3 in-house ligands that natively bind to the bombesin 3, orexin 2, and neuropeptide S receptors respectively, CRE-luciferase assays were performed in HEK293 cells transiently transfected with hβ_2-AR. Based on the results, the ligand search set was expanded: GPCR-CP-predicted hβ_2-AR neighbours from non-aminergic and non-muscarinic families were ranked by their GPCR-CP scores, and known or expected neighbours like adenosine receptors were iteratively removed based on literature searches. A database of relevant purchasable small molecules of the top 8 remaining neighbours (plus bombesin 3 and orexin 2 based on CRE-luciferase results) was obtained from Tocris Biosciences and hierarchically clustered by Tanimoto distance using Molsoft ICM v3.8 and above. Chemical centres were cherry-picked to maximise chemical and receptor diversity, totalling 11 compounds (including our in-house set) across 8 peptide GPCRs. Specific activity against hβ_2-AR was determined by competitive radioligand binding assays using [^{3}H]-dihydroalprenolol and crude membranes of COS-1 cells transiently transfected with hβ_2-AR.
Results. In CRE-luciferase assays, tested peptide receptor modulators appeared to be low-potency ligands of varying activities at the hβ_2-AR. Competitive radioligand binding assays confirmed the specificity of the identified off-target interactions, and further demonstrated that oxytocin, somatostatin 2, and melanin-concentrating hormone receptor modulators are moderate affinity ligands for the hβ_2-AR. This totals 4 out of 11 predicted ligands for a 36% hit rate.
Discussion. GPCR-CP can correctly predict novel pharmacological relationships in a manner applicable to orphan GPCR drug discovery. Future work involves computational docking to guide future site-directed mutagenesis studies to identify ligand-binding residues involved in the off-target binding of these peptide receptor modulators to the hβ_2-AR.

305

Formyl peptide receptor agonists as novel vasodilators and anti-inflammatories for pulmonary hypertension

William R. Studley[1], Katherine Martin[1], Rebecca H. Ritchie[2], Cheng Xue Helena Qin[2], Jane E. Bourke[1.] Department of Pharmacology, Biomedical Discovery Institute, Monash University[1], Melbourne, VIC, Australia; Monash Institute of Pharmaceutical Sciences, Monash University[2], Melbourne, VIC, Australia.

Introduction. Treatment of pulmonary hypertension (PH) with vasodilators, including sildenafil and riociguat, reduces pulmonary arterial pressure but not inflammation or vascular remodelling. In preclinical mouse models of cardiovascular disease, the small-molecule formyl peptide receptor (FPR) agonist, Cmpd17b, dilated blood vessels and attenuated cardiac fibrosis. Here, the precision-cut lung slice (PCLS) technique was used to investigate both the vasodilator and anti-inflammatory potential of Cmpd17b in the distal pulmonary vasculature relevant to PH.
Aims. 1) Compare vasodilator responses of FPR agonists and current treatments and 2) investigate mechanisms of FPR-mediated vasodilation in naïve mouse PCLS. 3) Assess Cmpd17b in a simple model of pulmonary inflammation by measuring dilator responses and its effects on secretion of PH-associated cytokines in TNFα-treated PCLS.
Methods. In PCLS prepared from 8-week-old male C57BL/6J mice, intrapulmonary arteries (<150µm diameter) were pre-contracted with 5HT (3µM), before concentration-response curves to FPR agonists Cmpd17b and Cmpd43 were compared to sildenafil and riociguat. Cmpd17b-mediated relaxation was also assessed in the presence of FPR antagonists, signalling inhibitors, and after overnight TNFα treatment. ELISA for inflammatory cytokines was performed on conditioned media from PCLS treated with TNFα in the absence or presence of Cmpd17b.
Results. Cmpd17b (n=9) elicited complete vasodilation with the same potency as riociguat (n=5) (both pEC_{50} 5.3±0.2) and 5-fold greater potency than Cmpd43 (n=6) or sildenafil (n=6) ($P<0.05$ *cf* Cmpd17b). Efficacy and potency of Cmpd17b were maintained in TNFα-treated PCLS. Relaxation was inhibited by antagonism of FPR1 but not FPR2, and maintained alongside treatment with inhibitors L-NAME (eNOS), ODQ (sGC) and indomethacin (COX). TNFα-induced secretion of the pro-fibrotic and pro-inflammatory cytokines IL-6, KC and MCP-1 was attenuated by Cmpd17b (n=4-6, $p<0.05$).
Discussion. Vasodilation of pulmonary arteries by Cmpd17b is largely mediated by FPR1, independent of endothelial-derived relaxing factors and sGC, and maintained under inflammatory conditions. Further research is required to define the mechanisms underlying the combined dilator and anti-inflammatory actions of Cmpd17b, and to confirm these in disease context, to support clinical translation of Cmpd17b as a novel dual-action therapy for PH.

306

Opioid Deprescribing Guidelines: Moving from Evidence to Practice

Aili V Langford[1], Danijela Gnjidic[1], Chung-Wei Christine Lin[2], Carl R Schneider[1]. School of Pharmacy, USYD[1], Sydney, NSW, Australia; Institute for Musculoskeletal Health, USYD[2], Sydney, NSW, Australia.

Introduction. Overprescribing of prescription opioids is a major international public health problem. The development and implementation of evidence-based opioid deprescribing guidelines is one strategy to improve judicious use, however, translation of guideline recommendations into clinical practice can be challenging.
Aims. To describe the contextualisation of opioid deprescribing guideline recommendations to enable implementation in clinical practice.
Methods. Opioid deprescribing guidelines were developed in accordance with validated methodologies, outlined by the National Health and Medical Research Council. Qualitative interviews were conducted with healthcare professionals and opioid consumers to inform guideline scope, content and context. Evidence synthesis and appraisal was undertaken, with the certainty of evidence determined using GRADE. Evidence-to-decision frameworks were developed by a multidisciplinary and consumer guideline group to systematically consider the acceptability, equity, feasibility and resource requirements of opioid deprescribing.
Results. Recommendations were formulated in response to three key questions; i) Does deprescribing of opioids result in benefits or harms? ii) What is the evidence of how to deprescribe opioids? and iii) Which interventions are effective in deprescribing opioids? A disconnect was revealed between the most effective strategies for opioid deprescribing and those which were acceptable to key stakeholders and feasible to implement in clinical practice. Multidisciplinary pain clinics showed the greatest evidence for opioid reduction, yet have poor implementability due to substantial accessibility, cost, equity and resource-related implications.
Discussion. Evidence alone is insufficient to facilitate opioid deprescribing in clinical practice. Contextualisation of evidence-based recommendations is necessary to ensure relevance and impact. Tailored implementation aids such as consumer resources and conversation guides may support translation of evidence into practice, however system-level changes are required to allow end-users to implement best-practice recommendations. Guidelines themselves need to be acceptable and feasible in professional communities. Therefore, in addition to the standard practice of public consultation, guideline usability testing should be conducted to ensure outputs are acceptable for end-users.

307

Pharmacological inhibition or genetic deficiency of insulin regulated aminopeptidase (IRAP) protects against UUO-induced renal injury in mice.

S Chelvaretnam[1], M Shen[1], SJ Mountford[3], PE Thompson[3], SY Chai[2], CS Samuel[1], RE Widdop[1], TA Gaspari[1], Biomedicine Discovery Institute and Dept of Pharmacol[1], Dept of Physiol[2]; Monash Institute of Pharmaceutical Sciences[3], Monash University, VIC.

Introduction. We have identified the enzyme insulin regulated aminopeptidase (IRAP) as a promising new anti-fibrotic target, with pharmacological inhibition of IRAP reversing age-induced cardiac fibrosis. However, little is known about its role in the setting of renal injury and disease.
Aims. This study aimed to (1) compare anti-fibrotic efficacy of the IRAP inhibitor, HFI-419 (HFI) and the IRAP knockout (KO) mouse strain in a murine model of unilateral ureteral obstructive (UUO)-induced renal fibrosis; and (2) investigate the role of IRAP substrates in mediating the protective effects observed with IRAP inhibition.
Methods. WT and IRAP KO male mice (8 weeks old, n=8-10/group) underwent either sham or UUO-surgery, with UUO-injured WT mice randomised to receive either vehicle or HFI-419 (0.72mg/kg/d). A separate group of IRAP KO mice were also treated with HFI to test inhibitor specificity. IRAP substrate involvement was evaluated in mice co-treated with HFI and one of the specific receptor blockers for oxytocin receptor (OTR), Angiotensin Type 2 Receptor (AT2R), Vasopressin Type 1a (V1R) or Type 2 Receptor (V2R). All treatments administered by osmotic mini-pump (s.c.)
Results. UUO-induced increases in interstitial fibrosis (UUO+Veh=6.9±0.3% vs Sham=1.0±0.3%, $p<0.05$), tubular damage and inflammation were all prevented to the same extent in HFI-treated and IRAP KO mice (Interstitial fibrosis: HFI=4.5±0.3%, IRAP KO=5.2±0.3%, $p<0.05$ vs. UUO+Veh), with no further reduction in fibrosis evident in IRAP KO mice treated with HFI (IRAP KO+HFI=4.6±0.3%).The anti-fibrotic effect mediated by IRAP inhibition was blunted in the presence of AT2R, OTR and vasopressin receptor blockers (AT2R=6.3±0.3%, OTR=6.0±0.3%, V1R=5.4±0.3%, V2R=5.7±0.3%, all $p<0.05$ vs HFI), with varying effects of the substrate inhibitors on markers of inflammation.
Discussion. These findings provide proof-of-concept that IRAP inhibition mediates anti-fibrotic and anti-inflammatory effects in a robust model of renal fibrosis. Furthermore, these protective effects were inhibited to varying degrees in the presence of IRAP substrate antagonists, highlighting a multi-modal mechanism of action of IRAP inhibition that involves AT2R, OTR, V1a and V2 receptor activation.

308

Small molecule Drp1 inhibitors for cardioprotection

Ayeshah A. Rosdah[1,2,3], William Smiles[1], Jarmon Lees[1], Jonathan Oakhill[1], Christopher Langendorf[1], Lea Delbridge[3], Jessica Holien[4], Shiang Y. Lim[1,3]. St Vincent's Institute of Medical Research[1], Melbourne, VIC, Australia; Universitas Sriwijaya[2], Palembang, Indonesia; University of Melbourne[3], VIC, Australia; RMIT University[4], VIC, Australia.

Introduction. Mitochondria are dynamic organelles constantly fusing and dividing in a balanced manner to maintain cellular health. In myocardial infarction, mitochondria undergo excessive fission, generating fragmented and dysfunctional mitochondria, leading to cardiomyocyte death and cardiac dysfunction. Inhibiting Drp1-mediated mitochondrial fission has been shown to improve cell survival and Mdivi-1 is the only known small molecule reported to inhibit Drp1. However, poor water solubility and lack of specificity towards human Drp1 protein render Mdivi-1 unsuitable for clinical applications.
Aim. To develop small molecule inhibitors that selectively target human Drp1 for cardioprotection.
Methods. Virtual screening was performed to identify small molecules that could bind to the active site of human Drp1. Direct binding to and enzymatic inhibition of Drp1 were assessed using surface plasmon resonance and GTPase activity assay, respectively. The effect of hit compounds on mitochondrial morphology was evaluated in Drp1 wild-type and knockout mouse embryonic fibroblasts. Finally, hit compounds were evaluated in *in vitro* injury models (HL-1 cell lines and human cardiomyocytes derived from induced pluripotent stem cells (CM-iPSCs)) and an *in vivo* murine model of acute myocardial ischaemia-reperfusion injury.
Results. Three hit compounds, namely DRP1i1, DRP1i2 and DRP1i3, displayed direct binding to human Drp1, inhibited GTPase activity of human Drp1 and suppressed Drp1-dependent mitochondrial fission in mouse fibroblasts. DRP1i1 exhibited cytoprotective effect in HL-1 cells and CM-iPSCs subjected to H_2O_2-induced oxidative stress and simulated ischaemia-reperfusion injury. Significant reduction in myocardial infarct size was also observed in mice treated with a single bolus dose of DRP1i1 (1 mg/kg) given at the onset of reperfusion.
Discussion. Collectively, we have shown that our novel Drp1 inhibitors are promising tool compounds to study Drp1-mediated mitochondrial fission and exhibit therapeutic potential for cardioprotection.

Rosdah AA et al (2016) Pharmacol Res Perspect 4(3): p. e00235

309

Insulin regulated aminopeptidase (IRAP) inhibition attenuates diabetes-induced cardio-renal pathology and vascular dysfunction in mice.

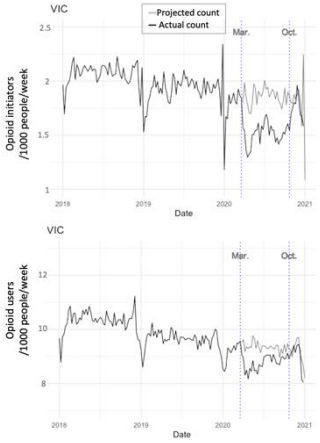
Cameron Hollands[1], Robert E Widdop[1], Dorota Ferens[1], Ekaterina Salimova[3], Iresha Spizzo[1], Siew Y Chai[2], Tracey A Gaspari[1], Dept of Pharmacol[1], Physiol[2], MBI[3], Biomedicine Discovery Institute, Monash University, Clayton, VIC.

Introduction. Diabetes is characterised by cardio-renal dysfunction and remodelling. Insulin regulated aminopeptidase (IRAP) inhibition is cardio- and vaso-protective in a number of CVD models, however the effect of IRAP inhibition in a diabetic model of cardiovascular disease has not yet been explored.
Aims. To determine whether IRAP inhibition improves cardio-renal pathology in a model of type 1 diabetes (T1D).
Methods. Male WT (C57Bl/6J; n=30; 8 weeks old) and $IRAP^{-/-}$ (n=14; 10-14 weeks old) mice received 5 x daily injections of Streptozotocin (STZ) (55mg/kg ip.) or citrate vehicle. Blood glucose and BP were measured fortnightly. After 8 weeks of diabetes, WT mice were administered vehicle or the IRAP inhibitor HFI-419 (HFI, 0.72mg/kg/day sc. via mini-pump, implanted under isoflurane inhalation (5%) anaesthetic) for a further 8 weeks. Diastolic and kidney function were assessed before and at the end of treatment, following which mice were euthanised and tissues collected.
Results. IRAP inhibition or genetic deletion did not alter BP or glucose handling. Cardiac function was not significantly altered in diabetes, however STZ significantly increased cardiac fibrosis (collagen % area 5.4±0.6, n=10) compared to citrate controls (2.9±0.5%, n=8; P<0.05), with HFI treatment attenuating the STZ-induced increase in fibrosis (3.9±0.5%, n=10). HFI reversed diabetes-induced increases in cardiac myofibroblast and superoxide expression, as well as reducing expression of the inflammatory markers phospho-IκBα, MCP1 and F480. In agreement, $IRAP^{-/-}$ mice administered with STZ were protected from developing these pathologies. Similar protective effects were observed in the kidney and vasculature. IRAP inhibition and genetic deletion preserved kidney function (albumin-creatinine ratio ug/mg: Cit Veh: 0.7±0.1, n=7; STZ: 9.9±3.8, n=9 P<0.05 Vs Cit Veh; HFI: 5.4±1.4, n=10; $IRAP^{-/-}$ Veh: 1.3±0.4 n=7; $IRAP^{-/-}$+STZ: 5.2±2.6, n=7) and significantly reversed diabetes-induced vascular dysfunction (ACh % R_{max}: Cit Veh: 68.6±3.9, n=6; STZ: 55.6±5.6, n=9; HFI: 74.1±4.0, n=6 P<0.05 Vs STZ; $IRAP^{-/-}$ Veh: 85.5±2.8, n=7; $IRAP^{-/-}$+STZ: 80.9±3.9, n=7).
Discussion. IRAP deficiency/inhibition exhibited cardio- and vaso-protective properties, as well as preserving kidney function in a mouse model of T1D. This study suggests that targeting IRAP may provide an effective therapy against diabetes-induced cardiovascular end-organ pathologies.

310

COVID-19 restrictions and the incidence and prevalence of prescription opioids in Australia

Monica Jung[1,2], Dickson Lukose[3], Suzanne Nielsen[1], J Simon Bell[1,2], Geoffrey I Webb[3], Jenni Ilomäki[2]. Monash Addiction Research Centre, Monash Univ[1], Melbourne, VIC, Australia; Centre for Medicine Use and Safety, Monash Univ[2], Melbourne, VIC, Australia; Monash Data Futures Institute, Monash Univ[3], Melbourne, VIC, Australia.



Introduction. The COVID-19 pandemic has disrupted seeking and delivery of healthcare, and COVID-19 restrictions may impact pain management.
Aims. To investigate the incidence and prevalence of opioid dispensing during COVID-19 restrictions in Victoria (VIC), New South Wales (NSW) and other Australian states.
Methods. We conducted time series analysis of people dispensed opioid analgesics for non-cancer pain between January 2018 and December 2020 using Australian Pharmaceutical Benefits Scheme data. Interrupted time series analyses were performed using Auto-Regressive Integrated Moving Average approach to examine changes in the trends for VIC, NSW and other Australian states at introduction of nationwide COVID-19 restrictions in March 2020, and at the end of lockdown in Victoria, the last state to ease restrictions, in October 2020.
Results. The sample comprised 626,163 people (54.0% female; mean [SD] age 51 [20] years) who were dispensed an opioid during the study period. Following COVID-19 restrictions, the incidence of prescription opioid use dropped by 0.38 (-0.49, -0.28), 0.33 (-0.46, -0.20) and 0.22 (-0.37, -0.07) /1000 people /week in VIC, NSW and other states, respectively. Incidence increased by 0.39 (0.20, 0.57) /1000 people /week in VIC post lockdown; no changes were observed in NSW and other states. A reduction in the prevalence was observed following COVID-19 restrictions by 0.54 (-1.03, -0.05), 0.62 (-0.97, -0.27) and 0.58 (-0.97, -0.18) /1000 people /week in VIC, NSW and other states, respectively, but no changes were observed in any states at the end of VIC lockdown.
Discussion. COVID-19 restrictions corresponded with a reduction in prescription opioid initiation and use. Further research may determine if lower opioid supply could be due to lower rates of help seeking, fewer injuries and/or reduced elective surgeries and other procedures during lockdown.

311

Development of a tool to evaluate medication management guidance provided to carers of people living with dementia at hospital discharge

Mouna J Sawan[1], Melissa Gench[1], Christine Bond[2], Yun-Hee Jeon[3], Sarah N Hilmer[4], Timothy F Chen[1], Danijela Gnjidic[1]. Sydney Pharmacy School, Faculty of Medicine and Health, Univ. of Sydney[1], Camperdown, NSW, Australia; Institute of Applied Health Sciences, Univ. of Aberdeen[2], Aberdeen, UK Charles; Sydney Nursing School, Faculty of Medicine and Health, The Univ. of Sydney, NSW, Australia[3]; Clinical Pharmacology and Aged care, Kolling Institute of Medical Research, Royal North Shore Hospital[3], St Leonards, NSW, Australia

Introduction. Medication management guidance for carers of people living with dementia at hospital discharge is important to prevent medication-related harm during transitions of care. Currently, there are no published validated tools that describe or quantify all aspects of medication management provided to carers of people living with dementia at discharge.
Aims. This study aimed to develop a tool to evaluate medication management guidance provided to carers of people living with dementia at hospital discharge.
Methods. The tool was developed using a multi-method two staged approach. Stage one involved item generation and content validation. Items were based on a previous qualitative study and systematic review. Content validation involved experts and consumers, with knowledge or experience of medication management guidance in the acute care setting, rating each item on importance and relevance. Stage two involved the conduct of cognitive interviews with carers of people living with dementia to pretest the tool.
Results. The final tool contained 30 items capturing information across five domains: 1) provision of medication management information at hospital discharge; 2) carer engagement in discussing the safe use of medications at discharge; 3) carer understanding of medication management guidance provided at discharge; 4) carer preparedness to conduct medication management activities after discharge; and 5) co-ordination of medication management after discharge.
Discussion. A tool to assess medication management guidance provided for carers of people living with dementia at hospital discharge has been developed. The next step is to explore the construct validity and reliability of the tool. The tool has the potential to fill an important gap in optimising care for people living with dementia.

312

Adverse drug reaction-related hospitalisations among people with dementia

Anum S Zaidi[1], Gregory M Peterson[1], Luke RE Bereznicki[1], Colin M Curtain[1], Mohammed S Salahudeen[1]. School of Pharmacy and Pharmacology, University of Tasmania[1], Hobart, TAS, Australia.

Introduction. Adverse drug reactions (ADRs) entail a substantial burden not only on patients but also on the healthcare system. Trends in the incidence of ADR-related hospitalisations have been studied in the general population, but not specifically in people with dementia.

Aims. This study investigated trends in the incidence of ADR-related hospitalisations among people with dementia, and identified the most frequently implicated drugs and diagnoses in these admissions.

Methods. Analysis of administrative data including all adults admitted to the four major public hospitals of Tasmania, Australia, with a primary or secondary diagnosis of dementia from July 2010 to December 2019. ADR-related hospitalisations were identified by using diagnosis-based and external cause codes. The Cochran-Armitage test was used to examine trends in the incidence of ADR-related hospitalisations.

Results. Of the 7,552 eligible people admitted to the hospital at least once, within the study period, 1,775 (23.5%) experienced at least one ADR-related hospitalisation. The annual incidence of ADR-related hospitalisation increased 18% (estimated 1,484 to 1,760 per 100,000 population with dementia, p for trend <0.05) from 2010 to 2019. Males accounted for 51% of patients with an ADR-related hospitalisation, but only 45% of those without (p <0.001). For those ADR-related admissions with a drug code recorded, 19.3% were due to antithrombotics and 11.5% to antihypertensives. The most frequent ADR-related admission diagnoses were renal diseases (72.9%). Length of hospital stay (median: 7 vs 5 days (ADR vs non- ADR)) and in-hospital mortality (11% vs 6.7% ((ADR vs non- ADR)) were both significantly greater for ADR-related hospitalisations.

Discussion. The annual incidence of ADR-related hospitalisations in people with dementia increased between 2010 and 2019. Antithrombotics were the most commonly implicated drug class. The ADR-related hospitalisations were associated with increased length of stay and greater mortality. Strategies focusing on identifying the risk of ADR-related hospitalisation and cautious prescribing of implicated medicines could help mitigate the burden of ADR-related hospital admissions.

313

Pilot study assessing gut toxicity in acute self-poisoning using a novel biomarker intestinal fatty acid binding protein

Varan Perananthan[1,2,3], Thilini Wijeyrathna[2], Indika Gawarammana[2], Andrew Dawson[1,2,3], Nicholas A Buckley[1,2,3] . Drug Health Services, Royal Prince Alfred[1], Sydney, NSW, Australia; South Asian Clinical Toxicology Research Collaboration[2], Peradeniya, Sri Lanka; Department of Pharmacology[3], University of Sydney, Sydney, NSW, Australia.

Introduction. Intestinal toxicity is expected following ingestion of various toxins. However, the extent of enterocyte damage has not been previously quantified. Intestinal fatty acid binding protein (I-FABP) is a cystolic protein specific to intestinal epithelial cells that are released into systemic circulation if direct intestinal injury occurs. Using this novel biomarker, this pilot study attempted to quantify the extent of intestinal injury by gloriosa superba, nerium oleander, organophospates (in various solvents), paracetamol, glyphosate, propanil and 2-methyl-4-chlorophenoxyacetic acid (MCPA) . Understanding intestinal toxicity in acute self-poisoning may be clinically useful as direct damage to the single layer of enterocytes may promote bacterial translocation leading to sepsis and worsening morbidity.

Methods. Twenty patients with serial plasma samples were retrospectively tested for I-FABP on healthy controls, gloriosa superba, nerium oleander, organophosphates (in its various solvents), paracetamol, glyphosate, MCPA and propanil. I-FABP was tested using the technique of ELISA with kits from Hycult Biotechnology, Netherlands.

Results. The median IFABP for healthy controls was 270.1pg/mL (IQR 153.5 – 558.0pg/mL) compared to gloriosa superba 1179.0pg/mL (IQR 393.1 – 3342.0pg/mL), nerium oleander 1216.0pg/L (IQR 731.2 – 2157.0pg/mL), organophospates 760.6pg/mL (IQR 437.7 – 1587.0pg/mL), paracetamol 432.5pg/mL (IQR 258.6 – 986.1pg/mL), glyphosate 477.0pg/mL (IQR 225.7 – 1804.0pg/mL), propanil 630.0pg/mL (IQR 23.5 – 1390.0pg/mL) and MCPA 424.5pg/mL (IQR 23.5 – 1136.0pg/mL). Median IFABP was significantly elevated compared to control in gloriosa superba (p<0.001), nerium oleander (p<0.001), organophosphates (p<0.001), paracetamol (p=0.03), glyphosate (p=0.04) but not significant for MCPA (p=0.22) and Propanil (p=0.77).

Conclusion. Gut toxicity following oral ingestion of toxins can be quantified with the novel biomarker I-FABP with significantly elevated levels shown in patients with self-poisoning of Gloriosa, Oleander, Glyphosate, Paracetamol and OP compared to healthy controls. Further research is required to determine the utility of I-FABP as a biomarker and whether the extent of reactive gastropathy can predict complications such as sepsis or worsening clinical outcomes.

314

Changes in tapentadol prescribing and harm relative to oxycodone in a tertiary healthcare setting

Frida Djukiadmodjo[1], Elizabeth Su[2], Christopher McMaster[1], David FL Liew[1]. Dept of Clinical Pharmacology and Therapeutics, Austin Health[1], Heidelberg, VIC; Dept of Pharmacy, Austin Health[2], Heidelberg, VIC.

Introduction. Since the approval of tapentadol in Australia in 2011, its market share of opioid sales has grown steadily. Tapentadol, a centrally-acting multimodal analgesic which acts through μ–opioid receptor (MOR) agonistic activity and inhibition of noradrenaline reuptake, may confer lesser abuse potential due to an 18-fold reduction in MOR affinity compared to morphine. Increased utilisation of tapentadol may have multiple implications, including increased medicine costs, but it is argued that this is justified by reductions in toxicity compared to other opioids. Despite this, relatively few real-world data exist examining the relative impact of tapentadol utilisation on opioid-related harm.
Aims. To evaluate local prescribing patterns of controlled release formulations (CR) of tapentadol from 2015 to 2019, compared to the prescribing patterns of oxycodone CR. Additionally, this study aims to provide an insight into the wider healthcare impacts of tapentadol treatment by assessing rates of hospitalisation relating to tapentadol toxicity.
Methods. This is a retrospective observational cohort study describing the temporal pattern of tapentadol and oxycodone CR prescribing in a tertiary hospital using pharmacy dispensing records, and corresponding harms based on administrative data, including ICD-10 coding. Cox proportional hazards was used to account for relevant confounders.
Results. Tapentadol CR was added to our hospital formulary in 2015 and a steady increase of 20-30% per year in utilisation of tapentadol CR was observed in the following two years. In 2018 there was a significant increase in tapentadol utilisation of 70% and the utilisation level remained steady in 2019. In contrast, the number of oxycodone CR prescriptions dispensed in our hospital has remained at a steady level between 2015 to 2019. Results will be presented from a comparative analysis of toxicity related to tapentadol CR versus oxycodone CR, using administrative ICD-10 coding related to opioid toxicity, and hospital admission data examining recurrent readmission.
Discussion. The utilisation of tapentadol in our institution is consistent with the observed increased trends in the volume of PBS-subsidised prescriptions for tapentadol in Australia, but has not led to a decrease in utilisation of oxycodone CR. Given tapentadol's higher cost, our analysis would need to show appropriately large reductions in tapentadol-related toxicity, relative to that from oxycodone, to support tapentadol's benefit as a non-inferior analgesic with a favourable safety profile and a lower potential for abuse relative to traditional opioids.

315

Efficacy and safety of Vedolizumab in older adults with Inflammatory Bowel Disease: A systematic review and meta-analysis

Clinical Pharmacology, Flinders Medical Centre and Flinders University[1], SA, Australia; Gastroenterology, Flinders Medical Centre[2], SA, Australia

Introduction. Vedolizumab, an anti-integrin monoclonal antibody, has established efficacy and safety in moderate to severe Inflammatory Bowel Disease (IBD). However, these outcomes have not been specifically determined in older adults as the majority of patients with IBD are diagnosed at a relatively young age. A systematic review focusing on older adults is lacking despite a number of recently published real-world studies.
Aims. We conducted a systematic review to assess the effectiveness and safety of Vedolizumab, as induction and maintenance therapy, in achieving clinical remission in older adults with IBD.
Methods. A structured search of MEDLINE, Embase, and CINAHL databases, trial registries and key conference abstracts screened from conception to April 2021, was conducted for studies evaluating Vedolizumab induction and maintenance of remission in IBD. All studies evaluating the adverse events of Vedolizumab in IBD were included. Studies involving older adults were defined as a mean or median >60 years at baseline.
Results. From a total of 160 studies initially identified, seven efficacy studies involved older adults (n=3,519, average age 68.3 years) with three retrospective cohort studies comparing Vedolizumab against another biologic, three retrospective non comparator studies, and one case-control study. 92 studies conducted in younger patient cohorts (n=27,848, average age 40.6 years) are being used as the comparator group. Safety data, in addition to the aforementioned studies, were reported in further 22 studies. Secondary outcomes being analysed involve 7 COVID-related safety issues, 12 studies on efficacy in extra-intestinal manifestations, 1 study in Peri-anal Crohn's, 3 studies in Primary Sclerosing Cholangitis-IBD, and 16 studies examining IBD-related surgical outcomes. Per PROSPERO protocol, separate meta-analyses are being performed according to age above and below 60 years, IBD subtype (Ulcerative colitis vs Crohn's Disease) and comparators. Meta-regression analysis are being used to identify the effects of age, IBD subsets, and concomitant use of immunomodulators on outcomes.
Discussion. Complete results and discussion to be presented at conference.

316

Cigarette smoking is associated with circadian rhythm disruption in mice.

Simone N. De Luca[1], Aleksandar Dobric[1], Stanley Chan[1], Kurt Brassington[1], Huei Jiunn Seow[1], Kevin Mou[1], Ross Vlahos[1]. [1]School of Health and Biomedical Sciences, RMIT University, Melbourne, VIC, Australia.

Introduction. Chronic obstructive pulmonary disease (COPD) is a major, incurable health burden, that is currently the 3rd leading cause of death globally, primarily caused by cigarette smoking. It is believed that the increased inflammation and oxidative stress in the lungs may 'spill over' into the systemic circulation, reaching and damaging other organs such as the brain to promote circadian rhythm disruption, which is a risk factor for neurocognitive impairment.
Aims. To investigate whether chronic cigarette smoke exposure causes disruption to physiological circadian rhythm and related signalling profiles.
Methods. We assessed physiological circadian rhythms by monitoring physical activity per hour, and the expression of circadian rhythm regulating genes by qPCR in male BALB/c mice exposed to cigarette smoke (9 cigarettes/day, 5 days a week) or room air (sham) for 24 weeks.
Results. Cigarette smoke exposure caused significant lung inflammation which consisted predominantly of increased neutrophils and macrophages ($p < 0.0001$; n=12). This lung inflammation was associated with a pronounced disruption in physiological rhythm with a 3-hour temporal forward shift in the total activity ($p = 0.001$; n=6) compared to sham mice. Molecular analysis found that cigarette smoking increased circadian rhythm-regulating genes, *Per1* and *Per2* (*Per1*: $p = 0.0009$; *Per2:* $p < 0.0001$; n=8) in the hypothalamic tissue containing the suprachiasmatic nucleus which is responsible for regulating circadian rhythms.
Discussion. Cigarette smoke exposure for 24 weeks leads to a strong disruption of physiological locomotor rhythm and does so in conjunction with a dysregulation of circadian rhythm genes in the hypothalamus. Future research is currently being undertaken to investigate the neuropathology and whether there are differences in key circadian rhythm proteins in the suprachiasmatic nucleus of the hypothalamus.

317

Effect of the ubiquitin E3 ligase NEDD4-1 on P-gp expression, activity and export of Alzheimer's Aβ peptides

Amanda B Chai[1], Richard Callaghan[2], Ingrid C Gelissen[1]. School of Pharmacy, Faculty of Medicine and Health, University of Sydney[1], Sydney, NSW 2006, Australia; Research School of Biology and Medical School, Australian National University[2], Canberra, ACT 2601, Australia.

Introduction. The ATP-binding cassette transporter protein, P-glycoprotein (P-gp), has been demonstrated to export neurotoxic amyloid-β (Aβ) peptides out of neurons (Chai et al 2021) and out of the brain via the blood brain barrier. However, its expression and activity are diminished with age and in Alzheimer's disease. P-gp is post-translationally regulated by the ubiquitin-proteasome system (UPS), with the specificity for targeting proteins for degradation determined by ubiquitin E3 ligases. NEDD4-1 is an E3 ligase that has previously been shown to ubiquinate P-gp, however whether this effect translates into altered P-gp functionality remains to be determined. Unravelling the mechanisms by which P-gp is regulated in the brain will improve our understanding of Alzheimer's pathophysiology and may offer novel therapeutic targets that ultimately facilitate removal of Aβ from the brain.
Aims. To establish the role of NEDD4-1 on P-gp protein expression, function, and P-gp-mediated export of endogenously expressed Aβ peptides from CHO-APP cells.
Methods. siRNA was used to knockdown NEDD4-1 expression in CHO-APP cells. Subsequent effects on P-gp were assessed using Western blot for protein expression, calcein-AM fluorescence assay for transport activity, and ELISA for cellular $A\beta_{40}$ secretion.
Results. Knockdown of NEDD4-1 expression in CHO-APP cells was associated with increased P-gp protein expression, in conjunction with increased function as determined by export of the P-gp substrate calcein-AM. Chemical inhibition of P-gp using verapamil and nicardipine reduced $A\beta_{40}$ secretion from CHO-APP cells in a concentration-dependent manner. Further analyses are underway to determine whether the effect of NEDD4-1 on P-gp expression confers altered Aβ export from CHO-APP cells.
Discussion. The ubiquitin E3 ligase, NEDD4-1, appears to be involved in the regulation of P-gp expression and activity. Understanding the NEDD4-1 regulation of P-gp-mediated clearance of neurotoxic Aβ peptides from the Alzheimer's brain may help to exploit such pathways for therapeutic targeting in future.

318

Novel antibodies targeting dopamine D_2 receptor phospho-sites allow evaluation of reported biased agonists.

Alastair C. Keen[1,2,3], Anika Mann[4], Stefan Schulz[4], J. Robert Lane[2,3. 1]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash Univ, Parkville, VIC, Australia, [2]School of Life Sciences, Queen's Medical Centre, Univ of Nottingham, Nottingham, United Kingdom, [3]Centre of Membrane Proteins and Receptors, Univ of Birmingham & Univ of Nottingham, Midlands, United Kingdom, [4]Institute of Pharmacology and Toxicology, Jena University Hospital, Friedrich Schiller Univ Jena, Jena, Germany.

Introduction. The dopamine D_2 receptor (D_2R) is a G protein-coupled receptor (GPCR) that is a drug target for several neuropsychiatric disorders. D_2R-G protein signalling is regulated by G protein-coupled receptor kinases (GRKs) that phosphorylate the intracellular loops of the D_2R, promoting interaction with arrestins. Recruitment of arrestins to the D_2R can lead to receptor endocytosis. Arrestins can additionally act as signalling scaffolds to elicit distinct physiological functions from those of G proteins. Recent studies have explored the action of signalling pathway-biased agonists as an avenue for the development of improved treatments. Despite this attractiveness, little is known about the patterns of D_2R phosphorylation that might control these actions. Antibodies that selectively bind intracellular phosphorylation sites have proved useful tools to investigate such mechanisms at other GPCRs.
Aims. We set out to understand how the phosphorylation induced by different D_2R agonists, including reported biased agonists, relates to their activation of other signalling pathways.
Methods. Specific phospho-peptides corresponding to regions within intracellular loops of the D_2R were used to raise rabbit antibodies. D_2R phosphorylation was determined via western blot. G protein activation, GRK2 recruitment and arrestin recruitment were carried out using bioluminescence resonance energy transfer.
Results. We identified a site within intracellular loop 3 of the D_2R that is phosphorylated by GRK2/3 upon agonist activation of the D_2R. Phosphorylation of this site predicted arrestin recruitment to the D_2R. Furthermore, canonical G protein mediated signalling measurements also appeared to correlate with phosphorylation within this site.
Discussion. This research highlights the utility of these phospho-site antibodies to characterise novel putative biased agonists in the future and hence better understand D_2R regulation.

Mann, A., Keen, A.C. *et al.* (2021). *Sci Rep* 11, 8288

319

Correlation between $GABA_A$ subunit variants and clinical phenotypes

Tsz Yuen Jonas Lok[1], Mary Chebib[1,2], Nathan Absalom[1,2], Katrine Johannesen[3], Rikke Moller[3]. [1]Sydney School of Pharmacy, Faculty of Medicine and Health, The University of Sydney, NSW, Australia; [2]Brain and Mind Centre, The University of Sydney, Sydney, NSW, Australia; [3]Filadelfia Epilepsy Hospital, Dianalund, Denmark.

Introduction. Genetic epilepsies have remained a challenge for the past decade due to its refractory nature. A range of clinical presentations with high individual variability were observed in patients. These include different seizure types, intellectual disabilities and psychiatric disorders. This study aims to delineate the correlation between genotype and clinical phenotypes of different $GABA_A$ receptor variants.
Aims. To investigate the correlation between functional types of GABRG2 and GABRB3 variants and their clinical features.
Methods. Patient data are collected through PubMed search with regards to GABRG2 variants. Unpublished data were acquired through GeneMatcher, genetic epilepsy centres in Australia, Europe and Canada. Functional data was performed by two-electrode voltage clamp electrophysiology. Graphpad prism and SPSS were utilized for analysis. The age of seizure onset, seizure phenotypes, inheritance pattern, cognitive function and patient response to first antiepileptic drug (seizure freedom) were compared in different variants. Mann-Whitney U test was used to investigate the correlation of age of seizure onset in different variants. Odds ratio and Fischer's exact test was calculated to delineate the complex relationship between genotype and phenotype in other parameters.
Results. The median age of seizure onset in patients with gain-of-function (GOF) variants was 1.5 months compared to 12 months for patients in the loss-of-function (LOF) variant group. The data suggest that patients with GOF variants have a much earlier seizure onset compared to LOF group (Mann-Whitney U = 27, p-value = 0.03). Patients with GOF variants were less likely to achieve seizure freedom compared to LOF variants (OR = 0.044, 95% CI = 0.0035 – 0, p-value = 0.035).
Discussion. Both GOF and LOF variants contribute to the formation of refractory seizures. However, GOF variants have a much diverse seizure phenotype and have an earlier seizure onset compared to LOF variants. These patients are less likely to achieve seizure free periods. Drug discovery programs targeting a reduction in GABAergic activity will likely be required to properly treat the GOF patients.

320

Habitual cigarette smoke exposure evokes anxiety-like behaviour in mice which is associated with neuroinflammation and oxidative stress of the amygdala.

Rana Alateeq[1], Simone N. De Luca[1], Aleksandar Dobric[1], Kurt Brassington[1], Alina Akhtar [1], Huei Jiunn Seow[1], Stanley Chan[1], Kevin Mou[1], Ross Vlahos[1]. [1]School of Health and Biomedical Sciences, RMIT University, Melbourne, VIC, Australia.

Introduction: Chronic obstructive pulmonary disease (COPD) is associated with several extra-pulmonary comorbidities. Of these comorbidities, mood disorders such as anxiety have detrimental effects on the overall health and quality of life of COPD patients, however they often remain underdiagnosed and undertreated. There is growing evidence that neuroinflammation may play a crucial role in the pathophysiology of mood disorders. Therefore, we hypothesised that lung inflammation and oxidative stress caused by cigarette smoking (CS) may "spill-over" into the systemic circulation, effecting the brain leading to the manifestation of anxiety disorders.
Aim: To examine whether chronic CS exposure causes anxiety-like behaviour associated with neuroinflammation and oxidative stress in the brain, and if so, determine whether inhibition of oxidative stress attenuates these changes.
Methods: Male BALB/c mice were exposed to CS (9 cigarettes/day, 5 days a week) or room air for 8 weeks in the presence or absence of apocynin (5 mg/kg, in 0.01% DMSO diluted in sterile PBS) administration (i.p.). We assessed anxiety-like behaviours using the elevated plus maze (EPM). qPCR was also conducted to assess neuroinflammation, oxidative stress and the neurotrophic marker brain-derived neurotrophic factor (*Bdnf*) in key anxiogenic brain regions.
Results: CS exposure induced a significant decrease in the percentage of open arm entries (n =10; p=0.02), which was alleviated by apocynin treatment (n =10; p=0.01). CS exposure significantly upregulated the expression of *Il1b* (n =6; p=0.002), *Tnfa* (n =6; p=0.002), *Nfkb* (n =6; p=0.001), *Nox2* (n =6; p=0.0006) and *iNos* (n =6; p=0.03) in the amygdala, while it supressed the expression of *Bdnf* (n =6; p=0.009) in the prefrontal cortex (PFC), however, these were not attenuated by apocynin.
Discussion: Habitual CS exposure evokes anxiogenic behaviours as assessed using the EPM and is associated with neuroinflammation and oxidative stress localised to the amygdala. Apocynin ameliorated CS-induced anxiety-like behaviour, suggesting the involvement of oxidative stress. Future work should focus on understanding how oxidative stress becomes established in the brain.

321

Apocynin treatment does not improve working memory impairments in a mouse model of COPD.

Alina Akhtar[1], Simone N. De Luca[1], Aleksandar Dobric[1], Kurt Brassington[1], Rana Alateeq[1], Huei Jiunn Seow[1], Stanley Chan[1], Kevin Mou[1], Ross Vlahos[1]. [1]School of Health and Biomedical Sciences, RMIT University, Melbourne, VIC, Australia.

Introduction: Chronic obstructive pulmonary disease (COPD) is currently the 3rd leading cause of death globally. Up to 61% of COPD patients suffer from cognitive impairments, reducing quality of life and increasing the risk of death. Despite the significant burden, the mechanisms underlying the molecular pathways remain largely unknown. We hypothesise that cigarette smoke (CS)-induced oxidative stress drives pulmonary inflammation, which may 'spill over' into the systemic circulation, leading to the development of cognitive impairments.
Aim: To examine whether attenuating oxidative stress may reduce CS-induced cognitive impairment.
Methods: Male BALB/c mice were exposed to room air (sham) or CS (9 cigarettes/day, 5 days a week) over 8 weeks with apocynin (5 mg/kg, i.p.) or vehicle (0.01% DMSO/sterile PBS) treatments daily 1h prior to the initial CS exposure. Working memory impairment was assessed using a Novel Object Recognition task, and synaptogenic markers were assessed by qPCR.
Results: CS-exposed mice had increased pro-inflammatory cell recruitment into the bronchoalveolar lavage fluid. Interestingly, neutrophilic infiltration was attenuated following apocynin treatment ($p<0.0001$, n=14). CS-exposed mice displayed significant working memory impairments (p=0.0002, n=12) when compared to sham counterparts but apocynin was unable to prevent this impairment. CS exposure suppressed expression of synaptogenic markers, *Slc17a7* and *Dlg4*, in the prefrontal cortex (PFC) compared to sham-exposed mice (p=0.0348; p=0.0136, n=6 respectively), and apocynin treatment prevented *Slc17a7* suppression.
Discussion: Exposure to CS impaired working memory and caused a downregulation of synaptogenic markers in the PFC, which is involved in cognitive function. Oxidative stress was also observed in the PFC as a result of exposure to CS. Apocynin treatment did not resolve working memory deficits but improved oxidative stress and expression of *Slc17a7* suggesting that memory impairments are independent of oxidative stress and presynaptic signalling.

322

Exploration of time-dependent clearance associated with busulfan treatment in paediatrics

Rachael A Lawson[1], Christine Staatz[2], Christopher J Fraser[1], Stefanie Hennig[3]. Blood and Marrow Transplant Service, Queensland Children's Hospital[1], Brisbane, QLD, Australia; School of Pharmacy, University of Queensland[2], Brisbane, QLD, Australia; Pharmacometrics, Certara Inc.[3] , Princeton, New Jersey, USA.

Introduction: Busulfan is an alkylating agent used within conditioning regimens prior to haematopoietic stem cell transplant. A structured literature review identified reports of a reduction in busulfan clearance (CL) over its four-day treatment course [1]. There is a paucity of non-linear mixed effects (NLME) models characterising the pharmacokinetics (PK) of busulfan with samples following each dose.

Aims:1) Characterise time-dependent CL of busulfan by developing a NLME model 2) Explore biological mechanisms for this observation 3) Observe the impact on busulfan exposure of using varied dose adjustment methods.

Methods: Data characterising the PK of busulfan across the four-day treatment course was collected in paediatric patients receiving busulfan. A NLME model was developed using NONMEM® to estimate typical PK parameter values and quantify how busulfan CL changed over time. Simulations were performed in R using RxODE package.

Results: A two-compartment population PK model was developed from 95 paediatric subjects (2491 concentration-time points). For CL, a continuous time-dependent equation described the data, with CL reduced by 12% from dose 1 to dose 2 and by 19% over the course of treatment in the average patient. Dose adjustment scenarios simulated showed that using non-compartment analysis to estimate exposure resulted in 86.7-100% of patients with exposures above target cumulative exposure. Scenarios that estimates exposures using the model to calculate subsequent doses and performed daily sampling achieved 70-99% of patients within 5% of target cumulative exposure.

Discussion: This unique dataset allowed quantification of the average reduction in CL (19%) over the course of treatment in paediatric subjects. Use of dose adjustment methods that assume CL remains constant over the course of treatment (as per Product Information leaflets) is likely to result in exposures above target, particularly if sampling is not repeated or maximum dose increase cap instituted. Using model based exposure to guide dose adjustments with sampling performed after each dose is recommended. Further investigation around the cause of time-dependant CL of busulfan after once daily dosing is warranted [1-3].

1. Lawson R et.al (2021) Clin Pharmacokinet 60:17-51; 2. El-Serafi I et al (2017) PlosONE 2017; 12:online; 3. Younis IR et al (2008) J Pharmacol Exp Ther 327:770-6.

323

Population pharmacokinetics and exposure-response analyses for allopurinol in patients with gout

Hailemichael Z. Hishe[1], Sophie L. Stocker[2], Lisa K. Stamp[3], Daniel F.B. Wright[1]. School of Pharmacy, Uni of Otago[1], Dunedin; School of Pharmacy, Uni of Sydney[2], Sydney. Department of Medicine, Uni of Otago[3], Christchurch.

Introduction. Allopurinol is commonly used for the management of gout. The relationship between oxypurinol plasma concentrations, the active metabolite of allopurinol, and urate-lowering is not well understood.

Aims. (1) To develop a population pharmacokinetic (PK) model for oxypurinol, and, (2) to determine the factors that predict oxypurinol exposure-response and the attainment of the serum urate concentration (SUA) target of < 0.36 mmol/L.

Methods. A population pharmacokinetic model for oxypurinol was developed with NONMEM (v7.3). The influence of creatinine clearance (CLcr), weight, sex, ethnicity, concomitant drugs, and renal transporter genotype, including ABCG2 (rs2231142), on oxypurinol pharmacokinetics was assessed. The final model was used to determine oxypurinol steady-state area-under the concentration-time curve ($AUC_{0\text{-}24h}$), pre-dose (C_{min}), and maximum concentrations (C_{max}) . These exposure metrics were compared to observed SUA graphically and by logistic regression. The reference oxypurinol exposure range was defined as the 5^{th} and 95^{th} percentiles of the predicted metrics that attained the SUA target.

Results. Data from n=300 gout patients included n=2842 oxypurinol concentrations and n=3209 SUA, of which 1574 were < 0.36mmol/L. Allopurinol doses ranged from 50-900 mg daily. A one compartment PK model with first-order absorption and elimination was the best fit to the oxypurinol data. CLcr, diuretic use, and weight were found to be significant covariates on clearance. Median (range) predicted $AUC_{0\text{-}24h}$, C_{min} and C_{max6h} were 2196 (101-16232) µmol*h/L, C_{min} 85.4 (0.8-725.1.2) µmol/L and 109.4 (4.7-747.8) µmol/L respectively. The median observed SUA was 0.35 (0.17-0.89) mmol/L. The Cmin reference range was 49-299 µmol/L. The odds of achieving the SUA target was increased by higher oxypurinol exposure, e.g. C_{min} (OR 3.7, 95%CI [2.9-4.5)]), ABCG2 GG genotype (OR 2.1, 95%CI [1.4-3.2]). Higher baseline urate (OR 0.007, 95%CI [0.0008-0.07]), reduced CLcr (OR 1.56, 95%CI [1.35-1.79]) and diuretic use (OR 0.5, 95%CI [0.3-0.8] reduced the odds of achieving the SUA target.

Discussion. Achieving SUA target appears to be more likely for patients with higher oxypurinol exposure and ABCG2 GG genotype. The risk of not achieving target was predicted by reduced CLcr, diuretic use, and higher baseline SUA.

324

A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients.

Ranita Kirubakaran[1,2], David W. Uster[3], Stefanie Hennig[4], Jane E. Carland[1,2], Richard O. Day[1,2], Sebastian G. Wicha[3], Sophie L. Stocker[1,2,5]. St. Vincent's Clinical School, The University of NSW[1], Sydney, NSW, Australia; Department of Clinical Pharmacology & Toxicology, St. Vincent's Hospital[2], Sydney, NSW, Australia; Department of Clinical Pharmacy, University of Hamburg[3], Germany; Certara Inc.[4], Princeton, NJ, USA; School of Pharmacy, The University of Sydney[5], NSW, Australia.

Introduction. Existing tacrolimus population pharmacokinetic models are unsuitable for guiding tacrolimus dosing in heart transplant recipients.
Aim. To develop and evaluate a population pharmacokinetic model for tacrolimus in heart transplant recipients, considering the tacrolimus-azole antifungal interaction.
Methods. Data from heart transplant recipients (n=87) administered the oral immediate-release formulation of tacrolimus (Prograf®) were collected. Routine drug monitoring data, principally trough concentrations, were used for model building (n=1100). A published tacrolimus model was used to inform the estimation of absorption rate constant [K_a], apparent central volume of distribution [V_2], apparent intercompartmental clearance [Q], and apparent peripheral volume of distribution [V_3]. Body weight was implemented as a covariate on apparent clearance [CL/F], V_2/F, V_3/F and Q/F on an allometry scale. The effect of concomitant azole antifungal use on tacrolimus CL/F was quantified. Subsequently, stepwise covariate modelling was performed. Significant covariates influencing tacrolimus CL/F were included in the final model. The robustness of the final model was confirmed using a prediction-corrected visual predictive check (pcVPC). The final model was externally evaluated for the prediction of tacrolimus concentrations of the fourth dosing occasion (n=87) from 1−3 prior dosing occasions.
Results. Concomitant azole antifungal therapy reduced tacrolimus CL/F by 80%. Haematocrit (changes in objective function value = -33, $p<0.001$) was included in the final model. The pcVPC of the final model displayed good model adequacy. One recent drug concentration is sufficient for the model to guide tacrolimus dosing.
Discussion. A population pharmacokinetic model that adequately describes tacrolimus pharmacokinetics in heart transplant recipients, considering the tacrolimus-azole antifungal interaction has been developed. Prospective evaluation is required to assess its clinical utility.

325

A Natural Language Processing Machine-Learning Model for the Detection of Adverse Drug Reactions

Julia TH Chan[1,2], Christopher McMaster[1,2] David FL Liew[1,2]. 1. Medicines Optimisation Service, Austin Health, Melbourne, VIC, Australia; 2. University of Melbourne, Melbourne, VIC, Australia.

Introduction. The detection of adverse drug reactions (ADRs) is critical to our understanding of the safety and risk-benefit profile of medications. With an incidence that has not changed over the last 30 years, ADRs are a significant source of patient morbidity, responsible for 5-10% of acute care hospital admissions worldwide. ICD-10 coding could help to automate ADR detection, however this is yet to be validated and is known to capture approximately only half of the adverse drug events identified from medical record reviews. Given that reviewing patient history is the most time-consuming process in maintaining a pharmacovigilance program, automated machine learning models have the potential to be trained to detect ADRs in real-time and flag potential high-risk cases for further review when required.
Aim. This study aimed to create a machine-learning model using natural language processing (NLP) to detect drugs and their associated adverse drug reactions within electronic medical record (EMR) discharge summaries.
Methods. Discharge summaries from the EMR of a 900-bed metropolitan tertiary teaching hospital in Australia were used to train a natural language processing model in Python version 3.9.6 with the Prodigy annotation tool. The model was built on top of the open-source Med7 model, and further trained with 3 NVIDIA 1080Ti graphics processing units. The model was evaluated using precision, recall, accuracy, and F-score metrics, and then compared against another machine-learning model trained to discriminate ADRs using the ICD-10 Y40-Y59.9 coding system.
Results. An initial model trained using 100 annotated discharge summaries achieved an ADR precision of 58.33, recall of 19.44 and F-score of 29.17. At 140 discharge summary annotations, the model showed ongoing improvement in accuracy (23% increase using all the data vs. 75% of the data), suggesting significant improvements can be made with further annotations.
Discussion. The use of NLP parameters for ADR detection bypasses the need for the pre-coding of clinical data for ADR detection; reduces inconsistencies, missed ADRs, and human errors which may arise with the use of the clinical coders and the ICD-10 system. Our study demonstrates that an NLP machine-learning model trained on EMR data for drug and ADR detection has a level of performance which approaches the ICD-10 trained model, with the additional capacity to pick up non-coded adverse events.

326

Psychometric evaluation of the Adherence to Refills and Medications Scale (ARMS) in Australians living with gout

Marcel Schulz[1], Richard O Day[1], Matthew J Coleshill[1], Nancy Briggs[2], Eindra Aung[1]. St. Vincent's Clinical School, UNSW Sydney, Sydney, NSW, Australia[1]; Stats Central, UNSW Sydney, Sydney, NSW, Australia[2].

Introduction. Gout is a debilitating inflammatory arthritis associated with high levels of serum urate (SU). Despite the availability of effective urate-lowering therapies (ULT), poor medication adherence is highly prevalent in gout. Existing questionnaires commonly used to assess medication adherence behaviours in people with gout have limited validity or have not been validated for their use in gout. The Adherence to Refills and Medications Scale (ARMS), designed to assess medication adherence behaviours, was validated in a few chronic diseases, but not in gout.
Aims. To examine, for the first time, the psychometric properties of the ARMS in people with gout.
Methods. We used data from the Gout App Study, a randomised controlled trial, to conduct exploratory factor analysis (EFA) and examine the scale's internal consistency (the Spearman-Brown coefficient) and agreement (the intraclass correlation coefficient) in ARMS scores across three timepoints (baseline, 6 and 12 months). We used the Kruskal-Wallis test and logistic regression to examine criterion-related validity [associations of ARMS score with 'the ULT taking and adherence status' and target SU (<0.36 mmol/L)], and predictors of optimal adherence (ARMS = 12).
Results. The mean age (SD) of 487 participants (in Australia) was 57.5 (12.84), 95.5% of them were male and 62.6% reported currently taking ULT. The median ARMS score (interquartile range) was 15 (13-19) at baseline. EFA suggested a one-factor structure. High internal consistency and moderate agreement in ARMS scores over time were observed. Differences in median ARMS scores of 13, 16 and 17, respectively in three participant groups: 1) those who reported taking ULT and always taking their gout medications, 2) those not taking ULT, and 3) those taking ULT but not always taking their gout medications, were statistically significant ($p<0.001$). Lower ARMS scores (indicating better adherence) predicted achieving target SU (odds ratio (OR) adjusted for age and sex: 0.89 ; 95% CI: 0.83-0.95; $p<0.001$). The odds of having an optimal medication adherence behaviour increased by 91% (OR: 1.91; 95% CI: 1.50-2.43; $p<0.001$) for every 10-year increase in age, the only variable which remained in the multivariable model.
Discussion. This study has confirmed that the ARMS is a reliable and valid measure of medication adherence behaviours in people with gout. Our work justifies use of ARMS in studies of medication adherence in people with gout, and we recommend its use as a single scale in this population.

327

Pharmacogenomic testing: perception of clinical utility, and enablers and barriers to adoption in Australian hospitals.

Angela Pearce[1], Bronwyn N Terrill[1,2], Jan-Willem C Alffenaar[3,4], Asad E Patanwala[3,5], Sarah Kummerfeld[1,2], Richard O Day[2,6], Mary-Anne Young[1,2], Sophie L Stocker[2,3,6]. Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research[1], Darlinghurst, Sydney, NSW, Australia; St Vincent's Clinical School, Faculty of Medicine, The University of New South Wales[2], Sydney, NSW, Australia; Sydney Pharmacy School, Faculty of Medicine & Health, The University of Sydney[3], Sydney, NSW, Australia; Pharmacy Department, Westmead Hospital[4], Sydney, NSW, Australia; Pharmacy Department, Royal Prince Alfred Hospital[5], Sydney, NSW, Australia; Department of Clinical Pharmacology & Toxicology, St Vincent's Hospital[6], Sydney, NSW, Australia.

Introduction. Pharmacogenomic (PGx) tests can help predict a patients' responses to select medications. Despite anticipated benefits to patient care, implementation of PGx testing in Australia is limited.
Aims. Assess healthcare professionals' (HCPs) perceptions of PGx testing and identify barriers to implementation.
Methods. An online survey (10-12 mins duration, 31 multiple choice and short answer questions) was used at 3 NSW hospitals (July 2020 and May 2021) to assess HCPs knowledge, usage, confidence and experience with PGx testing, and their perceptions of clinical utility, risks and barriers to its implementation.
Results. HCPs (n=107) were predominantly medical practitioners (70%) and pharmacists (23%). PGx testing was considered beneficial, particularly to identify risk of drug intolerance and side effects. Despite this, few HCPs reported past (23%) or intended future (26%) use of PGx testing. Few HCPs reported confidence in their ability to identify indications for PGx testing (13%), order tests (18%) and communicate results to patients (15%). Lack of clinical practice guidelines and knowledge were considered barriers to implementation of PGx. Reimbursement for testing, availability of guidelines and electronic clinical decision support, alongside models-of-care involving multidisciplinary teams and local clinical champions were suggested strategies to facilitate implementation of PGx testing into practice.
Discussion. Pharmacogenomic testing whilst important to guide drug selection and dosing decisions is infrequently used. Further education, development of guidelines, and onsite expert advice could help improve the implementation and adoption of pharmacogenomic testing into routine clinical care to inform prescribing decisions.