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

Radwan Ansaar[1], Lu Lu[1], Tran Nguyen[2], Robyn Meech[1]. Discipline of Clinical Pharmacology, College of Medicine & Public Health, Flinders University[1], Adelaide, SA, Australia; The Centre for Cancer Biology, University of South Australia[2], Adelaide, SA, Australia

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

401 Tacrolimus Dosing and Monitoring in Lung Transplant Patients

Yeseung J Kim[1,2], Ranita Kirubakaran[1,2], Sophie L Stocker[1,2], Fay Burrows[3], Lilibeth Carlos[3], Richard O Day[1,2], Jane E Carland[1,2]. Dept of Clin Pharmacol, St Vincent's Hosp[1], Sydney, NSW, Australia. St Vincent's Clinical School, University of New South Wales[2], Sydney, NSW, Australia. Dept of Pharm, St Vincent's Hosp[3], Sydney, NSW, Australia.

Introduction. Tacrolimus (TAC) is the first line immunosuppressant used in lung transplant (LTx). It is an ideal candidate for therapeutic drug monitoring (TDM) due to its narrow therapeutic window and high interpatient variability.
Aims. To assess dosing and monitoring of TAC in LTx patients at St Vincent's Hospital, Sydney (SVH) according to available SVH guidelines.
Methods. A retrospective cohort study (1 Jan to 31 Dec 2017) of TAC therapy in LTx patients was undertaken with a 6 month follow up period from the first TAC dose. In-patient data was collected from electronic medical records and analysed using IBM SPSS®. Trough concentrations were defined as ideal and adequate when collected within 1 and 2 hours prior to an administered dose, respectively. The target trough range was 12-15µg/L.
Results. The transplant cohort (n=30) comprised of 19 females (63.3%) and median age was 55.5 years (20 – 68 years). Indications for transplant included ILD (30.0%), COPD (23.3%), CF (23.3%), and other indications (26.7%). Median length of stay post-transplant was 15.5 days (8 – 68 days). Immunosuppression in all patients was maintained with a combination of TAC, prednisolone and mycophenolate mofetil. 50.0% of patients also received azole therapy. Of the blood samples collected (n=673), 175 (26.0%) were ideal troughs, 61 (9.1%) were adequate troughs and 425 (63.2%) were not troughs. Of the 236 trough samples, 156 (66.1%) were subtherapeutic, 39 (16.5%) were within target range, and 41 (17.4%) were supratherapeutic. Median time to therapeutic target was 8 days (4 – 21 days). The most common complications observed post-transplant were infection (76.7%), pneumothorax (40.0%), acute kidney injury (33.3%), new onset diabetes mellitus (33.3%), and rejection (23.3%).
Discussion. Our findings demonstrate the need to improve dosing and monitoring of TAC at SVH. However, several limitations to the study exist. Anecdotal evidence from the clinic suggests that actual dosing and sampling times may differ from the times recorded in patient charts used in the study. Complications reported include post-surgical effects and adverse effects of other drugs. PK models with Bayesian estimators may help optimise TAC therapy.

402 The effect of splitting a pill on dose accuracy: a systematic review

Kanika Chaudhri[1,2,3], Madeleine Kearney[1], Richard O Day[2,3], Anthony Rodgers[1,2], Emily Atkins[1,2,4]. Cardiovascular Div, The George Institute for Global Health[1], Sydney, NSW; Faculty of Medicine, Univ of NSW[2], Sydney, NSW; Dept of Clin Pharmacol and Toxicol, St Vincent's Hosp[3], Darlinghurst, NSW; Westmead Clin School, Univ of Sydney[4], Sydney, NSW.

Introduction. Physical alteration of original dose form prior to administration is a common practice, with almost a quarter of all drugs having their dose manipulated (Quinzler et al, 2006). Splitting a tablet allows for dose flexibility and facilitates swallowing for paediatric and geriatric patients. However, there are concerns these physical changes can lead to inaccurate dosing.
Aims. To summarise current literature assessing the effects of tablet splitting on dose accuracy of split drug.
Methods. A search of electronic databases for studies investigating effects of pill splitting was conducted. Databases searched included EMBASE (Ovid), MEDLINE (Ovid), CINAHL (EbscoHOST) and the Cochrane Library from the start of each database until August 2018. Data were included from pill splitting studies where the drug was not administered to a patient as these considered the weight or drug content of the split drug. Therefore, studies investigating any drug, where the pill has been split, were potentially eligible. Two reviewers independently assessed studies for inclusion. Data were extracted by one reviewer and checked by another. Registered on PROSPERO 2018 (CRD42018106252).
Results. Twenty-eight studies containing seventy-six different pills were identified. Overall, 14507 pills were split by either hand, knife or commercialized pill cutters. Dose accuracy was measured using weight or drug content. Weight variation between split pill and theoretical weight of half tablets varied from 0.22-36%. For studies which measured drug content, 40% met the required drug content regulations. One study reported on cost savings of split pill which varied from a saving of 19-50% of actual pill cost.
Discussion. Tablet splitting may lead to inaccurate doses. However, not all drugs are suitable for splitting. Careful consideration needs to be placed on formulation of tablets as well as physical characteristics. Accurate tablet splitting has the potential to decrease costs thus removing the economic constraints of marketed formulations that may not meet the needs of all patients.

Quinzler et al (2006) Eur J Clin Pharmacol 62:1065-1073.

403 Does statin use affect cognition in older adults? A pilot N-of-1 deprescribing trial

Alexander J Clough[1,2], Sarah N Hilmer[2,3], Sharon L Naismith[3,4], Danijela Gnjidic[1,2,3] School of Pharmacy, University of Sydney[1], Camperdown, NSW, Australia; Kolling Institute, Royal North Shore Hospital[2], St Leonards, NSW, Australia; Charles Perkins Centre, University of Sydney[2], Camperdown, NSW, Australia; Brain & Mind Centre, University of Sydney[4], Camperdown, NSW, Australia

Introduction. Evidence to support statin use in adults of 80 years of age with no indication of primary cardiovascular prevention is limited.
Aims. Primary: To determine the effect on cognition of discontinuation and rechallenge with statins. Secondary: To determine the effects on quality of life and functional status of discontinuation and rechallenge with statins.
Methods. Adults 80 years of age and above with dementia taking statins for at least 6 months were recruited from a geriatric outpatient clinic at Royal North Shore Hospital, NSW. A pilot N-of-1 study was conducted, with participants randomised to discontinue and restart statins over the course of 4-months. At baseline (0-weeks), recruited participants were randomised to their normal statin or placebo regiment for a period of 5 weeks. Participants were assessed and intervention switched 5, 10, and 15-weeks. Primary outcome was measured using rate of change in Alzheimer's Disease Assessment Score-Cognitive Subscale (ADAS-CoG), and secondary outcomes assessed using partient-relevant, carer-relevant, and physical measures.
Results. Over 6 months, 81 individuals were screened, 14 were deemed eligible, and 4 were recruited. Three potential participants ceased statin before baseline, and 1 participant (female, 88-years) completed all 4 assessments. Cognitive impairment, as measured by ADAS-CoG was minimally increased on placebo (15.5/70) compared to statin (15/70). A number of recruitment barriers were identified, including treating physician being unwilling to deprescribe statins, in-setting time constraints, and participant stress after clinical diagnosis of dementia.
Discussion. Out pilot study suggests there are major recruitment barriers to recruiting patients with dementia into deprescribing trials from outpatient settings. Moreover, no differences were found in cognition, nor in global functioning and functional status with the discontinuation and rechallenging of statins.

404 How are we using immunosuppressant medicines in Australasian Elderly Renal Transplant Recipients?

Amelia Cossart[1], Neil Cottrell[1], Megan Mcstea[2], Nicole Isbel[3], Scott Campbell[3], Christine E Staatz[1]. School of Pharmacy, University of Queensland[1], Brisbane, QLD, Australia; Centre for Health Services Research, University of Queensland[2], Brisbane, QLD, Australia; Department of Nephrology, University of Queensland at the Princess Alexandra Hospital[3], Brisbane, QLD, Australia.

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

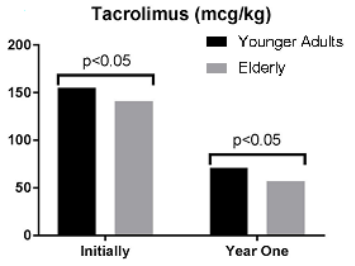


Figure 1: Median tacrolimus doses prescribed initially and at one-year post transplant in elderly and younger adult recipients

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

405 Supporting junior doctor prescribers' learning through self-assessment and feedback: positive impact of a mixed methods study

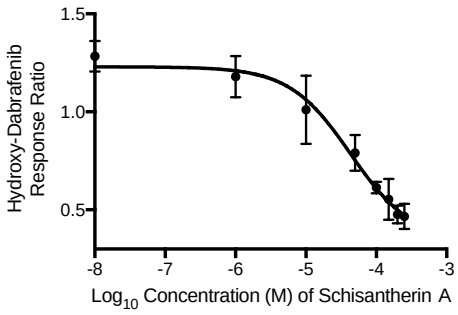
Ian Coombes[1,2], Christy Noble[3,4], Jenna Turkington[1], Brooke Myers[3], Peter Donovan[1,2], Charles Mitchell.[2] Royal Brisbane and Women's Hospital[1], Herston, QLD, Australia; University of Queensland[2], Brisbane, QLD, Australia; Gold Coast University Hospital,[3] Southport, QLD, Australia; Griffith University, Southport, QLD, Australia[4].

Introduction. Junior doctors find prescribing a challenging task. Prescribing errors are common and can contribute to patient harm. Many interventions to improve practice have been designed without considering important and relevant literature, such as learning and feedback.
Aims. To evaluate an innovative methodological approach to evaluating and developing junior doctor prescribing performance by supporting learning through evidence-based feedback processes.
Methods. Using a multifaceted educational approach, involving the principles of learner-centred feedback, junior doctors were invited to self-assess their performance using a competency tool. The self-assessment was triangulated with the ward-based pharmacist competency assessment and modified National Inpatient Medication Chart (NIMC) audit findings, using a minimum of 30 medication orders per prescriber. The findings from this assessment process were analysed and incorporated into a single one-on-one feedback session with either a clinical pharmacologist or pharmacist. Two sites Royal Brisbane and Women's Hospital (RBWH) and Gold Coast University Hospital (GCUH) undertook the study.
Results. A total of 89 interns (51 from RBWH and 38 from GCUH) were recruited during their Internal Medicine Term from March 2016 to March 2017. Eighty-eight had complete data and were included in the analysis. A total of 2750 orders were examined pre-intervention with 1598 individual errors (mean 0.56 ± 0.33 errors per order), with a significant reduction seen post-intervention (2694 orders, 1113 errors, mean 0.36 ± 0.30 errors per order, $p<0.001$). Rates of error reduction were similar if education were provided by either pharmacist or clinical pharmacologist. Improvements were seen in each of the five intern terms evaluated.
Discussion. This novel, systematic, multifaceted, evidence-based educational intervention that included self-assessment and structured feedback resulted in substantial reduction in prescribing errors in medical interns. Further research to examine generalisability and sustainability of this intervention are required.

406 The effect of the Wuzhi tablet on the metabolism of dabrafenib in human liver microsomes: a herb-drug interaction

Alia Fahmy[1], Xiaoman Liu[1], Alan V. Boddy[1,2]. School of Pharmacy, The University of Sydney[1], NSW, Australia; Current address[2]: School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia.

Introduction. Commercial extracts of *Schisandra sphenanthera* (referred to as Wuzhi tablets or capsules) are used in Traditional Chinese Medicine to treat hepatitis, inflammatory disorders, and cancer. *Schisandra sphenanthera* and several of its bioactive lignans, including Schisantherin A (SchA) and Schisandrol B (SchB), affect the *in vitro* and *in vivo* metabolism of substrates for drug-metabolising enzymes. Dabrafenib is a kinase inhibitor used in the treatment of $BRAFV^{600E}$-mutated metastatic melanoma. Dabrafenib is administered orally and chronically and undergoes CYP450-mediated metabolism. These factors make dabrafenib vulnerable to herb-drug interactions.



Aims. This study aimed to investigate the effect of Wuzhi tablet extract (WZE), SchA, and SchB on the metabolism of dabrafenib in human liver microsomes. Since SchA is reported to be the most abundant lignan in Wuzhi tablets, a secondary aim was to quantify the SchA content in WZE.

Methods. LC-MSMS was used to quantify the formation of hydroxy-dabrafenib from dabrafenib using human liver microsomes, including any inhibitory effect of WZE, SchA, or SchB. LC-MSMS was also used to quantify the amount of SchA in WZE.

Results. WZE and its lignans inhibited the metabolism of dabrafenib in a concentration-dependent manner. With a half-maximal inhibitory concentration (IC_{50}) of 0.36 µM, SchB was a more potent inhibitor compared to SchA (IC_{50} = 0.43 µM), and WZE (IC_{50} = 0.65 µM based on SchA content). Greater inhibition was observed when SchA and SchB were pre-incubated prior to the addition of dabrafenib, suggestive of mechanism-based CYP450 inhibition. Finally, SchA content was calculated to be 16 mg per gram of tablet powder (± 2.14 mg/g).

Discussion. The results of this study indicate that a herb-drug interaction between dabrafenib and Wuzhi is possible, and patients should be advised to avoid the combination until the clinical risk is evaluated.

407 NMBA-mediated anaphylaxis: can sugammadex act as an antidote?

Nithya A Fernandopulle[1], Paul F Soeding[1,2], Graham A Mackay[1], Dept of Pharmacology & Therapeutics[1], Univ of Melbourne, Parkville, VIC, Australia; Dept of Anaesthetics and Pain Medicine[2], Royal Melbourne Hosp, Parkville, VIC, Australia

Introduction: Neuromuscular blocking agents (NMBAs) are known to cause life-threatening anaphylaxis in approximately 1 in 10,000 patients. Symptoms of anaphylaxis are driven by the release of mediators from activated mast cells. Whilst this activation commonly involves IgE, new research has identified that a significant proportion of these events are caused by a non-IgE-dependent pathway involving the receptor MRGPRX2. Sugammadex, a drug that reverses neuromuscular blockade by some NMBAs, has been used as a treatment in these NMBA-mediated anaphylactic events with conflicting evidence of clinical efficacy (Clarke et al, 2012).

Aim: To investigate the utility of sugammadex as an antidote for NMBA-induced, non-IgE-dependent anaphylaxis.

Methods: We investigated the effects of pre- and post- treatment with sugammadex on MRGPRX2 activity using the NMBAs rocuronium, vecuronium and atracurium, alongside the MRGPRX2 agonist compound 48/80. We used the LAD2 human mast cell line, to measure calcium mobilisation, degranulation and cytokine release (MCP-1/CCL2).

Results: Compound 48/80 caused significant calcium mobilization, degranulation and cytokine release which was diminished when the cells were pre-treated with sugammadex. In contrast, NMBAs did not result in significant degranulation although they did cause calcium mobilization and modest MCP-1 secretion which was reduced with pre-treatment of sugammadex. Treatment of sugammadex post stimulation, which better mimics the clinical management of anaphylaxis, did not have an impact on degranulation but interestingly reduced MCP-1 secretion.

Discussion**:** We have shown that the LAD2 mast cell line is a representative model of a MRGPRX2-expressing, but functionally non-responding system and thus models the majority of patients where NMBAs are safe and effective. In addition, we have shown that sugammadex, even when given promptly after MRGPRX2-cell stimulation, is ineffective in halting degranulation. However, sugammadex-induced inhibition of the slower cytokine release produced by MRGPRX2 activation was evident, suggesting that it may have some benefit in controlling more chronic adverse effects produced by NMBAs.

Clarke RC, Sadleir PHM, Platt PR. (2012) Anaesthesia 63:266-273.

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

Gizem Gemikonakli[1,2], John Mach[1,2], Trang Tran[1,2], Susan Howlett[3], Rafael de Cabo[4], David G Le Couteur[2,5]& Sarah N Hilmer[1,2]. Lab of Ageing and Pharmacology, Kolling Institute, Royal North Shore Hosp, Sydney, NSW, Australia [1]. Northern Clinical School, Univ of Sydney, NSW, Australia [2]. Dalhousie University, Halifax, Canada[3]. Translational Gerontology Branch, National Institute on Aging, Maryland, USA[4]. ANZAC Research Institute, Sydney, NSW, Australia[5].

Introduction. Ageing, polypharmacy (use of ≥5 medications) and increasing DBI (measures total anticholinergic and sedative medication exposure) are associated with falls and impaired physical function. Preclinical ageing models can assess underlying mechanistic changes

Aims. We investigated whether chronic polypharmacy or monotherapy, with increasing DBI and/or cessation (deprescribing), affected physical function and/or muscle histology in mice.

Methods. 12-month-old male C57BL/6 mice received either control diet or study drug(s) at therapeutic doses. Polypharmacy diets consisted of Zero DBI (metoprolol, simvastatin, omeprazole, paracetamol, irbesartan), Low DBI (metoprolol, simvastatin, omeprazole, paracetamol, citalopram) and High DBI (metoprolol, simvastatin, citalopram, oxycodone, oxybutynin). Individual drugs (High DBI regimen) were tested as monotherapy. At 21-months, animals were randomised to continue treatment or gradual withdrawal. Rotarod performance was assessed at 12-24-months, and balance beam (6mm) at 24-months. Gastrocnemius muscle samples were collected at 26-months.

Results. Rotarod performance at 21-24-months indicated significantly increased endurance for metoprolol treated mice (n=15-36) compared to control (n=24-29) and to High-DBI (n=18-38), and for Zero-DBI mice (n=15-34) compared to Low-DBI (n=19-40); ($p<0.05$). Balance assessment showed deprescribed High-DBI (n=18) and metoprolol (n=16) mice performed significantly better than their continuously prescribed comparators (n=10-15; $p<0.05$). Preliminary histology results suggest a trend towards less muscle fibres per field in control (n=3), compared to High-DBI (n=3; $p=0.119$) and citalopram animals (n=2; $p=0.049$), while collagen quantification suggests no difference between control (n=5), High DBI (n=6) and High DBI deprescribed (n=4).

Discussion. Rotarod detected differences between drug treatments and deprescribing affected balance. Our preclinical results suggest polypharmacy and certain monotherapy drug regimens impact measures of muscle function and may affect structure. Future research will continue to characterise histological changes in muscle.

409 Update of perpetrators in the pharmacokinetic interaction screening (PKIS) database

Thomas M Polasek[1,2], Georgia E Glass[1]. Dept of Clinical Pharmacol, [1]Flinders University, Adelaide, SA, Australia; [2]Certara, Melbourne, VIC, Australia.

Introduction. The pharmacokinetic interaction screening (PKIS) database was first published in 2010. Since then, several new protein kinase inhibitors (KIs) and antivirals (AVs) have been released that may cause CYP-mediated pharmacokinetic drug-drug interactions (PK-DDIs).

Aims. To apply clinically relevant criteria to evaluate KIs and AVs as potential perpetrators of PK-DDIs.

Methods. Available KIs and AVs were identified and evaluated using the primary literature and prescribing information. The potential for these drugs to act as perpetrators at drug metabolizing CYP enzymes was assessed using the following criteria: available clinical PK studies in humans (n ≥ 6), including healthy volunteers and patients; the use of an appropriate *in vivo* CYP 'probe' (fmCYP ≥ 0.8); dosing until steady-state with a clinically relevant dose. Inhibitors were described according to the FDA classifications of *strong*, *moderate* or *weak*, whereas inducers were classified as *major* (≥ 2-fold decrease in area under the plasma concentration-time curve (AUC) of CYP probe) or *minor* (<2-fold decease in AUC). Data were entered in a catalogue of major CYP perpetrators based on 2-fold changes in the clearance of CYP probes (www.pkis.org).

	CYP1A2	CYP2C19	CYP3A
Strong inhibitors			Idelalisib Tipranavir/ ritonavir
Moderate inhibitors	Vemurafenib		Crizotinib Imatinib Nilotinib Darunavir/ ritonavir Fosamprenavir
Major inducers		Efavirenz	Dabrafenib

Results. There were 32 KIs and 22 AVs included in the assessment. Of the theoretical 324 CYP-perpetrator pairs (CYP-PPs), 66 (20.4%) had clinical PK interactions studies meeting the criteria. There were studies with 12 CYP-PPs that did not meet the criteria (3.7%), leaving 246 potential interactions between KIs or AVs and CYP probes that have not been studied *in vivo* (75.9%).Two strong inhibitors, 5 moderate inhibitors, and 2 major inducers were identified and added to the catalogue of major CYP perpetrators as shown in the table (16.6% of the perpetrators evaluated).

Discussion. Prescribers can commence, cease or change the dose of most KIs and AVs without perpetrating PK-DDIs with other chronically administered drugs.

410 Tacrolimus dosing and monitoring: a retrospective cohort study

Ranita Kirubakaran[1,2,3], Sophie L Stocker[1,3], Lilibeth Carlos[4], Fay Burrows[4], Richard O Day[1,3], Jane E Carland[1,3]. St Vincent's Clin Sch, UNSW[1], Sydney, NSW, Australia; Dept of Pharm, Sultan Abdul Halim Hosp[2], Sungai Petani, KDH, Malaysia; Dept of Clin Pharmacol and Toxicol, St Vincent's Hosp[3], Darlinghurst, NSW, Australia; Dept of Pharm, St Vincent's Hosp[4], Darlinghurst, NSW, Australia.

Introduction. Tacrolimus (TAC) is an immunosuppressant with a narrow therapeutic index and high PK variability. Therapeutic drug monitoring (TDM) of trough concentrations (C_0) is recommended to maintain TAC efficacy and safety.
Aims. To assess TAC dosing, TDM, and clinical outcomes in heart transplant (HTx) recipients at St Vincent's Hospital, Sydney.
Methods. A retrospective cohort study of all HTx recipients in 2017 was conducted. The guidelines recommend a starting dose of 0.075 mg/kg/day for oral TAC and the therapeutic target C_0 post-transplant is 8-12 µg/L for week 1, followed by 10-15 µg/L for up to 3 months. Electronic medical records were reviewed to collect relevant clinical data.
Results. There were 40 HTx recipients, 26 (65%) males and a mean (SD) age of 52 (14) years. Post-transplant doses were gradually increased from 0.011 mg/kg (Day 1) to 0.034 mg/kg (Day 6). Only 11% (90/805) of C_0 were classified as "true tough" (collected within 1 h prior to the next scheduled dose). Of these, 28 (31%) were subtherapeutic, 53 (59%) were therapeutic, and 9 (10%) were supratherapeutic. Median (IQR) time to target was 15 (8–22) days. TAC reported adverse effects included 16 (40%) instances of CNS effects (tremors and headaches) and 6 (15%) cases of acute kidney injury. There were 63 re-hospitalisation events post-transplant: 28 (44%) due to infection and 15 (24%) due to rejections. C_0 were similar across all endomyocardial biopsy grades of rejection ($P>0.05$).
Discussion. TAC dosing and monitoring are not concordant with the available local guidelines. Cautious dosing was observed and TDM samples were poorly timed. Bayesian dose prediction programs may provide an additional support required to optimise TAC dosing and monitoring of HTx recipients.

Baran DA *et al* (2002) Transplantation 74: 1136-1141.

411 Predictive performance of tacrolimus precision dosing software in heart transplant

Ranita Kirubakaran[1,2], Sophie L Stocker[1,2], Richard O Day[1,2], Jane E Carland[1,2]. St Vincent's Clin Sch, UNSW[1], Sydney, NSW, Australia; Dept of Clin Pharmacol and Toxicol, St Vincent's Hosp[2], Darlinghurst, NSW, Australia.

Introduction. Therapeutic drug monitoring (TDM) of tacrolimus (TAC) is recommended to maintain TAC efficacy and safety. However, cautious dosing in clinical practice, particularly to prevent TAC-induced nephrotoxicity, is not suitable to achieve therapeutic TAC concentrations in a timely manner.
Aims. To evaluate the predictive performance of a precision dosing software, DoseMeRx® (Brisbane, Australia) in predicting TAC concentrations (sampled at any point of time), accommodating concomitant azole therapy in heart transplant recipients (HTx) at St Vincent's Hospital, Sydney (SVH). DoseMeRx® employs validated population PK models and Bayesian forecasting to provide individualised dose predictions for patients.
Methods. A retrospective cohort study of all SVH HTx recipients in 2017 was conducted. Data inputted into DoseMeRx® included TAC concentrations and dosing regimen, and patient parameters such as hematocrit, height, weight and CYP 450 genotype (if available). The predictive performance of DoseMeRx® was evaluated by comparing the predicted concentrations to the observed concentrations using median prediction error (MPE, a measure of bias) and median absolute prediction error (MAPE, a measure of accuracy).
Results. There were 40 HTx recipients, 26 (65%) males and a mean (SD) age of 52 (14) years. Post-transplant TAC doses were increased from 0.011 mg/kg (Day 1) to 0.034 mg/kg (Day 6). All patients received itraconazole 200 mg BD as a prophylaxis for invasive fungal infection immediately post-transplant. During the first 2 weeks of TAC therapy, 329 blood concentrations were measured (n=38). DoseMeRx® demonstrated an overall MPE (95%CI) of –15% (-19,-12) and MAPE (95%CI) of 22% (19, 25). MPE reduced from -71% (Day 1 of TAC) to -14% (Day 7) and MAPE reduced from 71% (Day 1) to 16% (Day 7).
Discussion. DoseMeRx® was able to predict TAC concentrations across a heterogeneous patient population with a clinically acceptable bias and accuracy. The improvement in MPE and MAPE over a week aligns with the time for the full magnitude of interaction between itraconazole and TAC to be stabilised. The use of DoseMeRx® in dose guidance may provide significant benefits in clinical practice and potentially limit the need for frequent TDM.

412 Vancomycin – effects of obesity on drug exposure and outcome with Guideline based dosing

Mari Koyanagi[1], Rebecca Anning[1], Mark Loewenthal[1], Jennifer H Martin[1,2] John Hunter Hospital, Newcastle, NSW, Australia[1]; Hunter Medical Research Institute, Newcastle, NSW, Australia[2]

Introduction: Vancomycin, a glycopeptide with bacteriostatic and bacteriocidal activity has been the agent of choice since the 1950s against methicillin resistant *Staphylococcus aureus* (MRSA). Despite widespread use of vancomycin, dosing regimens and drug monitoring recommendations are poorly adhered to in many institutions internationally and in Australia, with concerns of propelling antibiotic resistance.
Aim: This study studied the administration practices of vancomycin in a single centre in Australia and focusing specifically on whether lower exposures and poorer outcomes were observed in a population with BMI ≥25kg/m2.
Method: Data were collected from September 2016 to March 2017 on all patients prescribed vancomycin.
Result: Of 488 patients screened, 107 patients had analysable data. Based on the local Hospital Guidelines, 62.6% were commenced on inappropriate vancomycin doses at the beginning of their therapy, and 60% of BMI ≥25kg/m2 were underdosed ($p = 0.007$). Length of hospital stay was longer in BMI ≥25kg/m2 compared to BMI <25kg/m2 (27.5 days versus 18 days), with a trend towards higher mortality (11.7% vs 8.5%). Overall 32.8% of the total 1145 vancomycin days given in the total population were subtherapeutic, with a majority (41.4%) involving patients who were underdosed initially.
Discussion: Patients who are initially underdosed have a high rate of total subtherapeutic vancomycin days, regardless of BMI, with on average to more than a third of their vancomycin treated days being subtherapeutic. Patient with BMI ≥25kg/m2 are more likely to be underdosed, with trends toward increased hospital LOS, mortality, and treatment failure.

413 Potential cytochrome-mediated drug interactions with cannabinoids

Catherine J Lucas A[1], Joanne Patel[1,2], Jessica Ryan[1,2], Peter Galettis[2], Jennifer H Martin[1,2]. John Hunter Hospital[1], Newcastle, NSW, Australia; University of Newcastle[2], Newcastle, NSW, Australia.

Introduction. There is increasing public interest in exogenous cannabinoid use. Clinicians should bear in mind potential for clinically significant pharmacokinetic or pharmacodynamic interactions between cannabinoids and other drugs.
Case report. A 37-year-old woman with a history of Eisenmenger syndrome, previous heart and dual lung transplants, acquired hypogammaglobulinaemia, hypertension, depression and gastro-oesophageal reflux, presented to hospital with altered mental state, limited verbalisation, hyper-reflexia, mydriasis and urinary retention. Medications prior to admission (many metabolised by the cytochrome P450 enzyme system) included fluoxetine (60 mg daily), tacrolimus, mycophenolate mofetil, prednisolone, pantoprazole, ranitidine, posaconazole, azithromycin, valganciclovir, sulfamethoxazole-trimethoprim, pregabalin and monthly intravenous immunoglobulin. Biochemistry, infection screen, electrocardiography and cerebral imaging were unremarkable. Urinary drug screen was positive for tetrahydrocannabinol. Collateral history elicited oral ingestion of an unknown quantity of "street" cannabis oil ~ 36 hours prior to presentation, and patient compliance with stable prescribed medication regimen until ~ 2 days prior to admission. One day post admission, plasma concentration of tacrolimus was 2.0 mg/L (trough target 5-15 mg/L). Plasma concentrations of fluoxetine and norfluoxetine (active metabolite), measured two days post admission (~ four days since last administration) were 729 mg/L and 712 mg/L, respectively (therapeutic range 120-500 mg/L).
Discussion. Anticholinergic toxicity has been reportedly associated with cannabis use. Additionally, cannabinoids undergo metabolism by cytochrome P450 enzymes, with delta-9-tetrahydrocannabinol mainly metabolised by CYP2C9, 2C19 and 3A4 and cannabidiol mainly metabolised by CYP2C19 and 3A4, and potential involvement of CYP1A1, CYP1A2, CYP2C9, and CYP2D6. Interactions may occur via coadministration of inhibitors of these cytochrome enzymes. There are case reports of mania resulting from co-administration of cannabis and fluoxetine. Supratherapeutic fluoxetine concentrations may have contributed to the patient's symptomatology (albeit concentration-toxicity relationship is uncertain). Adverse effects may be related to the cannabis product, and/or its effect on concurrent medications. Formal reporting of potential cannabinoid-drug interactions is vital to improve the data in this area.

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

Tamara Y Milder[1,2,3,4], Sophie L Stocker[1,3], Christina Abdel Shaheed[5], Lucy McGrath-Cadell[6], Dorit Samocha-Bonet[3,4], Jerry R Greenfield[2,3,4], Richard O Day[1,3]. Dept of Clin Pharmacol, St. Vincent's Hosp[1], Sydney, NSW; Dept of Endocrinol, St. Vincent's Hosp[2], Sydney, NSW; St Vincent's Clinical School, Univ of NSW[3], Sydney, NSW; Diabetes and Metabolism Division, Garvan Institute of Medical Research[4], Sydney, NSW; School of Public Health, Univ of Sydney[5], Sydney, NSW; Dept of Cardiol, St. Vincent's Hosp[6], Sydney, NSW.

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

415 Modulation of T- type Calcium Channels by Phytocannabinoids

Somayeh Mirlohi[1], Chris Bladen[1], Iain McGregor[2], Mark Connor[1]. Dep of Biomedical Sciences, Macquarie Univ[1], Lambert Initiative for Cannabinoid Therapeutics, Univ of Sydney, NSW[2]

Introduction: T-type calcium channels (I_{Ca}) are low voltage activated channels which can be opened by small depolarizations of the plasma membrane. They are encoded by the genes, $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$. T-type I_{Ca} are critical for many physiological processes, and their activity contributes to pathophysiological states including cardiac arrhythmia, epilepsy and pain. There is increasing interest in the use of cannabinoids as medicinal therapeutics, including for epilepsy and pain. Aim: Some cannabinoids can potently modulate T-type I_{Ca}, but there is less information about plant-derived phytocannabinoid effects on these channels, therefore we screened a library of phytocannabinoids to determine whether these compounds modulate T-type I_{Ca}. Methods: We used a fluorometric (FLIPR) assay to screen and phytocannabinoids for their ability to block T-type I_{Ca}, changes in intracellular Ca were measured following addition of 10 mM Ca after 5 minutes treatment with drug or vehicle. Then, several compounds were tested in HEK293 Flp-In-TREx cells stably expressing $Ca_V3.1$, using whole-cell patch clamp. Results: In general, phytocannabinoids most potently blocked Cav3.1 and Cav3.2, with less inhibition of $Ca_V3.3$. In the FLIPR assay, CBGA (cannabigerol acid) was the most potent compound, inhibiting $Ca_V3.1$ by 97 ± 1 at 10 µM, CBDV (cannabidivaran) and THCA (Tetrahydrocannabinol acid) ranked second and third. CBGA blocked $Ca_V3.2$ by 92 ± 3 with CBG (cannabigerol), CBN (cannabinol), THCA and CBDVA (cannabidivarinic acid) also inhibiting $Ca_V3.2$ by more than 50 % at 10 µM. THCA was the only compound that inhibited $Ca_V3.3$ by more than 50% (53 ± 7). In contrast, Δ^9-Tetrahydrocannabinol (THC) activated $Ca_V3.1$ and $Ca_V3.2$ but did not affect $Ca_V3.3$. Patch clamp recording of $Ca_V3.1$ showed THCA and THC potently blocked $Ca_V3.1$ with Log IC_{50} of -5.72 ± 0.07 and -6.07 ± 0.06 respectively. THC and THCA produced a significant hyperpolarizing shift in inactivation potential, likely accounting for inhibition of $Ca_V3.1$ current. THC also caused a negative shift in the $Ca_V3.1$ activation potential which was not seen using THCA, and this is probably responsible for increasing Ca entry seen in the FLIPR assay. Discussion: Phytocannabinoid modulation of T-type I_{Ca} extends beyond the previously reported effects of THC and cannabidiol, and $Ca_V3.X$ channels represent an interesting potential site of actions for these drugs in pain and epilepsy.

416 Preferred language for communicating deprescribing decisions in the discharge summary: a qualitative study

Brendan J Ng[1], Mai Duong[1], Natali Jokanovic[1], Melissa Baysari[2], David Le Couteur[3], Sarah Hilmer[1]. Clinical Pharmacology and Ageing Laboratory, Kolling Institute[1], St Leonards NSW; Australian Institute of Health Innovation, Macquarie University[2], Macquarie, NSW; Ageing and Alzheimers Institute, Concord Hospital[3], Concord, NSW.

Introduction. Communication of hospital deprescribing decisions to the general practioner (GP) is key for sustaining the deprescribing process into the community. Although improvements in medication list completeness and indications have occurred over time, no study has looked specifically at improving the quality of deprescribing communication in the discharge summary.
Aims. 1) Refine discharge summary language to effectively communicate deprescribing decisions in the discharge summary. 2) Inform the development of a standardised guide for communication of this information (content, phrasing, structure and method of entry).
Methods. Thirty participants (seven GPs, eight pharmacists, 15 hospital doctors) were recruited to nine interviews and four focus groups. Each participant was presented with 10 scenarios describing a deprescribing decision and with multiple language options for the discharge summary. Participants were asked for a preference, reasons why, any suggested alterations and questions on priorities, specific wording, and location. Final themes and framework for preferred language developed in data-analysis were agreed by consensus.
Results. Clinicians reported the importance of structured phrasing, with specific advice in a GP follow-up section. Communicating the decision and plan was important, but this structure also potentially facilitates further deprescribing discussion between the patient and GP. Based on participant suggestions, deprescribing decisions should be communicated via the following: '*Medication: Intention, Rationale. Clear plan (dose, duration, follow up). Patient agreement.*' The cohort gave mixed responses about including information on the drug burden index, monitoring or alternative strategies to manage the clinical issue. Using our results, a final 'fill-in-the-blank' template has been developed, the final design reviewed with local geriatricians, and integrated into point-of-care guidelines for further validation.
Discussion. A standardised preferred language guide for deprescribing has been developed from clinician preferences to improve sustainability of deprescribing decisions made in hospital.

417 Medication use in older inpatients with and without dementia

Jessica T V Nguyen[1,2], Danijela Gnjidic[1], Sarita Lo[2], Meggie Zhang[2], Sarah N Hilmer[2]. Syd Pharm School, Faculty of Med and Health, Univ of Syd[1], Sydney, NSW, Australia; Depts of Aged Care and Clin Pharmacol, Kolling Institute of Medical Research[2], Royal North Shore Hosp and Northern Clin School, Faculty of Med and Health, Univ of Syd, Sydney, NSW, Australia.

Introduction. Reducing the use of potentially inappropriate medications (PIMs) is a key aim of optimising medication use in the ageing population, which is particularly important among vulnerable older inpatients with dementia.
Aims. To compare use of PIMs in older inpatients with and without dementia.
Methods. A retrospective cohort study was conducted on 500 patients aged ≥75 years admitted consecutively to three Sydney metropolitan hospitals from 1st July 2016. Dementia was defined as a clinical diagnosis recorded in the electronic hospital records. PIM use was defined using the 2015 Beers Criteria and Drug Burden Index (DBI) was used to quantify the cumulative use of anticholinergic and/or sedative drugs. The differences in the numbers of patients exposed to medications with DBI>0, PIMs according to Beers Criteria and a total of ≥5 regular medications (polypharmacy) between admission and discharge according to dementia status were analysed.
Results. Dementia was identified in 20.4% (n=102) of participants. A higher proportion of patients with dementia had DBI>0 on admission (dementia: 68.6% (n=70); without dementia: 50.5% (n=201), P=0.002) and a similar trend was seen on discharge (dementia: 69.6% (n=71); without dementia: 58.8% (n=234), P=0.060). Any exposure to Beers Criteria medications trended towards being higher in patients without dementia on admission (dementia: 30.4% (n=31); without dementia: 38.2% (n=152), P=0.179) and on discharge (dementia: 29.4% (n=30); without dementia: 38.2% (n=152), P=0.126). A higher proportion of patients with dementia experienced polypharmacy on admission (dementia: 79.4% (n=81); without dementia: 75.9% (n=302), P=0.535) and a similar trend was observed on discharge (dementia: 86.3% (n=88); without dementia: 85.2% (n=339), P=0.902).
Discussion. Preliminary results show significant variations in medication use between patients with and without dementia admitted to hospital, with trends towards more anticholinergic and sedative use but less Beers Criteria PIMs in those with documented dementia than in those without.

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

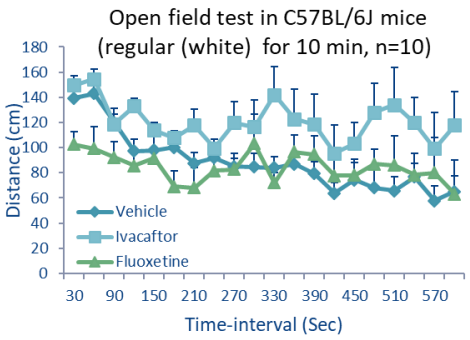
Mirjana Radovanovic[1,2], J Schneider[2,3,4], S Ackland [2,3,5], R LG Norris,[1,2] J H Martin,[1,2] P Galettis,[1,2]. Clin Pharmacol, Univ of Newcastle[1], Callaghan, NSW; HMRI[2], New Lambton Heights, NSW; HCRA, Univ of Newcastle[3], NSW; School of Biomedical Sciences and Pharmacy, Univ of Newcastle[4], Callaghan, NSW; Calvary Mater Newcastle[5], Waratah, NSW.

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

419 Investigation of the potentially beneficial CNS-activity of cystic fibrosis modulator drugs

Elena K. Schneider-Futschik[1], Daniel Hoyer[1,2,3], Tony Velkov[1]. [1]Department of Pharmacology & Therapeutics, School of Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, 3010, [2]The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC, 3052, Australia. [3]Department of Molecular Medicine, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA.

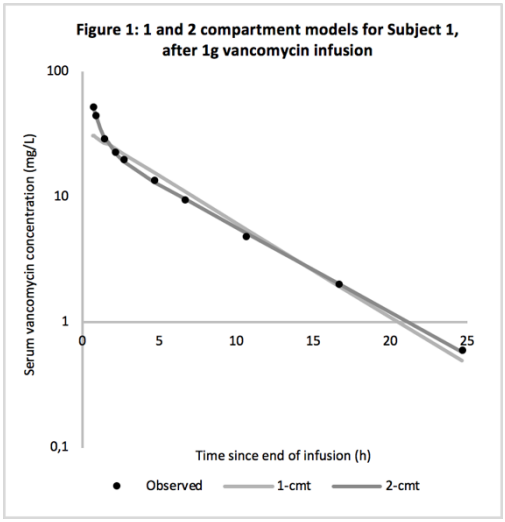
Introduction: Cystic fibrosis transmembrane conductance regulators (CFTR) are two new breakthrough treatment options for cystic fibrosis (CF).
Aims: The interactions of ivacaftor and metabolites with neurotransmitter receptors were investigated in radioligand binding, in a chronic mouse model of depression and evaluated with the Awescore questionnaire in patients.
Methods & results: Ivacaftor displayed significant affinity to 5-HT2C (pKi = 6.06 ± 0.03), β3 adrenergic (pKi = 5.71 ± 0.07), and δ-opioid (pKi = 5.59 ± 0.06) and the dopamine transporter (pKi = 5.50 ± 0.20); Iva-M1 to 5-HT2C (pKi = 5.81 ± 0.04), and the muscarinic M3 (pKi = 5.70 ± 0.10), whereas iva-M6 to 5-HT2A (pKi = 7.33 ± 0.05). The in vivo CNS-activity of ivacaftor (40 mg/kg, i.p. for 21 d) was assessed in a chronic mouse model of depression. In the forced swim test, the ivacaftor-treated group displayed decreased immobility (52.8 ± 7.6 sec), similarly to fluoxetine (33.8 ± 11.0 sec) and increased climbing/swimming activity (181.5 ± 9.2 sec). In the open field test, ivacaftor produced a higher locomotor activity than the fluoxetine group, measured both as mean number of paws touches (ivacaftor 81.1 ± 9.6 vs. fluoxetine 57.9 ± 9.5) and total distance travelled (ivacaftor 120.6 ± 16.8 vs. fluoxetine 84.5 ± 16.0) in 600 sec. Treatment of 23 CF patients with ivacaftor-lumacaftor resulted in significant improvements in the quality of life including anxiety in all 5 domains of the AweScoreCF questionnaire (p = 0.092-0.096).
Discussion: Our findings suggest ivacaftor displays potential clinical anxiolytic and stimulating properties and may have beneficial effects on mood.



420 Comparison of vancomycin area-under-the-curve using one and two-compartment pharmacokinetic models

Rashmi V Shingde[1], Garry G Graham[1,2], Jane E Carland[1,3], Kenneth M Williams[1,2], Stephanie E Reuter[4], Richard O Day[1,2,3] & Sophie L Stocker[1,3]. Dept of Clin Pharmacol, St Vincent's Hosp[1], Darlinghurst, NSW; School of Med Sci, Univ of NSW[2], Kensington, NSW; St Vincent's Clin School, Univ of NSW[3], Kensington, NSW; School of Pharm & Med Sci, Univ of South Australia[4], SA.

Introduction. The Bayesian forecasting program, DoseMeRx, used in the therapeutic drug monitoring (TDM) of vancomycin utilises a 1-compartment population model for vancomycin pharmacokinetics, despite the time course of plasma concentrations following a 2-compartment model (Figure 1). We aimed to analyse the difference in the AUC_{∞} calculated using either a 1 or 2-compartment model.



Methods. Twenty-eight adult patients with stable renal function were administered a single 1g dose of vancomycin (if weight <50 kg, 15 mg/kg), infused over at least 0.67 h. Patients were intensively sampled between 0.08-24 h post-infusion, and individual post-infusion datasets were computer-fitted (MINIM version 3.0.8), using weighted nonlinear least-squares regression. 'AUC_{TRUE}' was calculated from fitting all vancomycin concentrations to the 2-compartmental model. '$AUC_{1\text{-}cmt}$' was calculated from fitting all concentrations beyond the α-distribution phase (>1 h post-infusion) to the 1-compartment model.

Results. The AUC_{TRUE} (median (95%CI), 178.4 mg.h/L (122.2, 213.6)) was greater than the $AUC_{1\text{-}cmt}$ (144.5 mg.h/L (92.3, 178.2; $P < 0.001$). The median % prediction error (MDE%) between $AUC_{1\text{-}cmt}$ vs AUC_{TRUE} was -16.0 (95% CI -19.9, -13.3).

Discussion. In patients with stable renal function, a Bayesian forecasting program which utilises a 1-cmt population model for vancomycin pharmacokinetics will likely underestimate true AUC by approximately 16%, which may still be clinically acceptable.

421 Frailty and medication use in older inpatients: potentially inappropriate medications and polypharmacy

Meggie Zhang[1], Sarita Lo[1], Sarah N Hilmer[1,2], Peter R Carroll[2], Slade Matthews[2], Jessica T V Nguyen[1,2], Danijela Gnjidic[1,2], Ruth Hubbard[3], Brendan Ng[1], Mitchell Redston[1,2]. Kolling Inst of Med Research, RNSH and Univ of Syd[1], Sydney, NSW, Australia; Brisbane, QLD, Australia; Fac of Med and Health[2], Univ of Syd, Sydney, NSW, Australia; Southside Clin Unit, Fac of Med, PAH[3].

Introduction. Inappropriate medication use aggravates adverse outcomes in vulnerable multimorbid frail older people. Polypharmacy (regular use of ³5 medications) and the use of potentially inappropriate medications (PIMs) including those with anticholinergic and sedative medications are significant problems. A novel Frailty Index (FI) derived from routinely collected data was previously proposed to assess frailty in hospital. Identifying frailty, polypharmacy and PIMs in hospital can identify those most at risk of adverse outcomes and aid in the optimisation of medication use.

Aims. (i) To validate a novel FI in a large representative cohort of older inpatients; and (ii) To characterise and explore the association between medication use and frailty.

Methods. A retrospective cohort study was conducted on 500 inpatients aged ≥75 years admitted consecutively under General Medicine, Geriatric Medicine or Rehabilitation specialties for ≥48 hours in three metropolitan NSW hospitals from 1 July 2016. Patients were excluded if they died during admission. Data was manually collected from electronic hospital medical records. Frailty was assessed using the novel FI. PIMs were identified using Beers Criteria. Drug Burden Index (DBI) assessed the cumulative exposure to medications with anticholinergic and sedative effects. Descriptive statistics were reported. Spearman's Correlation Coefficient and the Mann Whitney U test were used.

Results. Patients had a mean age of 86.2±0.26 years and mean frailty score of 0.282±0.005. Polypharmacy was present in 76.6% of patients. 36.6% of patients used ≥1 PIM whilst 54.2% had a DBI score >0. There was a positive correlation between frailty and number of medications (ρ=0.328, n=500, $p<0.005$). Frailty was strongly negatively correlated with number of PIMs (ρ=-0.57, n=500, $p=0.204$) but positively correlated with DBI score (ρ=0.290, n=500, $p<0.005$).

Discussion. Polypharmacy is highly prevalent in hospital with frailer inpatients taking more medications overall and more with anticholinergic and sedative effects, potentially leading to adverse outcomes. However, they have less exposure to PIMs, suggesting a difference in prescribing pattern within the cohort that is related to frailty status. Future directions are to evaluate the association between frailty and adverse outcomes.

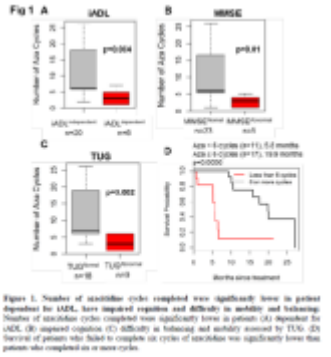
422 Simultaneous determination of the phenotyping cocktail drugs and their cytochrome P450-specific probe metabolites in human plasma and urine by liquid chromatography/tandem mass spectrometry

Mei Zhang [1,2], Grant Moore [2], Matthew Doogue[1], Matthew Strother [3] Department of Medicine, University of Otago - Christchurch [1]; Toxicology, Canterbury Health Laboratories [2], Department of Oncology, Christchurch Hospital [3], Christchurch, New Zealand

Introduction. Following partial hepatectomy, there is rapid regeneration of liver volume. However, functional recovery of hepatic drug metabolism is less well-studied than volumetric recovery. The cytochrome P450 enzyme superfamily (CYP), mainly expressed in the liver, is responsible for the majority of drug metabolism in human. *In vivo* CYP activity can be assessed through use of CYP-specific probe drugs. Combining probe drugs, or a probe "cocktail" allows concurrent phenotyping of multiple CYPs. Our group proposes to study phenotype changes in CYPs as a result of partial hepatectomy. For this work a phenotyping cocktail (Inje cocktail) was selected from established probe drugs previously used in "cocktail" studies.
Aims. To establish and validate a LC-MS/MS method for the simultaneous determination of the phenotyping cocktail drugs and metabolites in human plasma and urine.
Methods. Plasma and urine samples were pretreated with acetonitrile and then diluted with the mobile phase. The prepared samples were injected into the LC-MS/MS system. Cocktail drugs [caffeine (CYP1A2), dextromethorphan (CYP2D6), losartan (CYP2C9), midazolam (CYP3A4), omeprazole (CYP2C19), paracetamol (multiple enzymes)], CYP specific probe metabolites, and the corresponding isotopically labelled internal standards of all the compounds were resolved on a C8 column using gradient elution of 10 mM ammonium acetate and acetonitrile. All the compounds and internal standards were detected using electrospray ionisation in the positive mode.
Results and Discussion. The total analysis time was 10 min. All the analytes were well separated. The ranges of the standard curves in plasma and urine were 0.1 to 1000 ng/mL for midazolam and α-hydroxymidazolam, 0.1 to 2500 ng/mL for dextromethorphan, and 1.0 to 10000 ng/mL for caffeine, paraxanthine, losartan, E-3174, omeprazole, 5-hydroxyomeprazole, dextrorphan and paracetamol. The intra- and inter-day CVs over the concentration ranges for all the compounds were <10%. This method is simple, sensitive, specific and accurate for the simultaneous determination of the phenotyping cocktail drugs and their CYP-specific probe metabolites in human plasma and urine.

423 The GAP-MDS study: Geriatric Assessment in Older People with Myelodysplastic Syndrome is predictive of Azacitidine therapy completion and survival. A multidisciplinary translational research initiative at the Royal Adelaide Hospital.

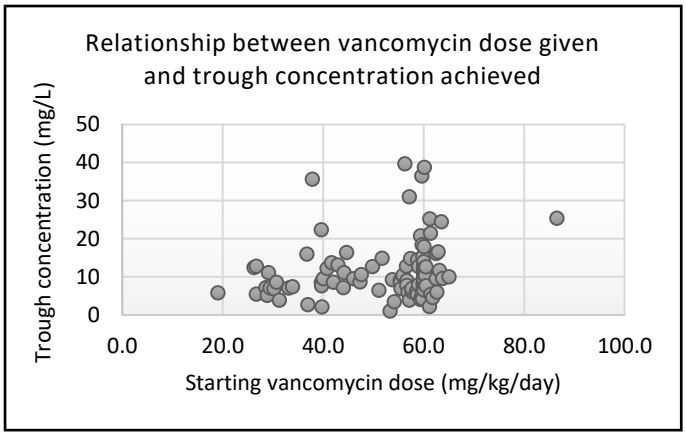
Angela Molga[1], Michelle Wall, Devendra Hiwase[2]. Dept Clinical Pharmacology, Royal Adelaide Hospital[1], Adelaide, SA, Australia; Department of Haematology, Royal Adelaide Hospital[2], Adelaide, SA, Australia.



Introduction. Standard oncological performance scores in older people are insufficient at identifying those who are unlikely to complete therapy.
Aims. To determine if deficits in ageing assessed using Geriatric assessments were associated with therapy completion rates and survival.
Methods. Ethics approval was obtained. After the treatment decision had been made by the haematologists, consented patients had geriatric assessments administered by a nurse case manager, followed by Geriatrician review.
Results. A total of 108 patients were enrolled into the study over a 4 year period. Although only 29 (27%) patients had an Eastern Cooperative Oncology Group score ≥2, 86 (79%) patients had deficits in at least one domain of ageing. Deficits were spread across all domains, including dependence for instrumental activity of daily living (iADL) (n=32, 29%). Patients who were iADL-dependent (3.2±5 vs. 10.8±15; p=0.004), were cognitively impaired (2.8±4 vs. 9.9±15; p=0.010) or had impaired mobility measured by the timed-up and got test (3.3±5 vs. 11.±15; p=0.002), completed significantly less cycles of azacitidine therapy than patients without deficits in these domains (Fig 1A-C). The patients who ceased therapy prematurely (less than 6 cycles) also had significantly poorer overall survival (OS) of patients compared to patients completing at least six cycles of azacitidine. Seventy patients were reviewed by the Geriatrician which led to the identification of a significant degree of multimorbidity (87%) and polypharmacy (73%).
Discussion. Deficits associated with ageing are associated with premature cessation of therapy and poorer survival. Geriatric assessments should form part of the assessment of older persons with the aim of reducing adverse outcomes and maintaining quality of life.

424 Vancomycin Therapeutic Drug Monitoring in Paediatrics

Joanne Patel[1,2], Catherine J Lucas[1,2], Jennifer H Martin[1,2]. John Hunter Hospital[1], Newcastle, NSW, Australia; Discipline of Clinical Pharmacology[2], University of Newcastle, NSW, Australia.



Introduction. Vancomycin is a commonly prescribed antimicrobial therapy in children. Therapeutic drug monitoring is recommended for courses beyond 48 hours, to monitor for efficacy and prevent toxicity.
Aims. To audit vancomycin dosing and monitoring in paediatric patients and to consider what changes are necessary to improve use.
Methods. Data was collated for all vancomycin prescriptions for under 18 year olds from two regional hospitals for a 16 month period. Patient age, gender, weight, admitting team, reason for admission, biochemistry and microbiology results were reviewed.
Results. A total of 133 vancomycin courses were audited. The average patient age was 6.5 years. The mean starting dose was 52.55 mg/kg/day (range 19.05 to 86.54). Trough concentrations were measured in 73% of vancomycin courses. Thirty five patients (26%) had their first trough concentration in the target range.
Discussion. Guidelines for weight based dosing are not routinely followed, and may require revision in obese adolescents. Therapeutic drug monitoring guidelines are poorly adhered to in the two centres audited. Studies suggest that trough concentrations are a poor predictor of $AUC_{0\text{-}24}$/MIC in the paediatric population, and trough concentration monitoring should be reviewed and potentially replaced with Bayesian modelling.

Le J, Bradley JS, Murray W et al. Improved Vancomycin Dosing in Children Using Area-Under-the-Curve Exposure. Pediatr Infect Dis J. 2013 April; 32(4): e155–e163.

425 Using population pharmacokinetics to guide vancomycin dosing in high flux haemodialysis; analysis of data from the VANISH study.

Sam Salman[1], Vanessa Sinclair[2], Sharon Ho[2], Kristen Watson[2], Frank Sanfilippo[3], Kevin Batty[4], Ramyasuda Swaminathan[5], Karen Siu[5], Paul Ingram[6], Matthew Rawlins[2] Medical school, University of Western Australia[1], Crawley, WA, Australia; Pharmacy Department, Fiona Stanley Hospital[2], Murdoch, WA, Australia, School of Population Health, University of Western Australia[3], Crawley, WA, Australia; Pharmacy and Biomedical Sciences, Curtin University[4], Bentley, WA, Australia; Nephrology, Fiona Stanley Hospital[5], Murdoch, WA, Australia; Department of Microbiology, PathWest[6], Murdoch, WA, Australia.

Introduction. Vancomycin is commonly used to treat resistant bacteria with therapeutic drug monitoring performed to improve efficacy and reduce toxicity. As high-flux haemodialysis removes a significant amount of vancomycin it adds complexity to dosing. Although previous publications have examined this complexity, including a previous population PK model (Ariano et al, 2005), none have provided a clear, adaptive dose regimen.
Aims. To develop a population PK model using data from patients on high flux haemodialysis receiving intermittent intravenous vancomycin for assessment of the sources of variability and development of dosing guidelines.
Methods. Patients with end-stage renal failure undergoing intermittent high-flux haemodialysis who were prescribed vancomycin were enrolled into the study. In addition to those obtained from usual care, additional vancomycin plasma concentrations pre- and post-dialysis were obtained and analysed using NONMEM.
Results. 269 concentrations from 14 patients over >100 dialysis sessions were included. The model comprised of a 2-compartment model with first order elimination and an additional clearance parameter during dialysis. Clearance during dialysis was ~30 times higher than intrinsic clearance, with inter-session variability accounting for most of the differences in this parameter. Simulations from the developed model were used to provide dose recommendations.
Discussion. The present population PK model is consistent with previous reports of vancomycin with high-flux dialysis. Dose recommendations in these patients incorporating therapeutic drug monitoring can be used to improve achievement of target concentrations in this complex situation.

Ariano et al. (2005) Am J Kidney Dis 46 681-687

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

Sam Salman[1], Julie Hibbert[2], Madhu Page-Sharp[3], Laurens Manning[1], Karen Simmer[2,4], Dorota A. Doherty[5], SanjayPatole[2,4], Kevin T. Batty[3], Tobias Strunk[2,4]. Medical School, University of Western Australia[1], Crawley, WA, Australia; Centre for Neonatal Research and Education, University of Western Australia[2], Crawley, WA, Australia; School of Pharmacy and Biomedical Sciences, Curtin University[3], Bentley, WA, Australia; Neonatal, King Edward Memorial Hospital[4], Subiaco, WA, Australia; Division of Obstetrics and Gynaecology, University of Western Australia[5], Crawley, WA, Australia

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

427 Designed with kids in mind: Pharmacokinetic and clinical assessment of a novel, palatable paediatric oral formulation of midazolam

Sam Salman[1], Edith KY Tang[2], Laurence C Cheung[3,4,5], Minh N Nguyen[2], David Sommerfield[6], Lliana Slevin[3], Lee-Yong Lim[2], and Britta S von Ungern Sternberg[1,6]. Medical School, University of Western Australia[1], Crawley, WA, Australia; Division of Pharmacy, University of Western Australia[2], Crawley, WA, Australia; Telethon Kids Institute, University of Western Australia[3], Crawley, WA, Australia; School of Pharmacy and Biomedical Sciences, Curtin University[4], Bentley, WA, Australia; Pharmacy Dept, Perth Children's Hospital[5], Crawley, WA, Australia; Dept of Anaesthesia, Perth Children's Hospital[6], Crawley, WA, Australia;

Introduction. Midazolam is one of many bitter drugs where provision of a suitable paediatric formulation, particularly in the pre-anaesthetic setting, remains a challenge. To overcome this problem a novel chocolate-based tablet formulation has been developed with positive pre-clinical results.
Aims. To further investigate the potential of this novel, paediatric formulation of oral midazolam in the clinical setting through analysis of PK and efficacy relative to current standard of care.
Methods. Children aged 3-16 years who were prescribed midazolam as a pre-medication were randomised to receive 0.5 $mg.kg^{-1}$ either as the novel formulation or an intravenous solution given orally (current standard in the study institution). Midazolam and 1-hydroxymidazolam concentrations were determined using HPLC. Population PK of parent and metabolite were modelled simultaneously using NONMEM. Tolerability was assessed by each child, parent and nurse using a five-point facial hedonic scale and efficacy was determined by the time to onset of sedation
Results. 150 children were enrolled in the study,76 received the novel formulation and 74 received IV solution orally. The PK of midazolam and 1-hydroxymidazolam were able to be suitably modelled simultaneously. The novel formulation had a higher estimated first pass metabolism (8.6 vs 5.0 %) and a significantly lower relative bioavailability of 82.1% (p=0.013) with no other significant differences. AUC relative to dose was in the range reported for midazolam syrup. The PK differences did not influence time to effect (p=0.14), while the novel formulation had significantly improved tolerability scores from children, parents and nurses (all p<0.001).
Discussion. We conclude that the novel chocolate-based formulation of midazolam provides improved tolerability while remaining efficacious with suitable pharmacokinetics for use as a pre-medication for children.

428 A population pharmacokinetic study of benzylpenicillin following benzathine benzylpenicillin administration in children and adolescents with rheumatic heart disease: new insights for improved secondary prophylaxis strategies

Sam Salman[1], Robert Hand[2], Nelly Newall[2], Amy Baker[2], Julie Vine, Madhu Page-Sharp[4], Asha Bowen[2,5], John Joseph[6], Julie Marsh[2], James Ramsay[7], Dianne Sika-Paotonu[2,8,9,10], Kevin T. Batty[4], Laurens Manning[1], Jonathan Carapetis[2] Medical School, University of Western Australia[1], Crawley, WA, Australia; Telethon Kids Institute, University of Western Australia, Crawley, WA, Australia; HiTH, Perth Children's Hospital[3], Crawley, WA, Australia; Pharmacy and Biomedical Sciences, Curtin University[4], Bentley, WA, Australia; Infectious Diseases, Perth Children's Hospital[5], Crawley, WA, Australia, Biochemistry, PathWest[6], Crawley, WA, Australia; Children's Cardiac Centre, Perth Children's Hospital[7], Crawley, WA, Australia; Pathology & Molecular Medicine, University of Otago[8], Otago, New Zealand.

Introduction. Benzathine benzylpenicillin (BPG) is recommended every 3-4 weeks as secondary prophylaxis to prevent recurrent episodes of acute rheumatic fever and subsequent RHD, an important global health challenge. Little is known of the PK of PEN when BPG as administered to populations at high risk of RHD (Currie et al, 1994).
Aims. To develop a population PK model of PEN after BPG injection in high risk children to better define PEN concentration profiles, determine factors that influence them and use for optimizing dose regimens.
Methods. This was a longitudinal, prospective PK study of children and adolescents receiving BPG as SP. Participants were followed throughout 6 monthly cycles of BPG. Dried blood spot samples were assayed with LC-MS. PEN concentrations were analysed by non-linear mixed effects modelling using NONMEM.
Results. 18 patients contributed 256 concentrations to the analysis. High body mass index (BMI) (≥ 25 kg/m^2, n=8) almost doubled (increase of 86%) the rate limiting absorption phase. No participant had PEN concentrations above 20 µg/L, the proposed target in the literature, for the full period between intramuscular injections. Simulations from the model indicate most will only achieve this target for short time, if any, between monthly doses.
Discussion. There is a discordance between the observed PK and the reported partial efficacy of BPG to prevent ARF pointing to a major knowledge gap in the PK/PD. This should be a critical component of future research into optimising SP with BPG, revising weight-based BPG dosage regimens in children and penicillin reformulation activities.
Currie et al (1994) Antimicrob Agents Chemother 38 1203-1204

429 Suboptimal prediction of serum vancomycin concentrations with Bayesian forecasting software using a 1-compartment model in haemodialysis patients

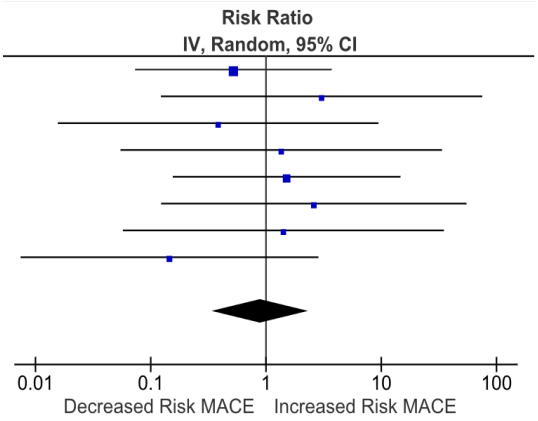
Maurizio Stefani[1,2], Darren Roberts[1,2], Department of Clinical Pharmacology and Toxicology, St. Vincent's Hospital[1], Sydney, NSW, Australia. St Vincent's Clinical School, University of New South Wales[2], Sydney, NSW, Australia.

Introduction. Vancomycin is commonly used for the treatment of infection in patients with end-stage kidney disease (ESKD). Vancomycin is predominantly excreted via the kidneys and variably eliminated by haemodialysis. A 2018 systematic review by Hui et al (2018) indicated that a low proportion of patients with ESKD achieved a target plasma vancomycin concentration with non-weight and weight-based dosing thus alternative approaches are required.
Aim. To assess if Bayesian forecasting software (DoseMeRx^{TM}) using a 1-compartment model derived from Buelga et al (2005) can assist clinicians in dosing patients on haemodialysis.
Methods. A retrospective analysis was conducted of patients from 2015 to 2017 with ESKD treated with haemodialysis and vancomycin. Pre-haemodialysis blood samples with measured serum vancomycin concentrations guided the doses administered towards the end of the haemodialysis treatment. Dosing regimens and all available serum creatinine concentrations were also collected and all data was entered into the model iteratively. Serum vancomycin concentrations predicted by DoseMeRx^{TM} were then compared to the measured concentrations.
Results. 26 patients received 34 courses of vancomycin, with 167 paired predicted and measured serum vancomycin concentrations. The median (range) number of doses per course was 4 (1 to 11). Median (range) dose was 1000 mg (250 to 2000). Mean bias was -0.54 mg/L and root mean squared error (RMSE) was 5.8 mg/L. In a subgroup of patients with serum creatinine measured within 72 hours of each vancomycin level, i.e. at each haemodialysis session, 103 paired observations were available, and mean bias and RMSE were -0.25 and 5.1 mg/L, respectively. The coefficient of variation of the vancomycin assay used ranged from 3.2 to 5%.
Discussion. Prediction of serum vancomycin levels was suboptimal in this patient population, likely secondary to violation of the assumptions underlying the model used, in particular, constant clearance.

Hui et al (2018) Int J Antimicrob Agents. 51(5):678-686.
Buelga et al (2005) Antimicrob Agents Chemother. 49(12): 4934–4941.

430 Systematic review with meta-analysis: risk of adverse cardiovascular events with proton pump inhibitors independent of clopidogrel

Riley Batchelor[1], Radya Kumar[1], Julia FM Gilmartin-Thomas[1,3], Ingrid Hopper[1], William Kemp[2], Danny Liew[1]. Department of Epidemiology and Preventive Medicine, Monash University[1], Melbourne, VIC, Australia; Department of Gastroenterology, Alfred Health[2], Melbourne, VIC, Australia; Research Department of Practice and Policy, University College London School of Pharmacy[3], London, United Kingdom.



Introduction: Clopidogrel's antiplatelet effects may be attenuated by a pharmacokinetic interaction with co-prescribed proton pump inhibitors, which inhibit oxidative pathways that convert clopidogrel into its active metabolites. Despite this, the impact of PPIs on cardiovascular risk in the absence of clopidogrel is not well defined.
Aims: To report a systematic review and meta-analysis of the association between PPIs and cardiovascular risk, independent of clopidogrel.
Methods: The databases of MEDLINE, EMBASE, CENTRAL, Scopus, Web of Science and ClinicalTrials.gov were systematically searched. The primary outcome was association between PPI monotherapy and any adverse cardiovascular event. The secondary outcome was association between proton pump inhibitor monotherapy and acute myocardial infarction. Studies were excluded if they reported or did not adjust for concomitant antiplatelet therapy.
Results: A total of 22 studies were included in the systematic review. Data from 16 studies were included in the meta-analysis. Of these, eight were randomised controlled trials. An increased risk of any adverse cardiovascular event with PPI monotherapy was observed using pooled data from observational studies (risk ratio 1.25, 95% confidence interval 1.11-1.42, I^2 81%), but not from randomised controlled trials (risk ratio 0.89, 95% confidence interval 0.34-2.33, I^2 0%).
Discussion: There is no clear evidence of an association between PPI monotherapy and increased cardiovascular risk.

431 The impact of diabetes on cardiac remodelling and the hexosamine biosynthesis pathway (HBP) in the human heart.

David M Nash. Heart Failure Pharmacology, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia; Department of Pharmacology, Monash University, Melbourne, VIC, Australia.

Introduction. Diabetes is associated with an increased risk of heart failure, independent of coronary artery disease, commonly known as diabetic cardiomyopathy (Jia et al, 2018). Studies in rodent models have shown that diabetes contributes to the up-regulation of the HBP, a minor glucose metabolic pathway which produces UDP-GlcNAc, the donor for dynamic post-translational modifications of proteins, known as O-GlcNAcylation. However, much less is known about the impact of diabetes on cardiac remodelling and the HBP in humans.
Aims. To gain an insight into 1) the extent of cardiac remodelling in the human heart in diabetes, 2) the association between the HBP/O-GlcNAcylation and diabetes, and 3) the impact of conventional glucose-lowering therapy on both cardiac remodelling and the HBP/O-GlcNAcylation in human diabetes.
Methods. Right atrial appendages (RAA) (n=54) were collected from consenting patients undergoing Coronary Artery Graft Surgery. Patients were grouped according to diabetic (D) or non-diabetic (ND) status, left ventricular ejection fraction (LVEF) >50% or <50% and whether diabetic patients were treated with metformin. RNA, protein and histological sections of human RAA were analysed for markers of cardiac remodelling and HBP/O-GlcNAcylation.
Results. Both RAA alpha-smooth muscle actin and atrial natriuretic peptide gene expression were higher in D/LVEF<50% (n=4) compared to ND/LVEF>50% (n=16, P<0.05) group. Diabetes was also associated with increased HBP/O-GlcNAcylation: total protein O-GlcNAcylation was higher in D/LVEF<50% (n=6) group compared to ND/LVEF>50% (n=20, P<0.05), while OGA protein content was lower in D (n=26) compared with ND (n=26, P<0.05). Conventional glucose-lowering therapy (metformin) (n=15) was associated with a trend for a lower expression of triggers of cardiac remodelling (NADPH oxidase and TNF-alpha), as well as expression of the key HBP enzyme, GFAT-1 (P<0.05).
Discussion. These data suggest that diabetes increased cardiac remodelling concomitantly with activation of the HBP in humans, particularly in those with a reduced ejection fraction. While these markers were less marked with the use of metformin in diabetic patients.

Jia G, Hill M, Sowers J (2018) Circ Res, 122(4): 624-638

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

Nimna Perera[1,2], Mitchel Tate[1,3], Minh Deo[1], Miles J De Blasio[1,4], Darnel Prakoso[1,4], Helen Kiriazis[1], Paul Gregorevic[1,4], Hong-Wei Qian[1,4], Marianne Tare[3], Rebecca H. Ritchie[1,3,4]. Baker Heart and Diabetes Institute[1], Melbourne, VIC; Dept. of Physiology[2] and Dept. of Medicine[3], Monash University, Clayton, VIC; University of Melbourne[4], Parkville, VIC.

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

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

Trang Tran[1,3], John Mach[1,2,3], Gizem Gemikonakli[1,2,3], Alexander Widiapradja[1,3], Scott P Levick[1,3], Susan Howlett[4], Rafael de Cabo[5], David G Le Couteur[3,6] & Sarah N Hilmer[1,2,3]. Lab of Ageing and Pharmacology, Kolling Institute, Sydney, NSW, Australia[1]. Clinical Pharmacology and Ageing, Royal North Shore Hosp, Sydney, NSW, Australia[2]. Northen Clinical School, Univ of Sydney, Sydney, NSW, Australia[3]. Dalhousie University, Halifax, Canada[4]. Translational Gerontology Branch, National Institute on Aging, Maryland, USA[5]. ANZAC Research Institute, Sydney, NSW, Australia[6].

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

434 Concatenated γ-aminobutyric acid type A receptors; creating order in chaos

Philip K Ahring[1], Vivian WY Liao[1], Han C Chua[1], Natalia M Kowal[1], Mary Chebib[1] and Thomas Balle[1]. [1]School of Pharmacy, Brain and Mind Centre, The University of Sydney, Sydney, NSW, Australia.

Introduction. The method of subunit concatenation has been used extensively in defining $GABA_AR$ stoichiometry and subunit arrangement. Theoretically, this technique allows precise experimental control at the single subunit level that would not be possible otherwise. However, while the concatenation technique is powerful, there are also caveats associated with its use. Recently, we discovered that the expression of published concatenated nicotinic acetylcholine receptor (nAChR) constructs in oocytes led to far more complex receptor pools than anticipated [1]. This was due to an ability of the linked nAChR subunits to orient themselves in both the clockwise and the counterclockwise directions.
Aim. The goal is to determine whether previously published concatenated $GABA_AR$ constructs give uniform receptor pools. Furthermore, we evaluate whether newly designed "optimized" constructs perform in a superior fashion.
Methods. Standard molecular biology techniques were utilized to build a range of dimeric and pentameric concatenated $GABA_AR$ constructs containing α1, β2 and γ2 subunits. *Xenopus* oocytes were injected with transcribed cRNA and resultant receptors were evaluated functionally using two-electrode voltage clamp methods.
Results. As expected, based on our previous work, published concatenated $GABA_AR$ constructs have the inherent ability to assemble in both the clockwise and the counterclockwise orientations. In an attempt to constrain this flexibility, we designed a range of dimeric constructs with different linker lengths. Crucially, we find that it is possible to obtain a uniform receptor expression using some of these new concatenated constructs.
Discussion. Our work imply that previous conclusions based on data from concatenated constructs need to be re-examined. Consequently, we further suggest that the science of $GABA_AR$ assembly may be less chaotic than previously proposed.

1. Ahring, P.K., V.W.Y. Liao, and T. Balle, *Concatenated nicotinic acetylcholine receptors: A gift or a curse?* J Gen Physiol, 2018.

435 Elucidating the structural basis of bias at the adenosine A_1 receptor

Anh Nguyen[1], Jo-Anne Baltos[1], Luigi Aurelio[2], Alaa Abdul-Ridha[1], Manuela Jörg[2], Leigh Ford[2], Shane Devine[2], Shane Hellyer[1], Paul White[1], Peter Scammells[2], Arthur Christopoulos[1], Lauren May[1]. Drug Discovery Biology[1] and Medicinal Chemistry[2], MIPS, Monash Univ, Parkville, VIC, Australia.

Introduction. The A_1 G Protein-coupled receptor (A_1AR) is a promising therapeutic target for the treatment of myocardial ischaemia-reperfusion injury; a condition that remains the leading cause of death and disability in Australia. Despite this, transition of A_1AR agonists into the clinic has remained elusive due to on-target adverse haemodynamic effects. We have demonstrated the utility of biased agonism in overcoming these limitations, with the biased bitopic agonist, VCP746, stimulating cardioprotection in the absence of bradycardia.
Aims. This study aimed to understand the structural basis of VCP746 bias at the A_1AR by using a combination of pharmacological, chemical and computational techniques.
Methods. Novel derivatives of VCP746 were synthesised to probe the structure-activity relationships of biased signalling, with modifications to the orthosteric pharmacophore, linker and allosteric pharmacophore. Derivatives were tested in A_1AR CHO cells to assess biased agonism and effects on isolated beating rat atria were subsequently determined. Single alanine substitutions were incorporated into the A_1AR and expressed in CHO cells to elucidate the influence of key amino acids on intracellular signalling and biased agonism. Interpretation of experimental findings were facilitated by docking VCP746 and derivatives into a recently solved active state human A_1AR (PDB ID: 6D9H).
Results. Derivatives with deletion of substituents in the allosteric pharmacophore exhibited reduction or abolishment in biased signalling. Loss of bias correlated well with effects on isolated atria, with non-biased derivatives stimulating a significant reduction in atrial beating. Alanine substitution of Glu172 and His264, residues present in extracellular loop (ECL) ECL2, significantly reduced VCP746 bias at the A_1AR. In support of this, the VCP746 binding pose within the A_1AR active structure predicted interactions between the allosteric pharmacophore and ECL2. In contrast, the allosteric pharmacophore of the non-biased VCP746 derivative was oriented towards ECL3.
Discussion. Collectively, these studies suggest that VCP746 bias results from interactions between the allosteric pharmacophore with ECL2. These findings will facilitate the rational design of future biased A_1AR compounds.

436 Investigating G protein-coupled receptor (GPCR) signalling using novel G protein biosensors

Martina Kocan[1], Adam L Valkovic[1], Maggie K Cao[1], Avanka Gunatilaka[1], Asuka Inoue[2], Daniel J Scott[1,3], Ross AD Bathgate[1,3]. Florey Instit of Neuroscience & Mental Health[1], Department of Biochemistry and Molecular Biology[3], Univ Melb, Parkville, VIC, Australia. Graduate School Pharmaceutical Sciences[2], Tohoku University, Sendai, MIYAGI, Japan.

Introduction. Recent advances in luminescence-based detection assays provide exciting new opportunities to access previously undetectable real-time GPCR signalling. Split-luciferase-based G-protein biosensors (NanoBiT-G protein assay) take advantage of the NanoBiT (NanoLuc binary technology, Promega) system and enable measurement of ligand-induced G-protein interactions with higher sensitivity as compared to recently described luciferase-based BRET sensors (Kocan et al (2017) Sci Rep 7:2968).
Aims. To utilise the NanoBiT-G protein assay to investigate ligand-induced G-protein activation of native and mutant prototype GPCRs including the neurotensin receptor 1 (NTS_1) and α_{1A}-adrenoceptor receptor (α_{1A}-AR).
Methods. HEK cells stably expressing rat NTS_1 (HEK-NTS_1) or a desensitization-incompetent mutant (HEK-NTS_1DCT) were transiently transfected with the G_q sensor components: $G\alpha_q$-LgBIT, Gβ and Gγ-SmBIT. COS-7 cells were transiently transfected with human α_{1A}-AR in addition to the G_q sensor. Cells were stimulated with NTS_1 agonist NT8-13 or α_{1A}-AR agonist A61603 and luminescence measured every 12 seconds for up to 55 mins.
Results. The NanoBiT-G_q sensor successfully detected rapid (reaching maximum within 5 mins and 10 mins, respectively) dose dependent agonist-induced real time Gq protein activation at both NTS_1 and α_{1A}-AR. Interestingly, NTS_1-Gq activation resulted in increasing luminescence signals, whereas A61603-induced α_{1A}-AR-Gq activation promoted a decrease in luminescence output over time. NTS_1 stimulated luminescence changes were transient and returned to baseline within 15-35 min following stimulation. In contrast, stimulation of NTS_1DCT resulted in sustained luminescence output over the 45min experiment. Luminescent changes after α_{1A}-AR stimulation were also transient but did not return to baseline within 55 mins following stimulation with A61603.
Discussion. The novel NanoBiT-G protein assays are excellent tools to assess real-time G-protein activation dynamics from the stimulation of any GPCR with high sensitivity. The negative A61603-induced luminescent signal may reflect traditional Gα and Gγ dissociation following α_{1A}-AR activation. However, the increase in luminescence signal induced by NTS_1 suggests G-protein association or recruitment that requires further investigation.

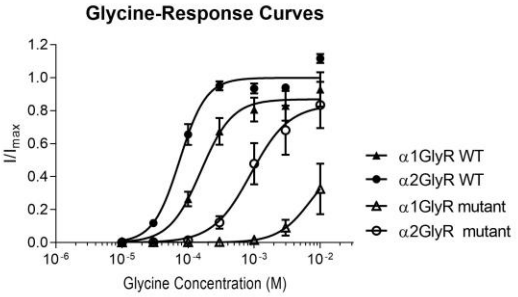
437 Expression of calcium pumps, channels and channel regulators in a model of therapy resistance in melanoma.

Chia C Chua[1], Heinz Hammerlindl[2], Melanie Robitaille[1], , Sarah J Roberts-Thomson[1], Helmut Schaider[2], Gregory R Monteith[1,3]. School of Pharmacy, University of Queensland[1], Brisbane, QLD, Australia; Diamantina Institute, University of Queensland[2], Brisbane, QLD, Australia; Mater Research Institute, University of Queensland[3], Brisbane, QLD, Australia.

Introduction. Targeted therapies for specific melanoma types, such as dabrafenib for melanomas with BRAF mutations, has revolutionised the treatment of this deadly skin cancer. However, such therapies are associated with intrinsic and acquired resistance resulting in disease relapse. This limits the long term benefits of many of these therapies. Identifying targets that when pharmacologically modulated could reverse or prevent acquired resistance is required. Specific calcium permeable ion channels and pumps can regulate therapy resistance in breast and prostate cancer, however, they have not been extensively assessed in models of melanoma resistance.
Aims. To assess the remodelling of expression of calcium pumps, channel and channel regulators in a novel model of BRAF inhibitor resistance in melanoma.
Methods. BRAF-mutated WM 164 melanoma cells were treated with sublethal concentrations of dabrafenib for up to 90 days. RNA was isolated from parental cells, induced drug tolerant cells (IDTCs) at 16 days, IDTC colonies at 45 days and resistant cells at 90 days. Levels of mRNA of calcium pumps, channels or channel regulators were assessed using a quantitative PCR method.
Results. Consistent with previous reports of significant epigenetic changes during resistance development in this model, most mRNA levels were significantly reduced compared to parental WM 164 melanoma cells. However, specific isoforms of the ORAI and transient receptor potential (TRP) channels were upregulated in IDTCs and IDTC colonies.
Discussion. Acquired resistance to BRAF inhibitors in melanoma cell may be associated with ORAI and TRP channel isoform specific mRNA increases. Further studies are required to define the potential targeting of specific ORAI and TRP channel to reverse or prevent BRAF inhibitor resistance in this and other models.

438 Towards developing a high throughput assay for Glycine receptors

Morgane Mazzarino[1], Vivian Liao[1], Nathan Absalom[1], Marika Heblinski[2], Philip Ahring[1], Mary Chebib[1]. [1] School of Pharmacy, Brain and Mind Centre. [2]Lambert Initiative for Cannabinoid Therapeutics, School of Psychology, Discipline of Pharmacology, the University of Sydney, Sydney, NSW, Australia.



Introduction. Glycine receptors (GlyRs) are members of the Cys-loop family of ion channels. Composed of homopentamers of α1/2/3 or heteropentamers of α1β, they are targets to develop agents to treat pain. One of the fastest techniques to identify lead molecules for any target is to screen molecules using a calcium-based fluorescent assay but GlyR conduct Cl^- ions.
Aim. The goal of this study is to find mutations within the selectivity filter region that would reverse the ion permeability of GlyR from a $Cl^-$to Ca^{2+} conducting channel, and so, enable us to develop a calcium fluorescent high throughput assay.
Methods. Molecular biology methods were employed to mutate the M1-M2 linker domain in α1 and α2 subunits, which was previously demonstrated [1] as crucial for ion selectivity. Mutated subunit cDNA was transcribed to mRNA and this was injected into xenopus oocytes. Effect of the mutations was assessed using two-electrode voltage clamp methods.
Results. Concentration-response curves to glycine were performed for wild-type and mutant homomeric α1 and α2 receptors. The EC_{50} values for glycine for both mutant subunits were 0.008±0.1563 M (n=6, P<0.05) for α1; 0.0009±0.0003 M (n=6, P<0.05) for α2, were significantly shifted to the right compared to WT, 1.8e-005±0.0001 M (n=7, P<0.05) for α1; 7.5e-005±7.3e-006 M (n=4, P<0.05) for α2.
Discussion. Changes in the receptor's response to glycine is most likely due to a change in conformation of the selectivity filter. Future studies will assess the current-voltage relationship to establish changes in reversal potential of the mutant receptors. Cell culture and a fluorescent assay will determine whether the mutations conduct calcium.

[1]Jensen et al (2005) Journal of Neurochemistry, 92, 962-972

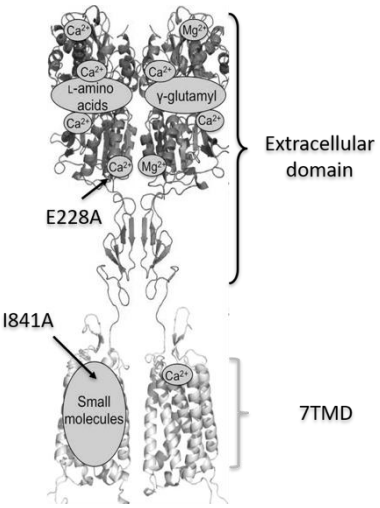
439 Characterisation of fluorescent ligands for use with NanoBRET technology.

Natasha C Dale[1,2,3], Elizabeth KM Johnstone[1,2], Angela Song[4], K Johan Rosengren[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]; School of Biomed Sci, Faculty of Med, Univ of Queensland[4], Brisbane, QLD, Australia; Dimerix Limited[5], Nedlands, WA, Australia.

Introduction. Nanoluc (Nluc) is an optimized luciferase enzyme subunit with advantageous experimental properties over previous luciferase enzymes. Along with the development of Nluc's optimized substrate furimazine, Nluc has enabled the development of the Nano-Bioluminescence Resonance Energy Transfer (NanoBRET) ligand binding assay, utilizing fluorescently-tagged ligands that themselves need to be characterised.
Aims. To characterise the pharmacology of BODIPY-tagged ligands using the NanoBRET ligand binding assay.
Methods. Fluorescently-tagged ligands were profiled for binding to Nluc-tagged receptors using the NanoBRET ligand binding assay. Ligand potency was also characterised utilizing a β-arrestin2 BRET assay.
Results. BODIPY-tagged ligands show successful binding to their respective receptors with Kd values in the nanomolar range. While some fluorescent ligands show equivalent affinity values to their untagged counterparts, others show differing affinity values from the untagged ligand. Subsequent β-arrestin2 BRET assays gave pEC_{50} values for the fluorescent ligands that are generally comparable to the untagged ligand, however for some fluorescent ligands increases in potency were observed.
Discussion. The addition of a BODIPY fluorophore to an array of ligands at times resulted in altered pharmacological profiles relative to untagged ligand. This is an important factor to consider when using fluorophore-tagged biological agents.

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

Jiayin Diao[1], Andrew Keller[1], Jane Bourke[2], Karen J Gregory[1], Katie Leach[1]. Drug Discov Biol, Monash Inst of Pharm Sci, Monash Univ[1], Parkville, VIC; Dept of Pharmacol, Monash Univ[2], Clayton, VIC.



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

441 Exploring the structural basis of biased allosteric modulation of metabotropic glutamate receptor 5

Karen J. Gregory[1], Kathy Sengmany[1], Shane D Hellyer[1], Sabine Albold[1], Andrew N. Keller[1], Katie Leach[1]. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences and Department of Pharmacology, Monash University[1], Melbourne, VIC, Australia.

Introduction. Metabotropic glutamate (mGlu) receptors mediate the activity of the major excitatory neurotransmitter glutamate and are promising target for various neurological and psychiatric disorders. Allosteric modulators that bind to sites within the 7 transmembrane-spanning domains, distinct from glutamate, offer the ability to fine-tune receptor activity and offer greater subtype. We have shown that positive allosteric modulators (PAMs) of mGlu receptor subtype 5 (mGlu5) can have differential apparent affinity or cooperativity with the same orthosteric agonist depending on the functional response measured, referred to as biased modulation (Sengmany et al., 2017).
Aims. We tested the hypothesis that allosteric modulators from different chemical scaffolds differentially engage amino acids within the common allosteric site to give rise to biased modulation.
Methods. Using stable HEK293A cell lines expressing single point mutations within the common allosteric site (Gregory et al., 2013) we assessed the pharmacology of four structurally distinct mGlu5 PAMs (DPFE, VU0409551, VU0424465, VU29). Allosteric modulation of glutamate or the surrogate orthosteric agonist DHPG was quantified using high throughput signalling assays (intracellular Ca^{2+} (iCa^{2+}) mobilisation, ERK1/2 phosphorylation and IP1 accumulation) by applying an operational model of allosterism. Allosteric modulators were docked into published mGlu5 crystal structures to aid interpretation of results.
Results. All PAMs had lower cooperativity with DHPG when measured in IP1 accumulation assays compared to iCa^{2+} mobilisation, and this was consistent across all mutations. The apparent affinities of VU0409551, DPFE and VU29 were differentially altered by mutations in a pathway dependent manner, whereas the influence of mutations on VU0424465 affinity was consistent across all assays.
Discussion. By understanding the key ligand-receptor interactions and networks that govern allosteric modulation, this study seeks to facilitate and inform future structure-based discovery of novel biased allosteric ligands with optimal therapeutic profiles.

442 Development of novel automated epifluorescence microscopy based assays for calcium signalling in breast cancer cells

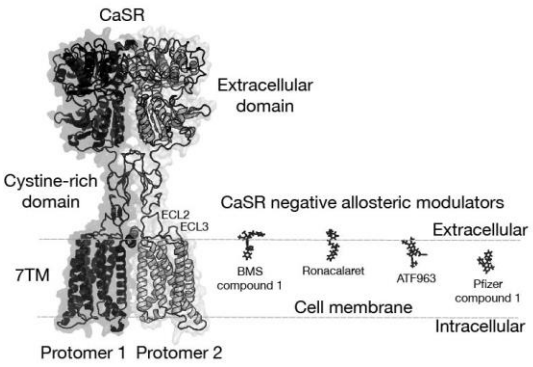
Ellen K Janke[1], John J Bassett[1], Francisco Sadras[1], Sarah J Roberts-Thomson[1], Gregory R Monteith[1,2]. School of Pharmacy, The University of Queensland[1], Brisbane, QLD, Australia; Mater Research Institute, The University of Queensland[2], Brisbane, QLD, Australia.

Introduction. Calcium signalling is a key mediator of a variety of cellular processes including proliferation and cell death, which may be deregulated in cancer. Genetically encoded Ca^{2+} indicators (GECIs) such as the GCaMP6 family of sensors enable sustained measurement of intracellular Ca^{2+}. There have been limited studies using GECIs in high throughput imaging environments, particularly in the context of assessing processes that occur in cancer cells over 6-24 hours.
Aims. To develop new methods to define calcium signalling in breast cancer cells using high-content, high-throughput automated epifluorescence microscopy and GECIs.
Methods. Breast cancer cell lines stably expressing GCaMP6m were seeded in 96-well plates. Changes in cytosolic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) levels were assessed after treatment with agents such as the purinergic receptor activator adenosine trisphosphate (ATP) and the transient receptor potential cation channel subfamily V member 4 (TRPV4) pharmacological activator GSK1016790A. Experiments were performed using an ImageXpress Micro (Molecular Devices) automated epifluorescence microscopy system with environmental control and advanced liquid handling instrumentation; the resulting data were analysed using MetaXpress software.
Results. Pronounced single cell heterogeneity in $[Ca^{2+}]_{CYT}$ was identified following treatment with most pharmacological agents. Using high-content imaging, distinct $[Ca^{2+}]_{CYT}$ changes with oncosis and apoptosis induced by GSK1016790A in MDA-MB-468 cells were identified.
Discussion. Automated epifluorescence microscopy based assays for the measurement of Ca^{2+} signalling in breast cancer cells provides new insights into the nature of changes in $[Ca^{2+}]_{CYT}$ in single cells across time scales which are relevant to crucial cellular process including proliferation and cell death.

443 Probing calcium sensing receptor negative allosteric modulator binding

Tracy M Josephs[1], Andrew N Keller[1], Elham Khajehali[1], Aaron Debono[1], Christopher J Langmead[1], Ben Capuano[1], Arthur D Conigrave[2], Irina Kufareva[3], Karen J Gregory[1], and Katie Leach[1], Drug Discovery Biology and Department of Pharmacology, Monash Institute of Pharmaceutical Sciences[1], Parkville, VIC, Australia; School of Life and Environmental Sciences, Charles Perkins Centre, University of Sydney[2], Sydney, NSW, Australia; Skaggs School of Pharmacy & Pharmaceutical Sciences, University of California[3], San Diego, CA, USA.

Introduction. Negative allosteric modulators (NAMs) that target the calcium sensing receptor (CaSR), a class C G protein-coupled receptor (GPCR), were originally developed to treat osteoporosis, but failed in human clinical trials. However, there is interest in repurposing these NAMs for the treatment of calcium handling disorders. A number of chemically and structurally distinct NAM scaffolds were discovered, but it is not known how these different scaffolds interact with the CaSR to inhibit receptor signalling in response to agonists.
Aims. To establish whether different CaSR NAM chemotypes bind to distinct CaSR allosteric binding sites.
Methods. We used a mutagenesis structure-function based approach combined with analytical pharmacology and computational modelling to probe the binding sites of four distinct NAM scaffolds (figure).

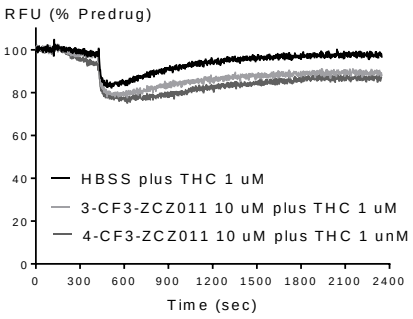


Results. We show that although all four NAM scaffolds bind to the 7 transmembrane (7TM) and/or extracellular loops (ECL), they occupy distinct regions in an extended cavity that accommodates multiple allosteric binding sites. Furthermore, different NAM scaffolds mediate negative allosteric modulation via distinct amino acid networks.
Discussion. Drugs that target distinct binding sites in the CaSR may stabilise distinct CaSR conformations, resulting in unique pharmacologies. Therefore, understanding how different NAM scaffolds bind to and inhibit the CaSR has implications for the future discovery of novel allosteric modulators in the treatment of osteoporosis and calcium handling disorders.

444 How ZCZ011 compare to its analogues on modulating and activating cannabinoid CB1 receptor

Marina Santiago[1], Shivani Sachdev[1], Mitchell Longworth[2], Michael Kassiou[2], Mark Connor[1]. Department of Biomedical Sciences[1], Macquarie University, NSW 2109, Australia; School of Chemistry[2], The University of Sydney, NSW 2006, Australia

Introduction. Allosteric modulators are promising drugs as they offer a new strategy for treating conditions in need of better treatments. ZCZ011 is a positive allosteric modulator (PAM) of CB1, thus binding at a different site to orthosteric agonists such as delta-9-tetrahydrocannabinol (THC). We previously suggested that ZCZ011 exhibits both PAM and agonist activity. By researching newly synthetised ZCZ011 analogues, we intend to determine it is possible to obtain a positive allosteric modulator without the agonist effect.



Aims. In this study we aimed to compare ZCZ011 to five analogues and determine if any of them are positive allosteric modulator without the agonist effect.

Methods. We used AtT20 FlpIn and CHO cells stably transfected with human CB1 receptors. Receptor activation was measured using FLIPR membrane potential assay in AtT20 CB1 cells. This real-time kinetics assay was also used to determine receptor signal desensitisation. Changes in intracellular calcium in CHO CB1 was obtained using Calcium 5 dye. Flexstation3 microplate reader was used to obtain fluorescence readings.

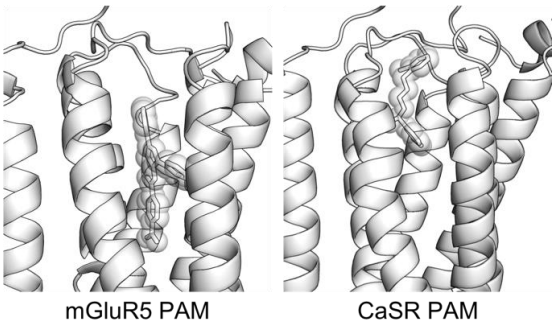
Results. Differently from ZCZ011, the analogues have very small if any agonist effect. Most drugs also lost the ability to allosteric modulate CB1 activation by CP5,940 and THC, which may indicate a decreased bind to the allosteric site. Preliminary data suggests two analogues, 3-CF3-ZCZ011 and 4-CF3-ZCZ011, have a much smaller agonist effect but can affect THC response and signalling desensitisation (figure - raw data).

Discussion. This study was able to identify ZCZ011 analogues which may be better options to use as allosteric modulator of CB1 receptor as they don't present as prominent agonist effect. Further research is needed to determine in vivo effects of this compounds with and without agonist treatment.

445 A structure-function approach to determine global and ligand-specific activation mechanisms in class C G Protein-Coupled Receptors.

Andrew N. Keller[1], Irina Kufareva[2], Tracy M. Josephs[1], Amy N.Y. Chen[1], Shane D. Hellyer[1], Jiayin Diao[1], Vyvyan T. Mai[1], Arthur Christopoulos[1], Karen J. Gregory[1], Katie Leach[1]. Monash Inst. Pharmaceut. Sci., Monash University[1], Parkville, VIC, Australia; Skaggs School of Pharm. & Pharmaceut Sci, Univ. Cali., San Diego[2], La Jolla, CA, USA

Introduction. Class C G protein-coupled receptors (GPCR) are important drug targets. They include the calcium sensing receptor (CaSR) and metabotropic glutamate receptors (mGluRs). Structurally, however, these receptors are poorly understood, hindering structure-based drug design.



Aims. To understand the structural basis of affinity and cooperativity of Class C GPCR allosteric modulators using CaSR and mGluR5 as model systems.

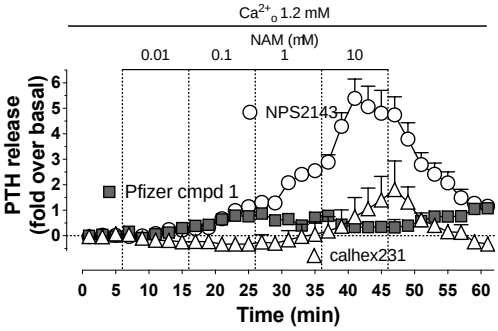
Methods. An operational model of allosterism was used to determine changes in affinity, efficacy and cooperativity of positive allosteric modulators (PAMs) and orthosteric agonists at select point mutations for the CaSR and mGluR5, based on Ca^{2+} mobilisation assays.

Results. We determined residues important for PAM affinity and cooperativity in the CaSR and mGluR5 7-transmembrane (7TM) domains and extracellular loops. These data guided *in silico* docking of PAMs, into either a homology model of the CaSR or the mGluR5 7TM crystal structure, providing a structural context for our mutagenesis studies. In both receptors, residues important for PAM affinity could be distinct from those mediating cooperativity. Moreover, select amino acids within the 7TM that included those outside of binding pockets, could influence CaSR and mGluR5 activation by both orthosteric and allosteric agonists.

Discussion. Comparisons between mGluR5 and CaSR revealed common residues mediating receptor activation and allosteric interactions within the 7TM. Our structure-functions studies are unravelling the structural elements important for activation and coupling of the class C 7TM domains.

446 Calcium sensing receptor negative allosteric modulators differentially stimulate parathyroid hormone release

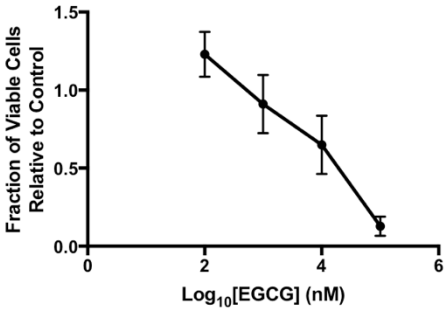
Katie Leach[1], Andrew Keller[1], Hee-Chang Mun[2], David Shackleford[1], Tracy Josephs[1], Ben Capuano[1], Arthur Conigrave[2], and Karen Gregory[1]. Monash Institute of Pharmaceutical Sciences[1], Parkville, VIC, Australia; School of Life and Environmental Sciences, Charles Perkins Centre, University of Sydney[2], NSW, Australia



Introduction. Calcium sensing receptor (CaSR) negative allosteric modulators (NAMs) were developed to stimulate transient release of parathyroid hormone (PTH) to promote bone formation in osteoporosis. However, three NAMs failed in human clinical trials, attributed to the fact they either stimulated prolonged PTH release (causing bone breakdown), stimulated insufficient PTH release, and/or had on-target CaSR effects in bone cells. Prolonged PTH release was successfully reduced by decreasing NAM half-life, and increasing potency for administration at lower doses that were metabolised more rapidly. Although on-target off-tissue effects would be difficult to circumvent, we hypothesise that insufficient PTH release may be overcome by enhancing NAM inhibition of CaSR signalling, governed by the magnitude of "negative cooperativity" with the CaSR's endogenous agonist, Ca^{2+}.
Aims. To compare the affinity and negative cooperativity of the CaSR NAMs, ronacaleret, NPS2143, ATF936, Pfizer compound 1, calhex231 and BMS compound 1, and to compare their ability to stimulate PTH release.
Methods. CaSR NAMs were evaluated in Ca^{2+}_i mobilisation and reporter gene assays in HEK293 cells, and in assays measuring PTH release from human parathyroid cells in culture.
Results. NAMs had distinct CaSR affinities and cooperativities at the CaSR. BMS compound 1, calhex231 and Pfizer compound 1 exhibited mixed positive and negative cooperativity via "biased modulation", modulation across the CaSR dimer, or an unknown mechanism, respectively. NPS2143 was the most efficacious NAM for stimulation of PTH release.
Discussion. There remains a need to understand allostery and CaSR-mediated signalling pathways coupled to regulation of PTH release to establish desirable NAM properties that stimulate maximum PTH secretion.

447 Effects of (-)-epigallocatechin-3-gallate on neuronal model PC12 cell differentiation: Implications for Aβ toxicity

Andrew Lesenko[1], Ian F Musgrave[1]. Dept of Pharmacol (FHMS)[1], Univ of Adelaide, Adelaide, SA, Australia.



Introduction. Accumulation of hyperphosphorylated tau, and aggregates of amyloid beta (Aβ) are characteristic of Alzheimer's disease (AD). The evidence indicative of AD being an amyloidopathy is the genetic predisposition in carriers of mutant variants of presenilin, the amyloid precursor protein, or having trisomy 21. The research undertaken pertains to the hypothesised ability of (-)-epigallocatechin-3-gallate (EGCG) to promote neuronal differentiation, and thus counteract Aβ neurotoxicity. EGCG (100μM) has been shown to be neuroprotective of neuronal model PC12 cells by inhibition of Aβ fibril formation (Harvey et al, 2011). Lower concentrations (0.1-10μM) have been demonstrated to possess a rescuing effect in serum-deprived PC12 cells undergoing intrinsic apoptosis (Reznichenko et al, 2005).
Aims. The aim of the experiment is to investigate the effects of optimised concentrations of EGCG (10^{-7}M) on PC12 cell viability and morphology in response to the induction of intrinsic apoptosis by serum starvation, and Aβ insult.
Methods. PC12 cells maintained in DMEM (5%FCS) were treated with decreasing serum, and under serum starvation with increasing EGCG concentrations; viability was determined by MTT assay. Cells were then exposed to increasing $Aβ_{1-40}$ concentrations with EGCG (0.2μM) to assess viability, and morphological responses by fluorescence imaging.
Results. EGCG treatment of serum-deprived PC12 cells shows a significant increase in the fraction of viable cells relative to an untreated control for the 100nM treatment group ($P<0.05$).
Discussion. The pro-survival signalling stimulated by low dose EGCG administration is likely a result of its protein binding properties, and this is expected to translate also to the stimulation of differentiation in PC12 cells. In turn, these events are speculated to ameliorate Aβ neurotoxicity by counteracting the induction of intrinsic apoptosis.
Harvey BS et al (2011) Food Chem 129:1729-1736.
Reznichenko L et al (2005) J of Neurochem 93:1157-1167.

448 *In vitro* synergism of Wnt inhibitor niclosamide and pyrvinium in combination with chemotherapy in colorectal cancer DLD-1 and SW620 cells

Johnson J Liu[1,2], Sonia Shastri[1,2]. Anna J Zhang[2], Nan Tian[2], Louise Nott[2], Dominic Geraghty[2], Dept of Pharmacol, School of Medical Sciences, UNSW Sydney[1], NSW; School of Medicine, Univ of Tasmania[2], Hobart, TAS[2].

Introduction. Aberrant Wnt signalling pathways play a critical role in the carcinogenesis of colorectal cancer that often carries APC and β-catenin mutations. Wnt inhibitors niclosamide (NIC) and pyrvinium (PYR) have shown preclinical anticancer activity, but their potential synergism with common chemotherapy has not been evaluated in colorectal cancer.
Aims. To determine the *in vitro* synergism between Wnt inhibitors and fluorouracil (5-FU) or irinotecan (IRI).
Methods. The synergisms between these agents were examined in human colorectal cancer DLD-1 and SW620 cells by measuring the combination index (CI) using the Chou-Talalay method and Compusyn program. mRNA of Wnt components were measured by RT-PCR, while total and active β-catenin protein levels by Western blotting.
Results. Combination of NIC and 5-FU displayed moderate synergistic effect in DLD-1 cells (CI, 0.76 ± 0.1, 0.71 ± 0.08 and 0.78 ± 0.15 at IC_{50}, IC_{70} and IC_{90}), and additive effect in SW620 cells (CI, 0.98 ± 0.11 at IC_{70}; 0.83 ± 0.2 at IC_{90}), but antagonistic effect at IC_{50} (CI, 1.25 ± 0.17). The IC_{50} value of 5-FU was reduced significantly by ~2.5-fold when combining with NIC in DLD-1 cells (P<0.05), whereas the reduction of IC_{50} in SW620 cells was not significant. Combination of NIC and IRI displayed synergistic effect in DLD-1 cells (CI, 0.84 ± 0.12 when Fa = 0.5), but antagonistic effect in SW620 cells (CI, 1.32 ± 0.11 at Fa = 0.5) (Fa, fraction affected). The IC_{50} of IRI was reduced significantly by ~2.0-fold in DLD-1, whereas increased by ~1.2-fold in SW620 cells. Combination of PYR with 5-FU or IRI displayed synergism or additive effect in DLD-1 cells. Wnt pathway components dvl1, LRP6, CTNNB1 and fzd7 were highly expressed in DLD-1 cells than SW620 cells. NIC and PYR caused reduction of total β-catenin and active β-catenin in SW620 cells, but not in DLD-1 cells after a 48-hour incubation at 0.1 to 1.0 μM.
Discussion. Combination of Wnt inhibitors NIC and PYR with chemotherapeutic drugs demonstrates cell type-specific synergisms, which could be attributable to the differential expression of Wnt components among different colorectal cancer cell lines. Supported by Royal Hobart Hospital Research Foundation and Cancer Council Tasmania.

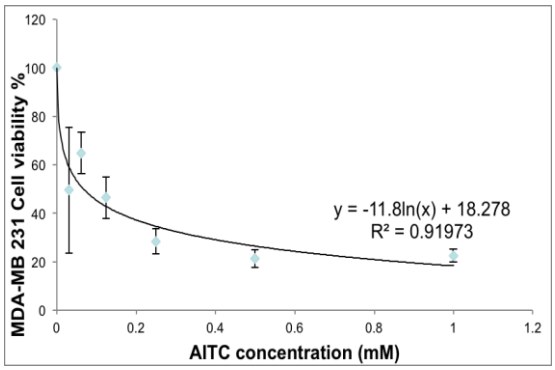
449 Exploring subtype differences in α_1-adrenoceptor ligand residence time

Samantha A Miles, Sean S So, Renate Griffith, Angela M Finch. School of Medical Sciences, UNSW Australia, Sydney, NSW.

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

450 Inhibition of human breast cancer cells MCF-7 and MDA-MB-231 growth and proliferation by allyl isothiocyanate from cruciferous vegetables

Suong NT Ngo[1], Sabah Butt[1], Robyn Meech[2]. School of Animal and Veterinary Sciences, The University of Adelaide[1], Adelaide, SA, Australia. College of Medicine and Public Health, Flinders University[2], Adelaide, SA, Australia.



Introduction. Cruciferous vegetables are a potent source of isothiocyanates. Allyl-isothiocyanate in particular has shown to be effective towards various cancers, however there is limited information at present regarding its anti-tumour effects in human breast adenocarcinoma cells.
Aims. The aim of this study was to examine whether allyl-isothiocyanate obtains anti-proliferative effects against human breast cancer cell lines MCF-7 and MDA-MB-231 and more specifically, whether it can inhibit self-renewal or indeed kill the breast cancer stem cell population within these lines.
Methods. Two main approaches were employed. They include crystal violet and mammospheres assays.
Results. Results indicate that allyl-isothiocyanate reduced cell proliferation MCF-7 line #1, #32 and MDA.MB.231 using crystal violet, producing IC_{50}s of 0.5, 0.75 and 0.4 mM, respectively. Allyl-isothiocyanate also found to reduce sphere size and number within a mammospheres assay, indicating that mechanisms involved in cell proliferation were blocked, and the stem cells were targeted. EGFP expression in the mammospheres formed by these transgenic breast cancer lines was also assessed an additional indication of the proportion of stem cells present, however it's unclear whether this assay provided a reliable indication of the effect of allyl-isothiocyanate on the cancer stem cell population.
Discussion. It is reasonable to conclude that AITC has potential for use as a chemotherapeutic agent, inhibiting progression, including proposed cancer stem cell-dependent behaviours such as relapse and metastasis, of human breast cancer by potentially targeting the stem cells within the breast cancer cell population.

451 Measurement of ligand-receptor interactions and internalisation of the CXCR4 chemokine receptor using high affinity Nanoluciferase complementation

C. W. White[1,2], K. D. Pfleger[2,3], S. J. Hill[1,2]. University of Nottingham[1], Nottingham, United Kingdom; Harry Perkins Institute of Medical Research[2], Perth, WA Australia; Dimerix Ltd[3], Nedlands, WA, Australia.

Introduction: The Nanoluciferase Nluc has been used to investigate many aspects of cell signalling including protein-protein interactions and ligand binding (1,2). Recently the Nluc has been engineered as two high affinity fragments HiBiT and LgBiT, which allow protein interactions to be investigated by luciferase complementation (3).
Aim: To investigate if the high affinity NanoBiT system could be configured to investigate ligand binding by NanoBRET and receptor internalisation using HiBiT-tagged CXCR4 chemokine receptors.
Methods: HEK293 cells were generated to stably overexpress CXCR4 tagged on the N-terminus with HiBiT or CRISPR/Cas9 engineered to express HiBiT/CXCR4 under endogenous promotion. For NanoBRET competition ligand binding studies, cells containing HiBiT/CXCR4 were incubated for 1 hour with CXCL12-AF647 (12.5nM) in the absence or presence of unlabelled ligands. For internalisation assays cells were incubated with unlabelled ligands only. Luminescence was generated by addition of cell impermeable LgBiT (10nM) and furimazine. Light emissions were measured using a PHERAstar plate reader (BMG).
Results: In NanoBRET competition ligand binding assays the pIC50 values of unlabelled AMD3100 and CXCL12 were determined using cells expressing transgenic or genome-edited HiBiT/CXCR4. In internalization experiments application of CXCL12 to cells expressing transgenic HiBiT/CXCR4 resulted in a concentration dependent decrease in luminescence suggestive of internalisation. However, the CXCR4 antagonists AMD3100 and IT1t resulted in a concentration dependent increase in luminescence indicating an increase in cell surface expression. This increase in luminescence by AMD3100 and IT1t was also seen in cells expressing genome-edited HiBiT/CXCR4 cells and in cells where endogenous CXCL12 had been knockout out.
Discussion: Cell surface expression and NanoBRET ligand binding can be investigated using HiBiT tagged CXCR4 receptors. However, in end point assays using HiBiT tagged receptors, constitutive trafficking and receptor recycling back to the plasma membrane should be considered when interpreting ligand-induced internalisation.
References: 1. Machleidt, T. et al. (2015) ACS Chem. Biol. 10: 1797–1804, 2. Stoddart LA et al. (2015). Nat Methods. 12: 661–663. 3. Dixon AS et al. (2016). ACS Chem Biol. 11:400-408.

452 Upregulation of cyclooxygenase-2 (COX-2) in MDA-MB-231 breast cancer cells by the arylurea-fatty acid CTU.

Md Khalilur Rahman[1], Tristan Rawling[2], Charlotte Smith[1], Yassir Al-Zubaidi[1], Hassan Choucair[1], Bala Umashankar[1], Kirsi Bourget[1], Yongjuan Chen[1], and Michael Murray[1]. Discipline of Pharmacology[1], University of Sydney; School of Mathematical and Physical Sciences, UTS[2], NSW, Australia.

Introduction: We identified an aryl-substituted fatty acid analogue, termed CTU, with selective cytotoxic actions in triple-negative breast cancer cells in vitro and in vivo (Rawling et al., 2017). More recently, CTU was found to elicit cell death by activation of endoplasmic reticulum (ER)-stress. In this study, we further characterised the cellular consequences of ER-stress activation by CTU.
Methods: mRNA-Seq expression profiling was conducted in CTU-treated MDA-MB-231 cells along with Ingenuity Pathway Analysis. Gene expression changes were corroborated by quantitative PCR and immunoblotting.
Results: Apart from ER-stress, a major regulated process in CTU-treated cells was activation of NF-κB and p38 MAP kinase signalling. COX-2 expression was strongly upregulated at the RNA level (to 230 fold of control; $p<0.01$) and at the protein level (to 80 fold of control; $p<0.05$) in cells treated with CTU (10 μM) for 24 hr. Co-treatment with an inhibitor of the IRE1α ER-stress pathway prevented the activation of COX-2 expression and impaired NF-κB and p38 MAP kinase signalling, as reflected by a decrease in phospho-p65 and phospho-p38 MAP kinase expression from that effected by CTU. Inhibitors of the IκB and p38 MAP kinases attenuated CTU-dependent activation of immunoreactive COX-2 expression.
Conclusion: Taken together, the present findings suggest that CTU upregulates COX-2 expression in MDA-MB-231 breast cancer cells by activating the IRE1α arm of the ER-stress mechanism, followed by activation of the p38-MAPK and NF-κB pathways. The optimal clinical use of CTU may involve a combination strategy that includes modulators of down-stream p38-MAPK and NF-κB signalling pathways.

Rawling T et al (2017) J Med Chem 69:8661-8666.

453 Cancer associated fibroblasts undergo a calcium channel switch

Francisco Sadras[1], Teneale A Stewart[2], Melanie Robitaille[1], Priyakshi Kalita-de Croft[3] Patsy Soon[4], Jodi M Saunus[3], Sunil R Lakhani[3,5], Sarah J Roberts-Thomson[1], Gregory R Monteith[1,2]. School of Pharmacy, The University of Queensland[1], Brisbane, QLD, Australia; Mater Research, Translational Research Institute, The University of Queensland[2], Brisbane, QLD, Australia; Faculty of Medicine, The University of Queensland, Centre for Clinical Research[3], Herston, QLD, Australia; South Western Sydney Clinical School, Bankstown Hospital, University of New South Wales[4], Bankstown, NSW, Australia; Pathology Queensland, The Royal Brisbane and Women's Hospital[5], Herston, QLD, Australia.

Introduction. Calcium (Ca^{2+}) signalling is remodelled in breast cancer cells and specific Ca^{2+} channels and pumps are potential therapeutic targets in breast cancer. Increasingly, research in cancer progression is focussing on the role of the tumour microenvironment, including cancer-associated fibroblasts (CAFs). However, whether these activated fibroblasts undergo Ca^{2+} signal remodelling during cancer progression is unknown.
Aim. To define the role of Ca^{2+} signalling in the induction of breast cancer CAFs.
Methods. Ca^{2+} channel mRNA levels in paired normal and CAF patient samples were assessed using qRT-PCR. Additionally, immortalised human mammary fibroblasts (HMF3S) cells were treated with transforming growth factor beta (TGFβ) to induce the alpha smooth muscle actin (αSMA)-positive CAF phenotype (HMF3S-CAF). Ca^{2+} channel mRNA levels and store-operated Ca^{2+} entry (SOCE) in HMF3S-CAF and HMF3S were compared using qRT-PCR and a Fluorescence Imaging Plate Reader. Pharmacological modulators of specific Ca^{2+} channels were used in combination with TGFβ to evaluate their ability to inhibit HMF3S-CAF induction.
Results. We identified substantial remodelling of Ca^{2+} channels in both the clinical samples and *in vitro* model, featuring induction of voltage-gated Ca^{2+} channel (VGCC) family members and downregulation of SOCE mRNA. Consistent with this, functional assays showed a significant decrease in SOCE in HMF3S-CAF compared to HMF3S cells. Finally, we found that treatment with nimodipine or ML218 significantly decreased TGFβ-mediated CAF activation.
Discussion. Ca^{2+} signalling appears to be remodelled in CAFs. Targeting specific Ca^{2+} channels could interfere with CAF induction and metabolic support of breast tumour cells. Currently, we are investigating these changes with immunohistochemistry on whole breast tumour sections; we also plan on performing *in vivo* experiments to assess the effect of VGCC inhibitors on tumour growth and metastasis.

454 Automated epifluorescence microscopy based imaging of breast cancer cells with fixed cellular geometry

Choon Leng So[1], John J. Bassett[1], Nam-Trung Nguyen[2], Sarah J Roberts-Thomson[1], Gregory R Monteith[1,3]. School of Pharmacy, The University of Queensland[1], Brisbane, QLD, Australia; Queensland Micro and Nanotechnology Centre, Griffith University[2], Brisbane, QLD, Australia; Mater Research Institute, The University of Queensland, Translational Research Institute[3], Brisbane, QLD, Australia.

Introduction. Cells must adopt specific shapes to perform many key physiological functions. The morphologies of cancer cells are correlated with invasive markers and changes in cell shape may alter the nature of responses to external stimuli such as growth factors. However, assessment of cellular signals in spatially defined cancer cells is challenging and it has not been widely applied to high throughput methods such as automated epifluorescence microscopy. Microplate based assays may help define the role of key processes related to changes in cancer cell shape and could be applied to high throughput screening for the identification of new agents that may act through pathways involved in cellular morphology.
Aims. To establish an automated epifluorescence microscopy method for the assessment of calcium signals in MDA-MB-231 breast cancer cells.
Methods. MDA-MB-231 cells stably expressing a genetically encoded calcium indicator (GCaMP6m) were cultured on 96-well plates with fixed geometry cell culture surfaces. Fluorescence intensity from GCaMP6m was assessed using an ImageXpress™ epifluorescence microscopy system.
Results. MDA-MB-231 cells exhibited concentration dependent cytosolic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) responses to external stimuli in all cellular geometries. The data generated was suited to automated assessment of $[Ca^{2+}]_{CYT}$ in 96-well plates with a temporal resolution appropriate for assessment of $[Ca^{2+}]_{CYT}$ waves.
Discussion. Microplates with cell surface micropatterning of cell culture surfaces offer an opportunity to define new aspects of Ca^{2+} signalling in cancer cells and may have novel applications for high throughput screening in cancer drug discovery.

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455 ZST316, a novel dimethylarginine dimethylaminohydrolase-1 (DDAH1) inhibitor, reduces angiogenic capacity in cultured endothelial cells

Negara Tajbakhsh[1], Elke M. Sokoya[2], Sara Tommasi[1], Arduino A. Mangoni[1], Clinical Pharmacology[1] and Human Physiology[2], College of Medicine and Public Health, Flinders University, Adelaide, SA, Australia.

Introduction. Enhanced angiogenesis is a critical step in the establishment and progression of solid tumour vascularisation and metastases. Anti-angiogenic therapy has therefore become a potentially attractive approach for cancer treatment. Nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) has been shown to be a potent angiogenic factor. Asymmetric dimethylarginine (ADMA) and L-monomethylarginine (L-NMMA) are endogenous inhibitors of eNOS that are mainly metabolised by dimethylarginine dimethylaminohydrolase-1 (DDAH1). Our group has recently synthesised a potent DDAH1 inhibitor, ZST316.
Aim. To assess the anti-angiogenic potential of the novel DDAH1 inhibitor, ZST316, in cultured endothelial cells.
Methods. *VeraVec*™ HUVECs were treated for 18 h with either ZST316 (5µmol/L; n=6), ZST316 (100µmol/L; n=6), or vehicle (water; n=6). Cell viability was assessed using the x-CELLigence® system (n=6). Immunoblotting was performed to measure DDAH1 and eNOS protein expression. Endothelial capillary-like structures were measured as an index of angiogenesis (n=5).
Results. DDAH1 protein expression was confirmed in *VeraVec*s. X-CELLigence® results revealed no ZST316-mediated cytotoxicity. ZST316 (5µmol/L and 100µmol/L) significantly reduced tube formation (26±5% and 25±4%, respectively) and loops (9±7% and 26±7%, respectively) relative to vehicle-treated cells ($P<0.05$, one-way ANOVA). In cells treated with 5µmol/L ZST316, there was a significant downregulation of eNOS protein expression (37±10%; $P<0.05$, one-way ANOVA), which was not observed in the presence of 100µmol/L ZST316.
Discussion. Our studies have shown that pharmacological inhibition of DDAH1 by ZST316 attenuates both endothelial angiogenic capacity and eNOS protein expression. Further studies are needed to establish whether the ZST316-mediated decrease in endothelial angiogenic capacity is facilitated by eNOS inhibition.

456 The AKT activator SC79 promotes Ca^{2+} influx in MDA-MB-231 breast cancer cells

Yin Y Tan[1], Alice H Bong[1], Sarah J Roberts-Thomson[1], Gregory R Monteith[1,2]. School of Pharmacy, University of Queensland[1], Brisbane, QLD, Australia; Mater Research Institute, University of Queensland[2], Brisbane, QLD, Australia.

Introduction. Breast cancers can be associated with the loss of activity of phosphatase and tensin homolog (PTEN) through mutations in the PTEN gene. PTEN loss is often associated with increased activity of the serine-threonine protein kinase AKT, as reflected in increased phosphorylated AKT (pAKT) levels. Enhanced calcium (Ca^{2+}) influx through specific pathways can enhance breast cancer cell proliferation and/or invasiveness. Recent studies in our laboratory have shown that PTEN silencing promotes Ca^{2+} influx in MDA-MB-231 breast cancer cells, however, the role of pAKT in this augmentation of Ca^{2+} influx in breast cancer cells is still unclear.
Aims. To assess the consequences of pharmacological activation of AKT on basal Ca^{2+} influx in MDA-MB-231 breast cancer cells.
Methods. The effects of the AKT pharmacological activator SC79 on pAKT and total AKT levels was assessed using immunoblotting. To assess the effect of SC79 on Ca^{2+} influx, MDA-MB-231 cells stably-expressing the genetically-encoded Ca^{2+} indicator GCaMP6m was used. Cells were pre-incubated in Ca^{2+} free buffer and Ca^{2+} influx was assessed -by the re-addition of extracellular Ca^{2+} (1.8 mM) and imaged using a fluorescence imaging plate reader (FLIPR).
Results. SC79 (30 μM) promoted the phosphorylation of AKT after 1 h incubation in MDA-MB-231 cells. SC79 significantly increased basal Ca^{2+} influx in MDA-MB-231 cells with 1 h (30 μM and 10 μM) and 24 h incubation (30 μM).
Discussion. These studies suggest that pharmacological activation of AKT phenocopies the effects of PTEN silencing on basal Ca^{2+} influx in MDA-MB-231 breast cancer cells. Further studies are required to determine how the loss of PTEN may contribute to tumorigenic pathways in breast cancer cells as a result of AKT-dependent enhancement of Ca^{2+} influx pathways.

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

Bui San Thai[1], Elizabeth A Vecchio[1,2], Dana S Hutchinson[1], Anh TN Nguyen[1], Paul J White[1], Lauren T May[1]. Drug Discovery Biology, Monash Institute of Pharmaceutical Science, Monash University[1], Parkville, VIC, Australia. Heart Failure Pharmacology, Baker Heart and Diabetes Institute[2], Melbourne, VIC, Australia.

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

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458 Investigating potential functional crosstalk of co-located metabotropic glutamate receptor 5 and adenosine A_1 receptors in primary neurons and glia

Phuc NH Trinh[1], Lauren T. May[1], Katie Leach[1] & Karen J. Gregory[1]. Drug Discovery Biology, Monash Inst Pharm Sci, Monash Univ[1], Parkville, VIC.

Introduction. Alzheimer's disease (AD) is the most prevalent cause of dementia in the elderly. Reactive astrocytes and activated microglia are associated with two AD pathological hallmarks: amyloid β plaques and neurofibrillary tangles. Emerging evidence suggests metabotropic glutamate receptor 5 (mGlu5) inhibition (Hamilton et al, 2016) and adenosine A_1 receptor (A_1R) activation (Angulo et al, 2003) reduces Aβ deposition and rescues memory deficits in animal models. Thus, mGlu5 and A_1R are promising therapeutic targets. However, mGlu5 and A_1R pharmacology is poorly characterised. Our preliminary data suggests mGlu5 and A_1R functionally interact in neurons.
Aims. We tested the hypothesis that mGlu5 and A_1R functionally interact in healthy neurons and glia, which is altered in AD pathological context.
Methods. Immunopanning and shake-off methods were employed to culture resting and activated forms of primary astrocytes and microglia. Gene and protein expression of mGlu5 and A_1R were confirmed by using qRT-PCR and western blotting. The purity of astrocytes and microglia cultures was assessed by immunocytochemistry with an Operetta High-Content Imaging System. Intracellular calcium mobilization (iCa^{2+}) was used to interrogate pharmacology and functional interactions in primary neurons and glia.
Results. mGlu5 and A_1R were detected in both astrocytes and microglia cultures for up to 6 days, with minimal levels of other adenosine receptor subtypes. VU04024465 (mGlu5 selective allosteric agonist) and MeCCPA, (A_1R selective agonist) mobilize iCa^{2+} in glia. Co-addition of MeCCPA significantly increased the maximal response to VU04024465 in neurons.
Discussion. Functional mGlu5 and A_1R are present in astrocytes and microglia. Coincident activation of A_1R enhances $mGlu_5$ activity in neurons. Future studies will investigate the effect of coincident activation and modulation of A_1R on mGlu5-mediated signalling in neurons and glia and determine molecular mechanisms governing A_1R/mGlu5 crosstalk.
Angulo E et al (2003) Brain Pathol 13:440-451; Hamilton A et al (2016) Cell Rep 15:1859-1865

459 Exploration of tricyclic TSPO ligands to overcome binding sensitivity to the A147T TSPO polymorphism

Eryn L Werry*[1], Renee Sokias*[2], Hei Wun Cheng[1], Tristan A. Reekie[2] and Michael Kassiou[2]. Faculty of Medicine & Health[1], and School of Chemistry[2] The University of Sydney, NSW, Australia

Introduction. The 18 kDa translocator protein (TSPO) is a target for development of diagnostic imaging agents for neuroinflammation. Clinical translation of TSPO imaging agents has been hindered by the presence of a polymorphism, rs6971, resulting in substitution of alanine for threonine at amino acid residue 147 (A147T TSPO). All disclosed second-generation TSPO ligands lose affinity at A147T TSPO, and efforts to develop a ligand that binds with similarly high affinity to TSPO WT and A147T have been hampered by a lack of knowledge about how ligand structure differentially influences interaction with the two forms of TSPO.
Aims. This study aims to explore how modifications of an *N*-*N*-disubstituted acetamide scaffold on dibenzodiazepine, pryazolobenzodiazepine, carbazole and pyrazoloindole head groups influence affinity at both TSPO forms.
Methods. Human embryonic kidney 293T cell lines stably over-expressing human WT and A147T TSPO were established, and the affinity of 16 *N*-alkylated acetamide derivatives were determined by competition radioligand binding using [^{3}H]-PK 11195.
Results. All ligands, apart from the *N*-*N*-dimethyl derivatives, bound to both TSPO forms. *N*-benzyl-*N*-methyl derivatives showed highest affinity to both WT and A147T TSPO, apart from within the dibenzodiazepine class. *N*-benzyl-*N*-methyl derivatives also showed the least amount of discrimination to A147T TSPO. In particular, the *N*-benzyl-*N*-methyl pyrazoloindole displayed high and equal affinity to both TSPO types (WT TSPO Ki: 10 ± 3 nM; A147T TSPO 11 ± 2 nM).
Discussion. Although some lipophilic bulk on *N*-alkylated acetamide tricyclic scaffolds is needed to ensure binding to WT and A147T TSPO, lowered discrimination at A147T TSPO is seen with *N*-benzyl-*N*-methyl derivatives, compared to *N*-*N*-diethyl and *N*-*N*-benzyl-*N*-ethyl derivatives.
*Equal first-authors

460 Mechanistic population in vitro-in vivo pharmacokinetic model of the effect of omeprazole on itraconazole pharmacokinetics

Ahmad Y Abuhelwa[1], Stuart J Mudge[2], Richard N Upton[1], David J.R Foster[1]. School of Pharmacy and Medical Sciences, University of South Australia[1], Adelaide, SA, Australia; Mayne Pharma International[2], Adelaide, SA, Australia.

Introduction. Sporanox and SUBA-itraconazole are two itraconazole (ICZ) oral capsule formulations each employing different formulation strategies to address the solubility limitation of ICZ. Drug-drug interactions of omeprazole, a proton pump inhibitor, have been reported in the literature. However, mechanistic understanding of the pharmacological and physiological interactions of omeprazole with orally-administered ICZ within a population modelling paradigm is lacking.
Aims. To mechanistically describe and quantify the effect of omeprazole on the pharmacokinetics of ICZ and its major metabolite, hydroxyitraconazole (HICZ), from the SUBA-itraconazole and Sporanox formulations of ICZ.
Methods. An in vitro-in vivo (IVIV) pharmacokinetic model of ICZ and HICZ was developed including data from an omeprazole interaction study with SUBA-itraconazole. Meta-models of gastric pH for healthy subjects and subjects receiving omeprazole was integrated into the IVIV model to capture omeprazole mediated gastric pH changes on ICZ dissolution and absorption.
Results. Omeprazole influenced the kinetics of itraconazole through: (1) altering the dissolution and absorption due to the pH-dependent solubility of itraconazole (2) and inhibition of efflux transporters; and (3) inhibiting the metabolism of ICZ and HICZ. Model predicted population effects of omeprazole on ICZ from SUBA-itraconazole was to increase $AUC_{0\text{-}24}$ and C_{max} by 35 and 31%, respectively, and to decrease $AUC_{0\text{-}24}$ and C_{max} from Sporanox by 68 and 76%, respectively.
Discussion. Unlike SUBA-itraconazole, which requires basic pH for ICZ release from the polymeric matrix, Sporanox dissolution is significantly reduced at higher gastric pH. The omeprazole-induced pH-mediated reduction in dissolution and absorption from Sporanox overrides any increased exposure from the drug-drug interaction at hepatic metabolizing enzymes or efflux transporters, resulting in an overall reduced exposure. The model herein is the most complete quantitative description of the clinical pharmacokinetics of ICZ and HICZ currently available.

461 Physiologically-based pharmacokinetic modelling of hyperforin to predict drug interactions with St John's wort

Jeffry Adiwidjaja[1], Alan V Boddy[2] & Andrew J McLachlan[1]. Sydney Pharmacy School, Univ of Sydney[1], Sydney, NSW, Australia; School of Pharmacy and Medical Sciences, Univ of South Australia[2], Adelaide, SA, Australia.

Introduction. Herb-drug interactions with St John's wort (SJW) have been well recorded in many clinical studies (Chrubasik-Hausmann et al, 2018). A physiologically-based pharmacokinetic (PBPK) model of hyperforin, the constituent of SJW responsible for interactions, will provide a greater insight into SJW interactions and allow prediction of the extent of interactions with SJW.
Aims. The aim of this study was to build and evaluate a PBPK model to predict SJW drug interactions based on previous knowledge of hyperforin pharmacokinetics.
Methods. A PBPK model of hyperforin accounting for the induction of cytochrome P450 (CYP)3A, 2C9 and 2C19 was developed in Simcyp Simulator v.17 and verified using published clinically-observed pharmacokinetic data. The predictive performance of this model was evaluated across a range of CYP substrates.

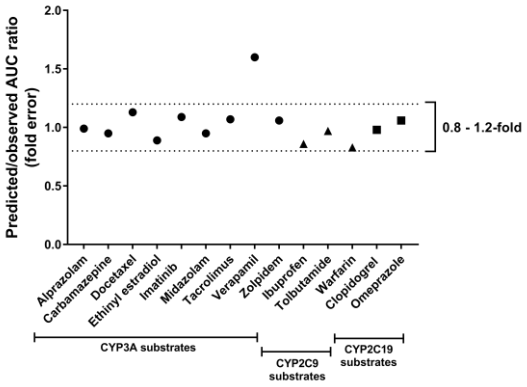


Figure 1

Results. The verified PBPK model predicted the change of drug exposure due to the induction by SJW (expressed as AUC ratio) within 0.8 to 1.2-fold of that reported in clinical studies, except for verapamil (Figure 1). The simulation indicates that the unbound concentration of hyperforin in the liver is far lower than in gut (enterocytes).
Discussion. The PBPK simulation suggested that the induction of intestinal CYP enzymes by hyperforin is more pronounced than the corresponding increase in liver. The PBPK model of hyperforin in this study has predictive capability for the interaction of SJW with different CYP3A, 2C9 and 2C19 substrates. The interaction of verapamil with SJW was underestimated, due likely to the lack of ABCB1 induction incorporated in the model.

Chrubasik-Hausmann S et al (2018) J Pharm Pharmacol doi: 10.1111/jphp.12858

462 Role of MRGPRX2 and evidence of biased agonism in drugs that cause non-IgE mediated mast cell activation

Nithya A Fernandopulle[1], Paul F Soeding[1,2], Graham A Mackay[1], Dept. Pharmacology & Therapeutics[1], Univ of Melbourne, Parkville, VIC, Australia; Dept. Anaesthesia & Pain Medicine[2], Royal Melbourne Hosp, Parkville, VIC, Australia

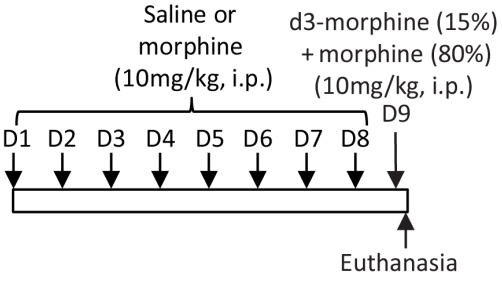
Introduction: Drug-related anaphylaxis is a growing concern with rising hospital admissions and death rates. Common drugs associated with this event include neuromuscular blocking agents (NMBAs), certain antibiotics and opioids. Symptoms of an anaphylactic reaction primarily result from the activation of mast cells followed by the release of mediators such as histamine. Whilst anaphylaxis is commonly considered as being IgE-driven, a significant proportion of these events occur in an IgE-independent manner. New research has identified the GPCR MRGPRX2 as the receptor responsible for this drug-induced, IgE-independent mechanism (McNeil et al, 2015). However, despite this, the question remains- why are only some individuals susceptible to these IgE-independent reactions*?*
Aims: This project aimed to characterise the role of MRGPRX2 in NMBA-mediated mast cell activation and investigate various components of the receptor's downstream signalling cascade.
Methods: We utilized the LAD2 wild type (WT) mast cell line that natively expresses MRGPRX2 and a comparator line where MRGPRX2 was knocked down (KD) through CRISPR cas9. The NMBAs rocuronium, vecuronium and atracurium and the well-established MRGPRX2 agonist, compound 48/80, were examined for their ability to induce degranulation (β-hexosaminidase release; CD63 expression), calcium mobilization and ERK-phosphorylation.
Results: All drugs tested caused calcium mobilization in the WT cells which was diminished in the MRGPRX2 KD cells indicating a critical role of MRGPRX2 in activation of LAD2 mast cells by NMBAs. However, not all drugs caused degranulation and ERK phosphorylation that was in keeping with their respective calcium mobilization responses. Whilst, compound 48/80 caused calcium mobilization, degranulation and strong ERK phosphorylation, NMBAs produced little to no degranulation and minimal ERK phosphorylation.
Discussion: Our data suggests some evidence for the presence of biased agonism in MRGPRX2 where different ligands of the same receptor promulgated different signalling pathways, only some of which resulted in productive cell degranulation. Further research is required to identify factors that influence an individual's susceptibility to NMBAs.

McNeil BD et al (2015) Nature 519:237-41.

463 Stable isotope-labelled morphine to study in vivo central and peripheral morphine glucuronidation and brain transport in tolerant mice

Yannick Goumon[1,2], Ivan Weinsanto[1], Jinane Mouheiche[1], AlexisLaux-Biehlmann[1], Marie-Odile Parat[3], CNRS UPR3212[1], Strasbourg, Bas-Rhin, France; Mass Spectrometry Facilities of the CNRS UPR3212[2], Strasbourg, Bas-Rhin, France; School of Pharmacy, University of Queensland[3], Woolloongabba, Australia.

Introduction. Chronic treatments can have a major impact on metabolic enzymes adaptations. Morphine metabolism in the liver has been extensively studied but morphine metabolic processes in the central nervous system (CNS) are poorly characterised. Long-term morphine treatment is limited by the development of tolerance.
Aims. Whether or not morphine analgesic tolerance affects *in vivo* brain morphine metabolism and blood-brain barrier (BBB) permeability remains a pending question. Our aim was to characterise the *in vivo* metabolism and BBB permeability of morphine after a chronic treatment.



Methods. Mice were injected i.p. with morphine or saline solution for 8 consecutive days to induce morphine analgesic tolerance. On the ninth day, both groups received a final injection of a mix of 85% morphine and 15% of d3-morphine (bearing three ^{2}H; w/w). Blood, urine, brain and liver samples were collected and LC-MS/MS was used to quantify morphine, its metabolite morphine-3-glucuronide (M3G) and their respective d3-labelled counterparts.
Results. We found no significant differences in morphine CNS uptake and metabolism between control and tolerant mice. Interestingly, d3-morphine metabolism was decreased compared to morphine without any interference with our study.
Discussion. Our data suggests that tolerance to the analgesic effects of morphine was not linked to increased glucuronidation to M3G or to altered global BBB permeability of morphine.

Weinsanto I et al. (2018) Br J Pharmacol. doi: 10.1111/bph.14454

464 The magnitude and time-course of hepatic and intestinal Cytochrome P450 3A4 induction and de-induction

Asha Kapetas[1], Andrew Rowland[1]. Clinical Pharmacology, Flinders University[1], Adelaide, SA, Australia.

Introduction. Cytochrome P450 (CYP) 3A4 is the drug metabolising enzyme of greatest clinical importance as it plays a major role in the clearance of more than 30% of clinically used small molecule drugs. Consistent with this important role, CYP3A4 is abundantly expressed in the liver and throughout the intestine with the exception of the colon, accounting for ~10% and >75% of total CYP expression in these organs, respectively. Metabolic drug-drug interactions (DDIs) that cause induction of CYP3A4 activity are an important source of variability in exposure for drugs metabolised by this enzyme. The characteristics of an induction DDI that determine the clinical importance are the magnitude of induction, and the induction and de-induction time-courses.
Aims. This study sought to characterise the magnitude and variability in induction of hepatic and intestinal CYP3A4 protein expression by rifampin. The time course of induction and de-induction as also evaluated.
Methods. A Simcyp model describing rifampin induction of intestinal and hepatic CYP3A4 was verified using reported *in vivo* data. This model was used to define a clinical study design to assess induction of intestinal and hepatic CYP3A4 by rifampin, and to evaluate the impact of participant characteristics including gender and ethnicity. Pre-specified criteria defining maximal induction and de-induction time-courses were >90% induction in at least 90% of participants for induction, and at least 90% of participants returning to within 20% of baseline activity for de-induction.
Results. The maximal induction of intestinal CYP3A4 (9.5-fold) was almost double that of hepatic CYP3A4 (5.5-fold). For both intestinal (10.5- versus 8.6- fold) and hepatic (6.1- versus 5.0- fold), the magnitude of induction was significantly higher in males compared to females ($P < 0.010$). Maximal induction of intestinal and hepatic CYP3A4 was achieved in > 90% of study participants within 5 and 10 days, respectively. Fourteen days after cessation of rifampin dosing induction of hepatic CYP3A4 persisted in 18% of participants. Intestinal CYP3A4 expression returned to baseline within 8 days in all participants. Differences in induction time course between males and females were not significant.
Discussion. Maximal induction of intestinal CYP3A4 is achieved faster than that of hepatic CYP3A4. In order to assess maximal induction of hepatic CYP3A4, rifampin (600mg daily) should be dosed for at least 10 days. Induction of hepatic CYP3A4 persists for greater than 7 days in more than 50% of healthy individuals. Simulations revealed a significant gender difference in the magnitude, but not time-course of CYP3A4 induction.

465 Quantification of serum levels of 8 drugs (11 metabolites) commonly taken by older people with polypharmacy

John Mach[1,2,3], Xiao Suo Wang[4] & Sarah N Hilmer[1,2,3]. Kolling Institute, Sydney, NSW[1]. Royal North Shore Hosp, Sydney, NSW[2]. Univ of Sydney, Sydney, NSW[3]. Laboratory of Ageing and Pharmacology, Kolling Institute, Sydney, NSW, Australia [1]. Clinical Pharmacology and Ageing, Royal North Shore Hospital, Sydney, NSW, Australia. Northern Clinical School, Univ of Sydney, Sydney, NSW, Australia [3]. Bosch Mass Spectrometry Facility, Bosch Institute, Univ of Sydney, Sydney, NSW, Australia [4].

Introduction. Polypharmacy (use of ≥ 5 drugs) is common in older people but has minimal pre-clinical or clinical evidence of safety or efficacy and is associated with adverse outcomes in older people. An efficient and sensitive method to measure multiple serum drugs and metabolites could inform drug dosing in the setting of polypharmacy.
Aims. Establish a method that can measure drugs (and their metabolites) in polypharmacy regimens commonly used by older adults using a mouse model.
Methods. Drug levels were determined using the Agilent 6460 Triple Quadrupole liquid chromatography (LC) mass spectrometry (MS) in 20ul of mouse serum. Mix of drugs in mouse serum were extracted via solid phase extraction followed by LC separation using a C18 Poroshell column. The jet stream electrospray was used on the triple quadrupole MS and ions were scanned and optimised before proceeding to Dynamic Multiple Reaction Monitoring and analysis.
Results. The lowest limit of detection (LOD) tested for the compounds ranged from 0.01-1ng/ml in serum. On positive ionization mode, the lowest LOD for majority of the compounds (metoprolol, α-hydroxymetoprolol, O-desmethylmetoprolol, omeprazole, 5-hydroxyomeprazole, omeprazole sulfone, paracetamol, Irbesartan, citalopram, N-desmethylcitalopram, oxybutynin, N-desethyl oxybutynin, oxycodone, noroxycodone and oxymorphone) was ≤0.05ng/ml. On negative ionization mode, the LOD tested for paracetamol-sulfate, tenivastatin and paracetamol-glucuronide were 0.05, 1, 1ng/ml, respectively. Preliminary results show that the recovery and matrix effect were acceptable. No carry over was observed at the concentrations tested.
Discussion. We have developed a method that should be sensitive enough to measure 8 drugs (and 11 metabolites) in serum from mice. The limit of detection is sufficient to detect these compounds at therapeutic concentrations in blood and is therefore suitable for testing in mice that have been chronically administered polypharmacy. The assay may ultimately be applicable to routine assessment to optimise drug dosing in the clinic.

466 Characterising the functions of UDP-glucuronosyltransferase (UGT) 2B15 and 2B17 in breast cancer cells

Quinn L Martin, Siti Mubarokah, Robyn Meech. Clinical Pharmacology, Flinders University, Adelaide, SA, Australia

Introduction. Steroids regulate breast cancer cell growth; estrogens promote growth of estrogen receptor (ER)+ cells and androgens show differential effects depending on receptor status. The expression of UGT2B15 and 2B17 enzymes is induced by steroids in breast cancer cells; in turn UGT2B15 and 2B17 inactivate steroids, predominantly androgens, via glucuronidation. However the consequences of this regulatory loop for cell growth are unknown.

Aims. To define the effects of UGT2B15 and 2B17 overexpression in MCF7 cells on proliferation and steroid-responsive gene expression.

Methods. MCF7 (ER+/AR+) breast cancer cells were transfected with vectors encoding UGT2B15 or 2B17 and stable over-expressing (O.E.) cell populations were generated. The proliferation rate of UGT O.E cells was compared to control (empty vector) cells in standard steroid-replete media or in steroid-depleted media with defined steroid treatments (estradiol, DHT) using a dye retention assay. Androgen responsive gene expression in UGT O.E. cells was quantified by RT-qPCR.

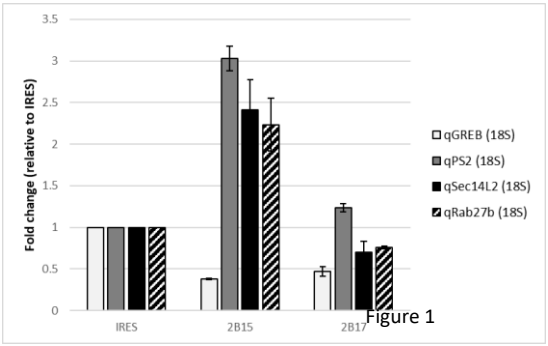


Figure 1

Results. Both UGT2B15 and UGT2B17 O.E. MCF7 cells proliferated faster than control cells in steroid-replete media. UGT2B17 O.E. cells proliferated more slowly in steroid-depleted media. Estradiol increased proliferation similarly in all cell lines. UGT2B15 and UGT2B17 O.E. MCF7 cells both showed altered expression of androgen-responsive genes; however while UGT2B17 O.E. reduced androgen-target genes, UGT2B15 O.E. increased expression (Figure 1).

Discussion. The increased proliferation of UGT2B15 and 2B17 O.E. MCF7 cells in standard steroid-replete media may reflect their abilities to metabolize growth-inhibiting androgens. This conclusion is consistent with the observation that UGT O.E. did not change the response to E_2 stimulation in steroid-depleted media. Moreover, the result is consistent with observations from our previous work that loss of UGT2B17 expression impairs MCF7 cell growth. The differential effects of UGT2B15 and UGT2B17 O.E. on androgen-regulated gene expression may reflect the different catalytic activities of these enzymes with the various androgenic steroids and metabolites present in standard serum.

467 Computational prediction of the sites of metabolism (SOM) of protein kinase inhibitors

Pramod C. Nair[1,2], Ross A. McKinnon[2] and John O. Miners[1,2]. Department of Clinical Pharmacology[1] and [2]Flinders Centre for Innovation in Cancer, College of Medicine and Public Health, Flinders University, Adelaide, SA, Australia.

Introduction. Small molecule protein kinase inhibitors (KIs) are an effective targeted therapy for multiple types of cancers. KIs are mainly biotransformed through oxidation reactions catalysed by CYP3A4. SOM prediction is a useful tool for identifying metabolically labile sites of KIs (and other drugs) in the drug discovery pipeline.

Aims. This study sought to predict the SOM of KIs using a range of computational methods and to identify amino acids important for KI binding within the CYP3A4 active site.

Methods. SOMs were collated for a dataset of 31 marketed KIs metabolised by human CYP3A4. A range of computational approaches were evaluated for SOM prediction: molecular docking (using three CYP3A4 X-ray crystal structure templates); molecular superpositioning (using 4 ligand templates); and Web-based methods (using three algorithms). Molecular docking additionally identified amino acids involved in KI binding within the CYP3A4 active site.

Results. Since CYP3A4 is known to exhibit plasticity in the catalytic site, three X-ray crystal structures were investigated as templates for molecular docking. Docking in the bromoergocryptine-bound structure (3UA1) provided superior SOM prediction (77%) compared to the unliganded and ritonavir-bound structures (74% and 68%, respectively). Of the various scoring functions investigated, the PMF-score showed more consistent SOM prediction. The web-based SOM prediction algorithms provided marginally better predictivity (77%-87%), whereas the substrate superpositioning (molecular overlay) approach using 4 different compounds as templates was less effective (42%-71% prediction accuracy). Docking of the KIs in the CYP3A4 active sites identified Glu37 Phe57, Asp76, Arg105, Arg106, Ser119, Arg212, Phe215, Thr224, Arg372, and Glu374 as important residues for substrate binding.

Discussion. The study demonstrated that SOM prediction of KIs was dependent on the CYP3A4 X-ray crystal structure employed as the template, consistent with the known plasticity of this protein. Web-based SOM algorithms provided the best predictivity. Hydrophobic, hydrogen-bonding, and charge interactions contribute to KI binding in the CYP3A4 active site. The approaches adopted here are likely to be applicable to SOM prediction of other CYP3A4 substrates.

468 Understanding variability in circulating extracellular nanovesicles: implications for biomarker analyses

Lauren Newman[1], Michael J Sorich[1], Andrew Rowland[1]. Clinical Pharmacology, Flinders University[1], Adelaide, SA, Australia.

Introduction. Extracellular nanovesicles (exosomes) have emerged as a potential rich source of biomarkers in human blood and present the intriguing potential for a 'liquid biopsy' to track disease and the effectiveness of interventions. Recently we have further demonstrated the potential for exosome derived biomarkers to account for variability in the activity of cytochrome P450 (CYP) 3A4 and hence drug exposure. In order to robustly utilise exosomes as a biomarker strategy it is essential to understand the underlying variability in exosome physiology.
Aims. This study sought to evaluate between-day and diurnal variability in circulating exosome abundance and cargo.
Methods. Blood samples were collected into Z serum tubes each morning (9:00am) and afternoon (3:00pm) for 5 consecutive days. Whole blood was spun twice at 2500g for 15min and exosomes were isolated from the resulting serum by precipitation using ExoQuick reagent. For comparison, exosomes were also isolated from serum by size exclusion using qEV columns and membrane affinity using ExoEasy spin columns. Exosome particle abundance and size distribution was assessed by nanoparticle tracking analysis (NTA). Variability in exosome cargo was assessed as differences in total RNA expression, total protein expression and expression of verified exosome cargo; GAPDH (mRNA marker), miR-16 (miRNA marker) and CD63 (protein marker).
Results. Up to 1.8-fold diurnal variability in exosome particle abundance was observed, with higher counts in pm versus am samples. The mean 1.47-fold higher expression in pm versus am samples (n=5) was statistically significant (p=0.015). Between day variability in exosome particle count for am and pm samples was 28% and 67%, respectively. Particle size distribution was comparatively consistent within- and between- days; mean (±SD) values for the mean particle size and 90% confidence interval were 126.3±15.9nm, 76.1±12.6nm and 182.9±25.2nm. Differences in the expression of exosome cargo were consistent with differences in particle count.
Discussion. Diurnal variability in circulating exosome particle abundance has potential implications for study sampling protocols and normalisation of biomarker data when considering expression of exosome derived cargo as a biomarker strategy.

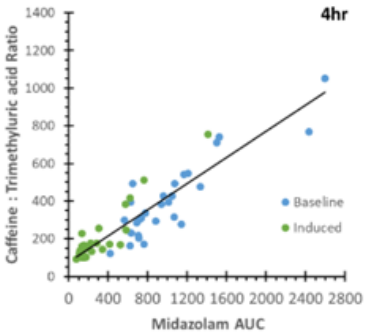
469 Detection of hepatic cytochromes P450 protein in plasma derived extracellular nanovesicles (exosomes)

Warit Ruanglertboon[1], Michael J Sorich[1], Andrew Rowland[1]. Department of Clinical Pharmacology and Flinders Centre for Innovation in Cancer, College of Medicine and Public Health, Flinders University[1], Adelaide, SA, Australia.

Introduction. Extracellular nanovesicles (exosomes) are small membranous vesicles that are released by organs including the liver into the blood and other bio fluids. Exosomes contain nucleic acids, proteins and small molecules derived from their cell of origin and are a potential source of biomarkers in the blood. Detection and quantification of cytochromes P450 (CYP) in exosomes presents the intriguing potential for a liquid biopsy for CYP expression.
Aims. This study sought to determine the array of CYP proteins present in exosomes isolated from human plasma.
Methods. Exosomes were isolated from human plasma by membrane affinity chromatography using ExoEasy spin columns and buffers. In-gel trypsin digestion was performed on 40 to 70kDa bands excised from 1D-SDS PAGE gels. Gel fragments were destained then dehydrated at room temperature using acetonitrile. Protein bands were reduced, alkylated, dehydrated and digested at an enzyme-to-protein ratio of 1:20. Peptides were extracted in acetonitrile, separated by liquid chromatography and detected using an ABSciex 5600+ triple time of flight mass spectrometer operating in positive ion mode. *De novo* sequencing was performed using Peaks Studio v7.0 software.
Results. Mass spectrometry based proteomic profiling of exosomes isolated from human plasma detected 188 unique peptides originating from CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 2J2, 3A4 and 3A5. The number of unique peptides detected for each protein ranged between 2 and 19, with a mean of 9.65. In addition, 5 unique peptides originating from NADPH-cytochrome P450 reductase (the redox partner required for CYP activity) were also detected. While cytochrome b5 (34.5kDa) was not detected in the current analysis as it was not contained within the window of protein bands analyzed, the presence of this protein in human derived exosomes has already been established.
Discussion. This study demonstrates the presence of protein for all key human drug metabolising CYP enzymes in plasma-derived exosomes. Building on recent work from this group demonstrating the strong concordance of exosome derived CYP3A4 markers and midazolam clearance, these data support the generalizability of exosomes as a strategy to account for variability in CYP activity.

470 Identification of the caffeine to trimethyluric acid ratio as a dietary biomarker to characterise variability in Cytochrome P450 3A4 activity

Madelé van Dyk[1], John O Miners[1], Jean-Claude Marshall[2], Linda S Wood[2], Michael J Sorich[1], Andrew Rowland[1] . [1]Department of Clinical Pharmacology, Flinders University, Adelaide, SA, Australia; [2]Precision Medicine, Pfizer Worldwide Research and Development, Groton, CT, USA



Introduction. Cytochrome P450 (CYP) 3A plays an important role in the metabolism of many clinically used drugs and exhibits substantial between subject variability (BSV) in activity. Current methods to assess variability in CYP3A activity have notable limitations highlighting the need for a non-invasive clinically translatable strategy to define CYP3A activity.

Aims. The purpose of this study was to identify a dietary biomarker to characterise variability in CYP3A4 activity.

Methods. In this study, the metabolic ratio of caffeine to 1,3,7-trimethyluric acid (TMU) was evaluated as a biomarker to describe variability in CYP3A4 activity in a cohort (n=30) of healthy males. Midazolam, caffeine, and TMU concentrations were assessed at baseline and following dosing of rifampin for 7 days.

Results. At baseline, correlation coefficients for the relationship between apparent oral midazolam clearance (CL/F) with Caffeine/TMU ratio measured at 3, 4, and 6 hr post dose were 0.82, 0.79 and 0.65, respectively. The strength of correlations was retained post-rifampin dosing; 0.72, 0.87 and 0.82 for the ratios at 3, 4, and 6 hr, respectively. Appreciably weaker correlations were observed between the change in midazolam CL/F and change in Caffeine/TMU ratios post-/pre- rifampin dosing; correlation coefficients ranged from 0.30 to 0.41.

Discussion. BSV in CYP3A activity was well described by Caffeine/TMU ratios pre- and post- induction. However, intra-subject variability caused by induction was poorly described. A dietary Caffeine/TMU ratio may be a convenient tool to assess BSV in CYP3A activity, but assessment of Caffeine/TMU ratio alone is unlikely to account for all sources of variability in CYP3A activity.

471 Pharmacy Intern Vaccination Training

Peter R Carroll, Yihua Chen, Pechdau E Vicheth, Patrick R Webber, Jane R Hanrahan. School of Pharmacy, Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia

Introduction: In Australia only registered and provisionally registered pharmacists are able to complete an accredited training program to administer influenza vaccine.[1] Therefore, pharmacy students are unable to complete accredited vaccination training during their degree. In 2016 and 2017 the University of Sydney School of Pharmacy offered Master of Pharmacy graduands the opportunity to complete the Pharmacy Guild of Australia (PGA) accredited influenza vaccination course shortly after gaining their provisional registration.

Aim: To evaluate these provisionally registered pharmacists' perceptions of the PGA vaccination course in preparing pharmacists to administer influenza vaccine, and their experiences in administering the vaccine.

Methods: Provisionally registered pharmacists were invited to take part in pre- and post-course surveys to assess their perceived influenza vaccination knowledge, and their confidence and skills to administer influenza vaccine. These pharmacists were then contacted 6-21 months later by telephone to assess their vaccination experience to date and asked their opinions of the timing and quality of the PGA training course in terms of preparing them to confidently administer vaccinations in the community pharmacy setting.

Results: Survey results showed significant increases in participants' confidence level of vaccine administration (47.5% increase, $p<0.001$), and their skills in (27.1% increase, $p<0.001$) and knowledge of (24.4% increase, $p<0.001$) influenza vaccination. Telephone interviews confirmed the survey results and showed that 69% of participants had administered influenza vaccine, with 55% doing so in their intern year. Moreover, 63% of participants agreed that the best time to undertake the vaccination training course was near the end of their studies, or during the intern year.

Discussion: The PGA vaccination training course significantly improved participants' confidence, skill and knowledge of influenza vaccination. However, the time between the training course and their first vaccination can affect their confidence in administering vaccines. This study will help determine the best time to implement training for university students should students be allowed in the future to complete accredited vaccination training during their degree.

1. www.pharmacycouncil.org.au/policies-procedures/standards/standards_vaccination_feb2015.pdf, accessed 18/10/18.

472 Delivery of flexible and effective curricula in pharmacology to diverse student cohorts

Elvan Djouma, Ross D. O'Shea. Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, VIC, Australia

Introduction. For the past six years, our team at La Trobe University has taught pharmacology to over 500 Health Science and Allied Health students annually. Foundations in Pharmacology is a core subject for students studying physiotherapy, podiatry and paramedicine, and is an elective for a number of biomedical and science-related degrees. We also teach pharmacology in other subjects to a diverse cohort, including students from speech pathology, orthoptics, human nutrition and exercise science. This diversity has presented a huge challenge in curriculum design.
Aims. While students shared common lectures, we sought to develop flexible, modular workshops that incorporated authentic case studies that would be relevant to students from different disciplines.
Methods. Weekly workshops involved two facilitators with pharmacology expertise to approximately 60 students, who worked in the same teams of 5-6 for the duration of the semester. Workshop notes were posted on the Learning Management System the week prior to the workshop and were designed to consolidate and extend blended content delivered in the previous week. The workshop notes encouraged students to use a variety of on-line resources, including MIMS and computer simulations of pharmacokinetics and pharmacodynamics. Questions consisted of MCQs, short answer, drawing or labelling diagrams in addition to interpreting graphs and clinical data. After completing the workshop activities, students were given a team submission sheet which incorporated questions taken from the workshop notes. Students were permitted to seek help from facilitators up until the team submission was handed out. These team submission sheets were worth 2% each week and contributed 20% of the overall mark for the subject.
Results. Quantitative and qualitative feedback indicated that students highly valued the workshops in assisting their learning and engaging with the content. Having a small assessable component assigned to each workshop also encouraged attendance and participation.
Discussion. Designing flexible workshop content enabled the teaching of pharmacology to large cohorts of students with diverse academic backgrounds and capabilities. We maximised the use of workshops that incorporated authentic learning and assessment tasks and relevant case studies which can easily be adapted to different drugs and diseases. Parts of this workshop model have now been adopted for subjects in the Bachelor of Pharmacy at La Trobe University.

473 Relationship between entry requirements and academic outcomes in pharmacology for nursing students

Sheila A Doggrell. Faculty of Health, Queensland University of Technology (QUT), Brisbane, QLD

Introduction. We have previously shown that nursing students are more reliant on ongoing assessment marks to pass a unit in pharmacology than paramedic or optometry students. This may be due to nursing students having lower entry requirements to attend university than the other allied health students.
Aim. At QUT, we have two cohorts of nursing students, and one has a lower entry score requirement than the other. The aim was to compare academic outcomes (failure rates, grades, and percentage marks in examinations and ongoing assessment) for nursing student in the higher- and lower- entry cut-off cohorts in 2014/5.
Methods. Failure rates and grades were determined. For students who passed the unit, marks for examinations and ongoing assessment were calculated as a percentage. In the Table, significance is at *P < 0.05 by Student's unpaired t-test
Results. In 2014 and 2015, the results were similar, and the results for 2015 are given in the Table. Failure rates and marks for ongoing assessment were not significantly different between the cohorts. Grades were lower for the lower-entry cut-off students, and this was due to lower marks in the examinations.

		For successful students		
Student cohort	Failure rate	Grade	Examinations (%)	Ongoing assessment (%)
Higher-entry cut-off	27 of 361 (8%)	4.87 ± 0.05 (344)	58.5 ± 0.7 (344)	80.7 ± 0.6 (344)
Lower-entry cut-off	11 of 96 (12%)	4.30 ± 0.09 (85)*	50.4 ± 1.2 (85)*	79.7 ± 1.3 (85)

For the students who passed the unit, the examination component was failed (≤ 50%) by significantly more low-entry (44/85; 51.8%) than higher-entry cut-off students (86/344; 25.0%); P < 0.05 by Odds ratio.
Discussion. Despite passing a pharmacology unit, nursing students do poorly in examinations, especially those with lower-entry cut-off. Additionally, some of the passing nursing students may have succeeded due to work done by others in ongoing assessment. Thus, nursing students, who failed the examination component, may not have assimilated the necessary knowledge to continue in their courses.
Doggrell SA & Schaffer S (2017) https://www.asceptasm.com/wp-content/uploads/2017/12/APSA-ASCEPT-poster-abstracts-51217.pdf

474 Student attitudes towards pharmacology practicals

Lynette B Fernandes[1], Anna-Marie Babey[2]. Pharmacology, School of Biomedical Sciences, University of Western Australia[1], Perth, WA, Australia; School of Medicine and Pharmacy, University of New England[2], Armidale, NSW, Australia.

Introduction. Laboratory practicals are under scrutiny with concerns they fall short of genuine enhancement of learning and the perceived cost- and resource-saving benefits associated with computer simulations. Further, students are more discerning and require that all teaching activities optimise their learning. Thus, it is essential to ascertain students' expectations of hands-on practicals and which features are perceived to facilitate learning.
Aims. To ascertain students' expectations of hands-on practicals and to investigate those features of laboratory practicals that are perceived to facilitate learning.
Methods. Paper-based surveys were administered to 2nd and 3rd year pharmacology students to obtain information about their expectations of laboratory practicals. Project approval was obtained from the University of Western Australia Human Ethics Committee (RA/4/1/9046).
Results. Approximately two-thirds of the 2nd year students were 19 years of age, 57% of whom were women. By contrast, the gender profile of 3rd year students was reversed with 44% being women. Both cohorts overwhelmingly agreed that staff preparedness and interest (96% agreement) and well-organised practicals (94% agreement) were essential to their learning. Third-year students (82%) were much more likely than second-year students (61%) to value practicals as a means of revising and reinforcing core content. Neither group expressed any substantial concern regarding any equipment problems, that protocols were recipe-based or that there was little opportunity to design their own practicals, although in each instance, the 3rd year students were slightly more concerned (2nd year: 25% agreement, 16% and 10% respectively; 3rd year: 37% agreement, 14% and 15%, respectively).
Discussion. There was near-unanimous agreement from students that engaged and engaging staff conducting well-organised practical activities enhanced their learning. Students were largely tolerant of problems that might arise during these practicals and activities that might seem repetitive, particularly when core concepts from lecture material were reinforced. Design and implementation of learning opportunities should take into account students expectations and learning needs.

475 Work integrated learning: Interdisciplinary, project-based learning as a means of developing employability skills.

Joanne Hart, Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia.

Introduction. Project-based learning has been used widely as a model for large scale work integrated learning experiences in Liberal Studies Degrees. This study aimed to evaluate the effectiveness of project-based learning in developing employability skills for students in Science Degree programs.
Methods. Education literature databases were systematically reviewed to source relevant research in project-based learning. The inclusion criteria were peer-reviewed journal articles (2008-2018) that discussed undergraduate science-based degree programs with project-based learning units involving students from multiple disciplines (majors). 47 studies were included in the analysis and assessed for quality. Articles were analyzed for evidence of a skill gain across six employability skills: Discipline knowledge; Communication; Teamwork; Interdisciplinary effectiveness; Problem solving and Self-management. Projects were assigned to categories based on (1) Interdisciplinarity: narrow (with closely associated disciplines); medium (within Faculty) or wide (across Faculties), and (2) Project authenticity: contrived (made-up) or authentic (real). Data analysis was carried out using Fisher's Exact test or by presenting odds ratios (OR).
Results. Student perception of a skill gain was significantly more likely to be reported than an objective measure of the skill gain (p<0.001). Students were more likely to achieve actual discipline knowledge gains in projects that were contrived (OR 12) or narrow in discipline mix (OR 6.6), however their perceived discipline knowledge gain was the same regardless of the project type. Projects that had wide interdisciplinarity were significantly associated with a perceived gain in interdisciplinary effectiveness (OR 32, p<0.05), and more likely to demonstrate a perceived gain in communication (OR 2.5) and teamwork (OR 3.4) skills.
Discussion. Designing objective tests for employability skills remains challenging. This study found skill gains in students completing project-based learning units were largely self-reported or based on perception. These data may be limited by factors including the risk of confirmation bias and the overall quality of the included studies.
Conclusions. When projects are more interdisciplinary, student perception of employability skill gains (interdisciplinary effectiveness, communication and teamwork) increase, perceived discipline knowledge gains are unaffected, however actual discipline knowledge gains are less likely. Further robust research in this area is warranted to ensure there is evidence that project-based learning is meeting the desired learning objectives of the curriculum.

476 Introducing project-based learning into the Pharmacology major at the University of Sydney; A case study.

Joanne Hart, Tina Hinton, Slade Matthews, Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia.

Introduction. The new curriculum being rolled out as part of the University of Sydney's strategic plan requires all liberal studies degrees to include project-based units of study in the 3rd Year of every major. Here, we present a case study on the introduction of an industry-connected, project-based unit of study with learning objectives aligned with the University of Sydney's Graduate Qualities into the Pharmacology major.
Methods. Academic teams in the Faculty of Science met to redesign their majors to deliver a 6cp, project-based unit of study at the 3rd year level. Ethics approval to evaluate project-based units was obtained. PCOL3911 Toxicology (Advanced) was used to pilot a project-based unit of study in collaboration with an industry partner, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS), offering toxicology-focused projects.
Results. The project task comprised 10% of the final grade and was assessed by the coordinator (Matthews). 20 students were involved in the project, working in groups of 4, on project work for a total of around 20 hours. Projects included toxicological assessments of compounds not currently assessed by the regulator. The chemicals assessed in these projects were among the thousands of existing chemicals not yet assessed for chemical safety included in the Inventory Multi-tiered Assessment and Prioritization (IMAP) framework. The real-world relevance and obvious importance of the task is intended to be motivational for the students. Students expressed high levels of satisfaction with the project which culminated in a visit to NICNAS offices and a chance to meet regulatory scientists. Feedback from the staff at NICNAS, including both the director and principal scientist, was also extremely positive.
Discussion. This initiative successfully introduced students to an environment where they could apply their developing skills and knowledge to an industry setting. While it is possible that discipline-specific content may be lost by introducing project-based units, the tasks undertaken by these students closely aligned with learning objectives of PCOL3911 while also extending the students. Extra time commitment from both academic and industry staff was required and this must be considered when proposing project-based units. Making the initial connection with an industry partner and negotiating the collaboration is the most complicated aspect of staging industry-connected units, and there is a need for further staff development to support staff wanting to make this commitment to their students.

477 Transforming education through cloud-based technologies for authentic and adaptive learning

Tina Hinton[1], Brooke Storey-Lewis[1], Nicholas Randal[1], Melissa Cameron[2], Michael Morris[2], Sharon Herkes[2], Brent McParland[1], Hilary Lloyd[1], Kellie Charles[1], Tara Speranza[2], Vanessa Gysbers[1] and Margot Day[2]. [1]School of Medical Sciences (Pharmacology), The University of Sydney NSW 2006 Australia, [2]School of Medical Sciences (Physiology), The University of Sydney NSW 2006 Australia.

21st century learning needs to be flexible, authentic and adaptive. Cloud-based technology permits these key elements. Kuracloud by ADInstruments supports active learning by scaffolding instruction and bench-top activities around data acquisition, simulation and analysis, along with rich multimedia resources and self-assessment tools, providing truly hybrid synchronous and asynchronous learning for individuals and groups. At the University of Sydney we have adopted Kuracloud to develop new modules to replace out-dated software, transform current paper-based practicals, and create all new learning activities and assessments. In doing so we have improved the quality and delivery of units of study for multiple cohorts across multiple degree programs and years of study. Transitioning our practicals to Kuracloud has provided a consistent, user-friendly and sustainable learning platform that increases student preparedness and engagement. Analysis of unit of study surveys, bespoke and ASELL survey results, and staff and student outcomes from a learning space evaluation show that use of Kuracloud has improved student learning experiences, particularly around laboratory and group-work, that students prefer to use Kuracloud over other technologies, and that staff have been able to transform curriculum design using Kuracloud. These outcomes are in alignment with research showing that facilitated hybrid learning increases learning efficacy and maximises concept and skill development[1].

[1]Van Doorn, JR & Van Doorn, JD (2014). Front. Psychol., 5: 324. doi: 10.3389/fpsyg.2014.00324

478 Design and development of a new oncology unit of study in the new integrated pharmacy curriculum

Rebecca H Roubin, The University of Sydney School of Pharmacy, The University of Sydney, NSW

Introduction: A discipline-based approach to the learning and teaching of pharmaceutical sciences (including pharmacology) to pharmacy students has faced years of perceived lack of relevance to their chosen profession. This was despite improvements in case examples and contextualising lectures, labs and workshops.
Methods: A new integrated curriculum approaches the teaching of pharmacy from a more integrated perspective, rather than the previous discipline based approach. It is structured by themes and underpinned by a detailed set of learning outcomes, which describe the knowledge, skills and attitudinal milestones to be achieved each year and by the time of graduation. We have designed a new integrated oncology unit of study that covers the therapeutics of immunology & cancer including the pharmacology and pharmaceutical sciences that underpin such drug therapies. Through the use of case-based learning, students participated in the interpretation, application and dissemination of pharmaceutical and pharmacotherapeutic concepts and knowledge. On completion of this unit of study students were able to apply an understanding of the pharmaceutical sciences to optimising the drug and non-drug therapy of patients with cancer and immunological disorders. Students were able to apply also interprofessional communication and the application of specialist knowledge to implementing pharmacist cognitive services such as clinical oncology interventions and/or medication management review. Interprofessional learning role-plays were used to develop students' communication skills for interaction between pharmacists and their clients (patients, doctors, nurses, other health professionals).
Results: This study aimed to examine the effectiveness of the new integrated Master of Pharmacy curriculum and the development of the new oncology unit of study. Unit of Study surveys (USS) collected feedback on the student experience at the unit of study level. The new integrated Master of Pharmacy curriculum demonstrated favourable results quantitatively and qualitatively compared to the previous discipline based curriculum, with improved student perceived relevance and engagement.
Discussion: The new integrated curriculum approach of the teaching of pharmacology and pharmaceutical sciences from a more integrated perspective demonstrated favourable student perceived relevance to their pharmacy profession and greater engagement.

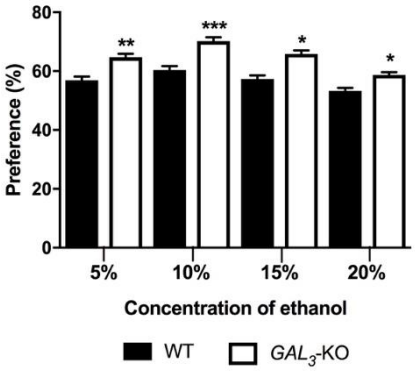
479 Experiential training courses for medical technology and pharmaceutical translation

R Seeber[1], M Eijkenboom[1], K Houston[1], M Oldakowski[1], I Oldakowska[1], J Harries[1], C Williams[1], C Bass[1], E Villaceran[2], L Meagher[2], A Lee[3], K Bechta-Metti[3], P Rolan[3], M Wallach[4], K Pfleger[1]. Accelerating Australia, WA[1], VIC[2], SA[3], NSW[4], Australia.

Introduction. Medical technology and pharmaceutical innovation/translation is a highly complicated process requiring multidisciplinary teams to work together in a stringent regulatory environment. To boost the translation of biomedical research in Australia, a gap was identified in the course offerings to higher degree and postdoctoral innovators, as well as professionals in the ecosystem. Experiential training was identified as a need, ie. provision of real-life biomedical enterprise environments to develop confident, action-orientated, adaptable and impact-driven skills and mindsets.
Aims. To empower biomedical innovators to confidently take ownership of their invention, career path and ability to change the world. To support development of future leaders that will create successful Australian companies and patient benefits through the translation of Australia's world-class medical research.
Methods. Experiential training courses, based on international best practice, have been adapted to suit local ecosystems across four states in Australia. Entrepreneurial Mindset Boot camps help participants to identify their vision for the future, and utilise their exceptional expertise and/or inventions to their advantage using lean methods. The Concept-to-Creation course assists innovators at company startup phase to test the market, form a team and develop a strong business model. SPARK Monash, Perth Biodesign and Adelaide Biodesign form multidisciplinary and cross-sector teams who undertake clinical engagement/immersion to identify unmet clinical needs, invent a new technology concept and shape the early stage business case. SPARK Global bioinnovation courses provide a 2 week hands-on experience of solving healthcare problems in international, multidisciplinary teams.
Results. Since 2017, over 300 participants have completed Accelerating Australia-supported courses nationally and internationally. Trainees have since won commercial grants, seed funding and placements in accelerator programs. Surveys suggest substantial mindset shifts, enhanced confidence and increased adaptability in participants of courses.
Discussion. A wide variety of integrated, experiential training courses were applied with great results in instilling both new translational skills and a can-do attitude in higher degree and postdoctoral innovators, along with professionals from the broader ecosystem.

480 Characterisation of alcohol-seeking behaviour in galanin receptor-3 knockout mice

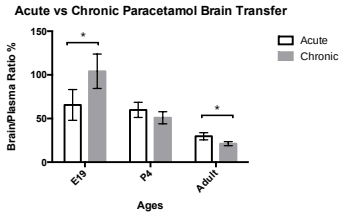
Shannyn G Genders, Elvan Djouma. Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, VIC, Australia.



Introduction. Galanin is a neuropeptide that has been critically implicated in mediating addiction. Allelic variation in the galanin receptor-3 (*GAL₃*) gene has been associated with an increased risk of alcohol use disorders in diverse human populations while administration of the GAL_3 selective antagonist, SNAP 37889, reduces alcohol self-administration in animal models.
Aims. To characterise alcohol-seeking behaviour in *GAL₃* knockout (KO) mice.
Methods. *GAL₃*-KO mice and wildtype (WT) littermates underwent two-bottle free choice testing to determine preference for ethanol, sucrose and saccharin, in addition to investigating dietary preference. Alcohol metabolism was also assessed, and an operant self-administration paradigm was utilised to determine the effect of the non-selective GAL receptor antagonist, M35, on alcohol-seeking behaviour.
Results. The two-bottle free choice paradigm revealed *GAL₃*-KO mice consistently show a significantly increased preference for ethanol when compared to WT littermates at concentrations of 5%, 10%, 15%, and 20% (5.8-9.8% increase; $p<0.05$). No genotype differences were observed in preference for sucrose, saccharin or a high fat diet, indicating the increased consumption by *GAL₃*-KO mice may be specific for ethanol. Additionally, alcohol metabolism was comparable between genotypes and a battery of behavioural tests revealed no genotype difference in cognition and locomotor behaviours, suggesting that the results obtained were not due to any non-specific behavioural deficits. Preliminary findings showed no genotype difference for operant self-administration of alcohol in mice treated with vehicle or M35.
Discussion. Overall, our results show that deletion of *GAL₃* in mice increases alcohol consumption which is in contrast to the previous effect observed with pharmacological studies using the selective GAL_3 antagonist SNAP 37889.

481 Paracetamol entry into the developing brain

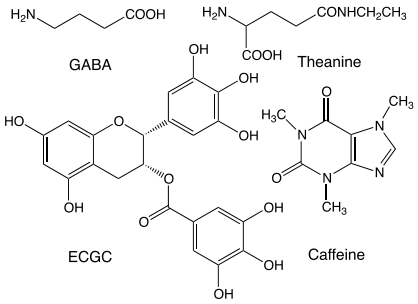
Yifan Huang[1], Liam M Koehn[1], Kate Dziegielewska[1], Mark D Habgood[1], Norman R Saunders[1]. Department of Pharmacology and Therapeutics, The University of Melbourne[1], Melbourne, VIC, Australia



Introduction. Paracetamol is one of the most commonly used drugs with over 70% of women taking it during pregnancy (Werler et al., 2005). It is also the only analgesic recommended for babies under 3 months (WHO, 2012). However, it is not known how much of the drug can reach the developing brain both during pregnancy and in the postnatal period.
Aims. To determine the entry of paracetamol and expression of ABC efflux transporters at the brain barriers in the rat brain during development under acute (single dose) and chronic (multiple doses) conditions.
Methods. Sprague Dawley rats were injected IP with 15mg/kg of paracetamol containing a radioactive tracer (^{3}H[acetaminophen]) at 3 ages (E19, P4 and adult). In acute experiments one dose was given 30 minutes before sample collection and in chronic experiments the drug was given twice daily for 4 days, then sampled 30 minutes after a final dose on the 5th day. The transfer of paracetamol into the brain, CSF and plasma was measured using liquid scintillation counting. For RT-qPCR brains, choroid plexuses and placenta (E19 only) were used and expression of ABC efflux transporters analysed.
Results. In acute experiments, an age dependent decrease in the permeability of paracetamol into the brain and CSF was observed, with permeability into the adult brain being much lower than E19 and P4 (Figure above). In chronic experiments the transfer of paracetamol into the E19 brain, compared to the acute group, increased (65.57% ± 17.68; n=10 and 104.10% ± 19.71; n=10). In contrast, in the adults, less drug entered the brain (29.69% ± 4.11; n=4 and 21.19% ± 2.31; n=4) following chronic exposure compared to an acute dose. Placental transfer of paracetamol did not change between experimental groups (acute: 42.11% ± 6.09, n=11; chronic: 43.06% ± 5.04, n=11). In the adult brain, the ABC transporter MRP5 increased its expression in chronic experiments compared to the acute group.
Conclusion. ABC efflux transporters in the early developing brain lack the adult's ability to efficiently upregulate their expression.
Organisation WH (2012); Werler et al (2005) Am J Obstet Gynecol 193:771-777

482 Neuroactive constituents of GABA-enriched oolong tea compared to regular oolong tea

Tina Hinton[1], Vincent Viengkhou[1], Sin Yoo Kam[1], Sandra Kindaro[1], Herbert F Jelinek[2], Slade Matthews[1], Graham AR Johnston[1,] [1]School of Medical Sciences (Pharmacology), The University of Sydney NSW 2006 Australia, [2]School of Community Health, Centre for Research in Complex Systems, Charles Sturt University, Albury NSW 2640 Australia.

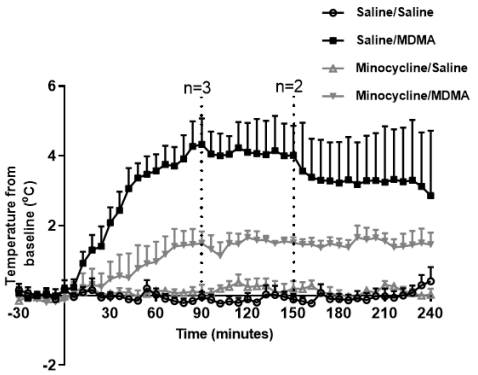


Tea (brewed from the leaves of *Camellia sinensis*) contains neuroactive constituents that are known to influence GABA receptors and to have beneficial effects on stress. In addition to GABA itself, these constituents include theanine, caffeine and polyphenols such as epigallocatechin gallate (EGCG) (Hinton and Johnston, 2018). Exposure of oolong tea to a nitrogen-enriched environment during processing enhances the content of naturally occurring GABA in the tea by up to ten times. Such "GABA tea" is highly prized in Japan and Taiwan for its calming effects.
Reverse-phase high performance liquid chromatography was used to quantify GABA, theanine, EGCG and caffeine in cups of regular and GABA-enriched oolong tea brewed to manufacturer's instructions. Teas were prepared by steeping 5 g dry leaves in 200 mL water at 90˚C for 10 min. As expected GABA tea showed significantly increased levels of GABA (2.01 vs. 0.25 mg/200 mL serving) compared to regular oolong tea. In addition, there were significant changes in other neuroactive constituents. Caffeine levels were also increased (20.13 vs. 13.38 mg/200 mL), while theanine (4.12 vs. 8.26 mg/200 mL) and ECGC (0.13 vs. 17.68 mg/200 mL) were decreased. Thus, the anaerobic fermentation process required for the production of GABA-enriched oolong tea alters the concentration of other neuroactive constituents of oolong tea.

Hinton T and Johnston GAR (2018) GABA, the major inhibitory neurotransmitter in the brain, *Research Module in Biomedical Sciences*, Elsevier, editor M Caplan, in press)

483 MDMA-induced hyperthermia: the effects of minocycline on brain hyperthermia at high ambient temperature

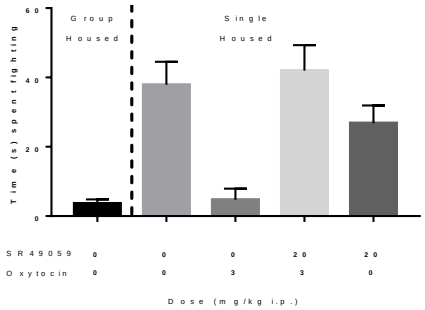
Stefan T Musolino[1,2], Mark R Hutchinson[1,2], Erik P Schartner[1,3], and Abdallah Salem[1,2]. ARC Centre of Excellence for Nanoscale BioPhotonics[1], Adelaide, SA, Australia. Discipline of Pharmacology, Adelaide Medical School[2], Adelaide, SA, Australia. School of Physical Sciences [3], Adelaide, SA, Australia.



Introduction. Brain hyperthermia is the major complication associated with MDMA administration. Minocycline has previously been shown to have potent neuroprotective effects in the brain, but its effects related to hyperthermia in the brain are unknown.
Aims. To determine the therapeutic effects of minocycline on MDMA-induced increases in brain temperature.
Methods. Prior to saline (10ml/kg, i.p) or MDMA (10 mg/kg, i.p) administration, rats were pre-treated with 3 doses of saline (10ml/kg, i.p) or minocycline (50mg/kg, i.p) 12 hours apart. Optical fiber temperature sensors were implanted into the right striatum and brain temperature was recorded for 4 hours post-MDMA administration.
Results. MDMA at high ambient temperature caused significant increases in striatal brain temperature at 30 minutes post-MDMA administration ($p<0.0246$) and this peaked at 90 minutes post-MDMA ($p<0.0001$). Minocycline significantly attenuated MDMA-induced hyperthermia in the striatum. Striatal temperatures remained significantly reduced after 30 ($p<0.05$), 60 ($p<0.0002$) and 90 minutes ($p<0.0001$) post-MDMA. Minocycline also delayed the onset of hyperthermia with no significant increase in brain temperature observed until 60 minutes ($p<0.05$) post-MDMA compared to the saline controls.
Discussion. We have successfully demonstrated the therapeutic effects of minocycline on MDMA-induced hyperthermia in the brain. Further investigation of minocycline and the pathways underlying the MDMA-induced hyperthermic response may provide for a future pharmacological intervention to treat acute MDMA toxicity in a clinical setting.

484 Oxytocin and vasopressin inhibit hyper-aggressive behaviour in mice via actions at the V1A receptor

Oliver Tan[1,2], Hande M Musullulu[2], Bianca B Wilson[1,2], Joel S Raymond[1,2], Michael T Bowen[1,2]. [1]The University of Sydney, Faculty of Science, School of Psychology, Sydney, NSW, Australia; [2]The University of Sydney, Brain and Mind Centre, Sydney, NSW, Australia.



Introduction. Despite the high prevalence of aggression across a wide range of disorders, there are a lack of viable treatments. Recent studies have shown oxytocin to be effective in reducing aggression in mouse models. However, these studies often relied on models of territorial aggression and our understanding of the molecular mechanisms driving oxytocin's anti-aggressive effects remains poor.
Aims. Over a series of experiments, the current study aimed to test oxytocin and vasopressin in a model of non-territorial aggression and examine the involvement of oxytocin and/or V1A receptors.
Methods. Male Swiss mice were either isolated (N=128) or group housed (N=32). Experiments began six weeks into isolation and examined the acute anti-aggressive effects of oxytocin (0.03, 0.1, 0.3, 1, 3, 10 mg/kg i.p.), vasopressin (0.01, 0.03, 0.1 mg/kg i.p.), and the selective oxytocin receptor agonist TGOT (1, 3, 10 mg/kg i.p.) on isolation-induced hyper-aggressive behaviour. Subsequent experiments examined whether the anti-aggressive effects of oxytocin and vasopressin could be blocked by pre-treatment with either a selective oxytocin receptor antagonist (L-368,899, 10 mg/kg i.p.) or V1A receptor antagonist (SR49059, 20 mg/kg i.p.).
Results. Oxytocin produced dose-dependent anti-aggressive effects, which were blocked by pre-treatment with SR49059, but not L-368,899. Consistent with its ~20-fold greater affinity for the V1A receptor, vasopressin was much more potent at reducing aggression than oxytocin and these effects were also blocked by SR49059. Surprisingly TGOT was also found to be anti-aggressive.
Discussion. Overall, this study showed that oxytocin and vasopressin are able to reduce isolation-induced hyper-aggressive behaviour via activation of the V1A receptor.

485 Prevalence and comorbidities associated with analgesic prescribing for poly-medicated elderly patients

AA Al-Qurain[1], L Gebremedhin[1], MS Khan[1,4], MD Wiese[1], DB Williams[1], L Mackenzie[1], C Phillips[2], P Russell[3], MS Roberts[1,4]. School of Pharmacy and Medical Sciences, University of South Australia, and Basil Hetzel Institute for Translational Research, The Queen Elizabeth Hospital[1]; School of Nursing and Midwifery, University of South Australia[2]; Royal Adelaide Hospital, Adelaide SA[3]; Therapeutics Research Centre, Diamantina Institute, The University of Queensland, Translational Research Institute, Brisbane, Qld, Australia.[4]

Introduction. Pain is common in older patients and management guidelines rarely consider the effect of polypharmacy or multiple comorbidities.
Aims. To identify patterns, prevalence and factors associated with analgesic prescribing in older patients.
Methods. Older patients (aged ≥ 75 years) admitted to the Royal Adelaide Hospital between September 2015 and August 2016 and on polypharmacy were included, and their comorbidities and medications prescribed at discharge were recorded. Drug burden index (DBI) and Charlson comorbidities index (CCI) were calculated. Number of prescribed medications (NPP) and number of medications increasing the risk of orthostatic hypotension (OD) were recorded. Logistic regression was used to compute the association between analgesic use and participant characteristics, and results were presented as an odds ratio (OR) and 95% confidence interval (95% CI), adjusted for age, gender, CCI, DBI and OD.
Results. From 1192 patients, 824 (69%) patients were prescribed analgesic medications. Paracetamol (used by 61.2% of cohort), opioids (23.7%) and adjuvants (11.4%) were used more frequently than NSAIDs (5.5%). Analgesic users had a higher median DBI (OR = 3.03, 95% CI 2.36-3.88), were prescribed more medications (OR = 1.2, 95% CI 1.15-1.26), and were less likely to be male (OR = 0.62, 95% CI 0.48-0.79) compared to non-users. Musculoskeletal diseases (OR = 2.2, 95% CI 1.6-2.8), hypertension (OR = 1.5, 95% CI 1.1-2) and falls (OR = 1.9, 95% CI 1.3-2.7) were more prevalent with analgesic users. Opioid use was associated with DBI (OR = 5.4, 95% CI 4.3-6.8), while adjuvant use was associated with OD (OR = 1.3, 95% CI 1.1-1.4). Opioid use was associated with osteoporosis (OR = 1.6, 95% CI 1.1-2.4) and falls (OR = 1.5, 95% CI 1.02-2.1) while NSAID use was associated with pulmonary diseases (OR = 1.9, 95% CI 1.1-3.4).
Discussion: Poly-medicated elderly patients are presented with multi comorbidities and concurrent medications that can increase risk of analgesic adverse effects.

486 Body mass impacts survival in non-small cell lung cancer patients

Hannah Rillstone[1], Benjamin Harris[1], Euan Walpole[2], Jennifer Martin[3], Sallie Pearson[4], Connie Diakos[5], Kellie Charles[1]. Discipline of Pharmacology, The University of Sydney[1], Sydney, NSW, Australia; Cancer Serviced Division, Princess Alexandra Hospital[2], Brisbane, QLD, Australia; Clinical Pharmacology, The University of Newcastle[3], Newcastle, NSW, Australia; Pharmacoepidemiology and Pharmaceutical Policy Research Group, The University of Sydney[4], Newcastle, NSW, Australia; North Sydney Cancer Centre, Royal North Shore Hospital[5], St leonards, NSW, Australia.

Introduction. Non-small cell lung cancer (NSCLC) is an incredibly heterogeneous disease. Treatment modalities include chemotherapy agents such as carboplatin, paclitaxel and gemcitabine. These regimes have highly unpredictable efficacy and response and therefore prognostication is challenging. Such variability among populations demands further improvements in the treatment of disease with respect to drug utilization.
Aims. This study aimed to investigate the influence of patient characteristics on the drug utilization and subsequent outcomes using a linked dataset of a real-world population of advanced NSCLC patients.
Methods. The Chemotherapy Dosing in Cancer Related Inflammation dataset comprised of Queensland NSCLC patients treated between 2009–2014 was used. Univariate and multivariate statistical analysis was used to investigate the influence of sex, BMI, age and geographical remoteness on drug utilization and clinical outcomes.
Results. 631 patients with NSCLC were investigated, who were mostly male (57%), residing mostly in urban areas with a median age of 65 yrs and BMI of 25. Regimen selection was not different between age, sex, BMI and geographical remoteness. 75% of patients had advanced disease and 85% received platinum-based chemotherapy. Starting dose reductions were common in the elderly (>70 yrs) and overweight patients but did not influence survival. Overall survival was similar regardless of age, sex and remoteness. However univariate analysis showed overweight patients had improved survival compared to normal weight counterparts (median survival months (95 % CI) 12 (9.5-11.45) vs 7.5 (6-9)). Multivariate analysis suggested BMI maintained influence on survival.
Discussion. BMI is an important factor that can be used to help influence clinical decisions and suggests overweight patients are receiving appropriate chemotherapy doses. This data-set shows elderly patients are being appropriately dose reduced at the start of chemotherapy without impacting survival. Regional and gender differences did not impact survival outcomes. However, body size does influence survival and needs further investigation.

487 Effect of early adverse events on response and survival outcomes of advanced melanoma patients treated with vemurafenib or vemurafenib plus cobimetinib: A pooled analysis of clinical trial data

Ashley M Hopkins[1], Madele Van Dyk[1], Andrew Rowland[1], Michael J Sorich[1]. Flinders Centre for Innovation in Cancer | Discipline of Clinical Pharmacology, College of Medicine and Public Health, Flinders University[1], Adelaide, SA, Australia

Introduction. Adverse events with use of vemurafenib or cobimetinib may result in dose adjustments, the therapeutic impact of which is currently unknown. Adverse events have also been identified as predictors of therapeutic outcomes to several targeted medicines but have been minimally investigated for vemurafenib / cobimetinib.
Aims. Explore the association of early adverse events with overall survival (OS) and progression-free survival (PFS) in advanced melanoma patients treated with vemurafenib or vemurafenib plus cobimetinib.
Method. A pooled secondary analysis of participants treated with first-line vemurafenib or vemurafenib plus cobimetinib from trials BRIM3 and coBRIM. Adverse events were defined by requirement for dose adjustment (reduction/interruption or treatment withdrawal) or by grade of severity and were limited to occurrence within the first 28 days of therapy. Associations were evaluated by Cox proportional hazards regression for OS and PFS.
Results. 583 participants received vemurafenib monotherapy and 247 received vemurafenib plus cobimetinib. Adverse events requiring vemurafenib/cobimetinib dose adjustment within the first 28 days of therapy occurred for 293 (36 %) participants and were significantly associated with OS (HR [95%CI]: Dose reduced/interrupted = 0.79 [0.65-0.96]; Drug withdrawn = 1.18 [0.71-1.96]; P = 0.032), and PFS (HR [95%CI]: Dose reduced/interrupted = 0.82 [0.67-0.99]; Drug withdrawn = 1.58 [0.97-2.58]; P = 0.017). At day 43, plasma trough concentrations of vemurafenib and cobimetinib were similar between individuals with and without an early dose reduction/interruption. Arthralgia occurring within the first 28 days of vemurafenib or vemurafenib plus cobimetinib therapy was significantly associated with favorable OS (HR [95%CI]: Grade 1 = 0.92 [0.73-1.16]; Grade 2+ = 0.67 [0.49-0.91]; P = 0.026), and PFS (HR [95%CI]: Grade 1 = 0.80 [0.63-1.01]; Grade 2+ = 0.76 [0.57- 1.01]; P = 0.042). Early-onset rash, photosensitivity, nausea and vomiting, diarrhoea, fatigue or pyrexia were not significantly associated with OS (P>0.05).
Discussion. For advanced melanoma patients treated with vemurafenib monotherapy or vemurafenib plus cobimetinib, early adverse events requiring dose reduction/interruption and grade 2+ arthralgia were associated with improved survival outcomes.

488 The risk of head trauma associated with antipsychotic use among community dwellers with Alzheimer's disease

Vesa Tapiainen[1,2], Piia Lavikainen[1], Marjaana Koponen[1,2], Heidi Taipale[1-4], Antti Tanskanen[3-5], Jari Tiihonen[3,4], Sirpa Hartikainen[1,2], Anna-Maija Tolppanen[1,2]. School of Pharmacy, University of Eastern Finland[1], Kuopio, Finland; Kuopio Research Centre of Geriatric Care, University of Eastern Finland[2], Kuopio, Finland; Department of Clinical Neuroscience, Karolinska Institutet[3], Stockholm, Sweden; Department of Forensic Psychiatry, Niuvanniemi Hospital, University of Eastern Finland[4], Kuopio, Finland; Impact Assessment Unit, National Institute for Health and Welfare[5], Helsinki, Finland.

Introduction: Antipsychotic use is associated with an increased risk of falls in older population. However, there are no previous studies concerning antipsychotic use and risk of head traumas. We studied the association between antipsychotic use and head traumas among community-dwelling persons with Alzheimer's disease (AD).
Methods: A matched cohort study comparing new antipsychotic users with matched nonusers in the MEDALZ cohort include all Finnish community dwellers with clinically verified AD in 2005-2011. Antipsychotic use was extracted from the Prescription Register and one-year washout period were used to identify new users. Antipsychotic users were matched with nonusers by age, sex and time since AD diagnosis. The number of user-nonuser pairs was 21,795. Head traumas (ICD-10 S00-S09) and traumatic brain injuries (ICD-10 S06.0-S06.9), were extracted from the Hospital Discharge and Causes of Death Registers. The association was investigated using Cox proportional hazard models. Propensity scores were derived from comorbidities, medication use and socioeconomic position and used for inverse probability of treatment (IPT) weighting.
Results: Antipsychotic use was associated with an increased risk of head traumas [event rate per 100 person-years 1.69 (95% CI 1.53-1.86) for users and 1.26 (1.16-1.37) for nonusers, IPT-weighted HR 1.29 (1.14-1.47)] and TBIs [event rate per 100 person-years 0.91 (0.80-1.04) for users and 0.73 (0.65-0.81) for nonusers, IPT-weighted HR 1.22 (1.03-1.45). Quetiapine, compared to risperidone raised risk for TBIs [IPT-weighted HR 1.59 (95% CI 1.15-2.21)] and for head traumas [IPT-weighted HR 1.27 (1.00-1.63)].
Conclusions: Antipsychotic use was associated with an increased risk of head traumas and TBIs among community dwellers with AD. Therefore, use of antipsychotics should be carefully considered in this vulnerable population.

489 Trajectories of work disability before and after opioid initiation for non-cancer pain: 10-year population-based study

Samanta Lalic[1,2,3], J Simon Bell[1], Hanna Gyllensten[3], Natasa Gisev[4], Emilie Friberg[3], Jenni Ilomaki[1] Janet K Sluggett[1], Ellenor Mittendorfer-Rutz[3], Kristina Alexanderson[3]. Centre for Medicine Use and Safety, Monash University[1], VIC, Australia; Pharmacy Department, Austin Health[2], VIC, Australia; Division of Insurance Medicine, Karolinska Institutet[3], Stockholm, Sweden; National Drug and Alcohol Research Centre, UNSW Sydney[4], NSW, Australia.

Introduction. Non-cancer pain is a leading cause of work disability (WD).
Aims. To identify trajectories of WD before and after opioid initiation for non-cancer pain and the factors associated with these trajectories.
Methods. A longitudinal population-based study of 201,641 people (24-59 years) without cancer initiating opioid analgesics in 2009 in Sweden. WD was defined as the combined annual number of compensated net absence days from work due to sick leave and disability pension for each person. Trajectories of net annual WD in the 5 years before/after opioid initiation were estimated with group-based trajectory modelling. Multinomial logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for factors associated with trajectory groups.
Results. Among the 6.9% of people initiating strong opioids, 12.5% had persistent high WD (estimated 320 days/year) before and after opioid initiation and 72.9% had persistent low/minimum WD (estimated 30 days/year). 8.6% of people had increasing WD and 6.1% had decreasing WD after opioid initiation, although this appeared to reflect continuation of pre-initiation patterns. Trajectories were similar at lower WD days/year among those initiating weak opioids. Persistent high WD among strong opioid initiators were associated with ≥5 comorbidities (OR=8.72, 95%CI 5.61-13.56), ≤9 years of education (OR=5.83, 95%CI 4.84-7.03), and prior use of antidepressants (OR=4.57, 95%CI 3.89-5.37) and antipsychotics (OR=4.49, 95%CI 2.93-6.88).
Discussion. Three-quarters of people initiating opioids for non-cancer pain had persistent low/minimum levels of WD five years before and after initiation. Increasing and decreasing WD after opioid initiation appeared to reflect a continuation of pre-initiation patterns. Our findings highlight the complex range of sociodemographic and medication-related factors associated with persistent WD.

490 Prevalence and incidence of prescription opioid analgesic use in Australia

Samanta Lalic[1,2], Jenni Ilomaki[1], J Simon Bell[1], Natasa Gisev[3]. Centre for Medicine Use and Safety, Monash University[1], VIC, Australia; Pharmacy Department, Austin Health[2], VIC, Australia; National Drug and Alcohol Research Centre, UNSW Sydney[3], NSW, Australia.

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

Indicator	Sensitivity	Specificity
PPI	0.9217 (0.8822-0.9491)	0.9464 (0.9114-0.9686)
Antipsychotics	0.9170 (0.8742-0.9467)	0.9188 (0.8811-0.9458)
More than 4	0.6321 (0.5724-0.6882)	0.9293 (0.8913-0.9552)

Discussion. Rates of opioid use have remained high since 2013, with approximately 3 million adults using opioids and over 1.9 million adults initiating opioids each year in Australia. Between 2013-2017, opioid prevalence has slightly increased but incidence has decreased. People without cancer account for the majority of opioid use and are more likely to be initiated on short-acting and weak opioids. Initiation of strong opioids has increased over time, reinforcing concerns about increased use and harms associated with strong opioids in the community.

491 Validation and implementation of three new medicines-related quality indicators in residential aged care facilities

Leonie Picton[1], Taliesin Ryan-Atwood[1], Michael Dooley[1,2], Carl Kirkpatrick[1], Natali Jokanovic[1], Jenni Ilomaki[1], J. Simon Bell[1]. Centre for Medicine Use and Safety, Monash University[1], Parkville, VIC; Pharmacy Department, Alfred Health[1], Prahran, VIC

Introduction. Polypharmacy is an established quality indicator in public sector residential aged care services (PSRACS) in Victoria. Longitudinal data from this quality indicator shows polypharmacy is increasing in PSRACS. Our research on behalf of the Victorian Government Department of Health and Human Services (DHHS) has identified and prioritised 16 strategies to address polypharmacy (Jokanovic et al, 2017). One of these strategies is to implement three new medicines-related quality indicator measures (1: PPI use 2: Antipsychotic use, and 3: More than 4 daily administration times).
Aims. To validate the data collection tools for 3 new medicine-related quality indicator measures and then implement the new measures in four regional health services in western Victoria.
Methods. Indicator specifications for each measure were developed in consultation with expert panels and DHHS. Data collection tools and a supporting educational package for nurses were produced. Nurses from 27 PSRACS in 4 regional health services took part in an educational session followed by a simulated audit using 22 mock charts.
Results. Feedback from the educational sessions and the mock audit results have informed the final educational package: three professionally produced training videos supporting written indicator specifications and data collection tools.
Discussion. The new quality indicator measures implemented in the four participating health services will inform state-wide adoption. Data collection will occur quarterly alongside the established PSRACS quality indicators. The health services will receive facility level feedback.

Jokanovic N et al (2017) RSAP 13(3):564-74

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

Leonie Picton[1], Malcolm Clark[2], Peter Jenkin[3], Laura Dean[1], Rob Sutherland[4], Esa Chen[1] Michael Dooley[1,5], Carl Kirkpatrick[1], J Simon Bell[1]. Centre for Medicine Use and Safety, Monash University[1], Parkville, VIC, Aus; Camberwell Road Medical Practice[2], Camberwell, VIC, Aus; Resthaven Inc.[3], Wayville, SA, Aus; Audio Visual Department[4], Monash University, Parkville, VIC, Aus; Pharmacy Department, Alfred Health[5], Prahran, VIC, Aus.

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

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

493 An assessment of the safety and quality of nurse-initiated and pro re nata (PRN) medication use in Victorian aged care services: A study protocol

Leonie Picton[1], Taliesin Ryan-Atwood[1], Bev Adams[2], Carl Kirkpatrick[1], Jenni Ilomaki[1], Claire Keen[1], J. Simon Bell[1]. Centre for Medicine Use and Safety[1], Monash University, Parkville, VIC, Australia; Ballarat Health Services[2], Ballarat, VIC, Australia.

Introduction. Resident medication needs are complex. Aside from regularly administered medication, nurse-initiated and PRN medications are also used. The decision to administer nurse-initiated and PRN medications is often made by nursing staff. Little is known about how nurse-initiated and PRN medications are currently used.
Aim. The aim of this project is to better understand the prevalence and safety of PRN and nurse-initiated medication (NIM) charting and administration and the factors influencing their use in Victorian Public Sector Residential Aged Care Services (PSRACS) over a 12-month period.
Methods. This will be a retrospective longitudinal audit of the medication records of residents of 10 PSRACS in Victoria. All residents living in the selected aged care services at the index date, July 1st 2016, will be included. Residents who die during the follow-up period will be censored. Baseline characteristics at the index date (including age, sex, length of stay, comorbidities, charted regular and PRN medication) will be extracted from the medical records. PRN and NIM administration records will be extracted for the study period 1st July 2016 to 30th June 2017 or until the date of death. Facility characteristics including size and staffing mix will also be collected.
Analysis. Baseline data collected at the index date will be analysed using descriptive statistics. Rates of PRN and NIM administration will be estimated. Potential predictors of PRN and nurse-initiated medication administration will be investigated using Poisson regression. The model will be adjusted for resident and facility level factors. Variability across different PSRACS may be explored in stratified analyses.
Discussion. An expert group will be convened to assess quality and safety issues arising from the audit. The group will then make recommendations regarding the safety and quality of PRN and NIM use in Victorian PSRACS. This may guide the possible development and implementation of a novel quality indicator pertinent to PRN medications in Victorian PSRACS.

494 Attitudes towards deprescribing: results of a nationally representative sample of older adults in the United States

Emily Reeve[1,2], Jennifer Wolff[3,4], Maureen Skehan[3], Elizabeth A Bayliss[5,6], Sarah Hilmer[1,7], Cynthia Boyd[3,4]. NHMRC CDPC, Kolling Inst of Medical Research, Fac of Med and Health, Univ of Sydney[1], Sydney, NSW, Aus; Geriatric Med Research and Coll of Pharm, Dalhousie Univ and Nova Scotia Health Auth[2], Halifax, NS, Can; Dept of Health Policy and Management, Johns Hopkins Bloomberg Sch of Public Health[3], Baltimore, MD, US; Center for Transformative Geriatric Research, Div of Geriatric Med and Gerontol, Johns Hopkins Univ Sch of Med[4], Baltimore, MD, US; Inst for Health Research, Kaiser Permanente Colorado[5], Aurora, CO, US; Dept of Family Med, Univ of Colorado Sch of Med[6], Aurora, CO, US; Depts of Clinical Pharmacol and Aged Care, Royal North Shore Hosp[7], Sydney, NSW, Aus

Introduction. Use of harmful and/or unnecessary medications in older adults is prevalent. Primary care clinicians report that patient resistance to medication withdrawal is a significant barrier to deprescribing. Although patient engagement is critical in safe and effective medication use, nationally representative data regarding patient perspectives on medication use and willingness to consider discontinuation of medications are notably absent.
Aims. To describe the attitudes of older adults towards deprescribing and determine whether individual characteristics are associated with these attitudes.
Methods. Population-based observational study of US Medicare beneficiaries 65 years and older. Data were taken from the Medication Attitudes module (n=2124) in round 6 of the National Health and Aging Trends Study. The questions in this module were drawn from the Patients' Attitudes Towards Deprescribing questionnaire. Sampling weights were applied to take into account survey nonresponse and differential probabilities of selection.
Results. Ninety-two percent of older adults reported being willing to stop one or more of their medicines if their doctor said it was possible and 67% wanted to reduce the number of medicines that they are taking. Older adults taking six or more medications had greater odds than those taking less than six medications of being willing to stop taking one or more of their medicines (adjusted odds ratio (aOR)=2.90, 95% CI=1.74-4.82) and wanting to reduce the number of medicines that they are taking (aOR=2.31, 95% CI=1.71–3.13).
Discussion. Healthcare providers considering deprescribing as part of comprehensive, patient centered care, should be reassured that the vast majority of older adults are open to having one or more of their medicines stopped if their doctor said it was possible, and more than two-thirds want to reduce their number of medicines.

495 Proton pump inhibitor use in residential aged care services: does it pass the acid test?

Ivanka Hendrix[1], Amy Page[2], Maarit Korhonen[2,3], J Simon Bell[1,2,4], Edwin CK Tan[2], Renuka Visvanathan[1], Tina Cooper[5], Leonie Robson[5], Janet K Sluggett[2,4]. NHMRC CRE in Frailty & Healthy Ageing, The University of Adelaide[1], Adelaide, SA, Australia; Centre for Medicine Use and Safety, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University[2], Melbourne, VIC, Australia; Institute of Biomedicine, University of Turku[3], Finland; NHMRC Cognitive Decline Partnership Centre, Hornsby Ku-ring-gai Hospital[4], Hornsby, NSW, Australia; Resthaven Inc.[5], Adelaide, SA, Australia.

Introduction. While proton pump inhibitors (PPIs) are generally considered safe and well tolerated, frail older people who take PPIs long-term may be susceptible to dose-dependent adverse events.
Aims. To determine the prevalence of PPI use in residential aged care services (RACSs) and factors associated with high dose PPI use in this setting.
Methods. A cross sectional study of 383 residents in six Australian RACSs was conducted. Clinical, diagnostic and medication data were collected by study nurses. The proportions of residents who took PPIs for more than eight weeks, those with documented indications and residents receiving a concurrent medication associated with increased bleeding risk were calculated. Age and sex-adjusted logistic regression models were used to identify factors associated with high-dose PPI use compared to standard/low doses.
Results. 196 (51%) residents received a PPI, with 46 (23%) prescribed a high dose. Overall, 173 (88%) PPI users had documented clinical indications or received medications that can increase bleeding risk. Three quarters of PPI users with gastro-oesophageal reflux disease or dyspepsia had received a PPI for more than eight weeks. High dose PPI use was associated with increasing medication regimen complexity (odds ratio (OR) 1.02, 95% CI 1.01-1.04 per one point increase in Medication Regimen Complexity Index score) and a greater number of regular charted medications (OR 1.11, 95% 1.01-1.21 per additional medication).
Discussion. Half of all residents received a PPI, of whom nearly nine in ten had documented clinical indications or received medications that may increase bleeding risk. Residents who received a high dose PPI were more likely to take multiple medications or have complex regimens. Most PPI use was consistent with guidelines but confirmation of clinical indications for residents taking PPIs >8 weeks and 'step-down' approaches for high dose PPI users may reduce the likelihood of adverse events.

496 Multiple antihypertensive use and the risk of mortality in residents of aged care services: a prospective cohort study

Miriam Kerry[1], J Simon Bell[1], Claire Keen[1], Janet K Sluggett[1], Jenni Ilomäki[1], Natali Jokanovic[1], Tina Cooper[2], Leonie Robson RN[2], Edwin CK Tan[1]. Centre for Medicine Use and Safety, Monash University[1], Parkville, VIC, Australia; Resthaven Incorporated[2], Adelaide, SA, Australia

Introduction. The risk-to-benefit ratio for treating hypertension in older people with functional and cognitive impairment is widely debated.
Aims. To investigate the association between multiple antihypertensive use and mortality in residents with hypertension, and the moderating effects of dementia and frailty on this association.
Method. This was a two-year prospective cohort study involving 239 residents with diagnosed hypertension receiving antihypertensive therapy across six residential aged care services in South Australia. Data were obtained from electronic medical records, medication charts and validated assessments. All-cause mortality was the primary outcome. Inverse probability weighted Cox proportional hazard regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Covariates included age, sex, dementia severity assessed using the Dementia Severity Rating Scale, frailty status assessed using the FRAIL-NH scale, Charlson's Comorbidity Index and cardiovascular comorbidities.
Results. The study sample (mean age of 88.1±6.3 years; 79% female) included 70 (29.3%) residents using one antihypertensive medication and 169 (70.7%) using two or more antihypertensives. The crude incidence rates for death were higher in those who used two or more antihypertensives compared with monotherapy (173 and 251/1000 person-years, respectively). After adjusting for potential confounders, residents who used two or more antihypertensives had a greater risk of mortality compared with monotherapy (HR 1.41, 95%CI 1.03 – 1.93). After stratifying by dementia diagnosis and frailty status, the risk remained significant only in those with diagnosed dementia (HR 1.83, 95%CI 1.16-2.89) and who were most frail (HR 2.74, 95%CI 1.15-6.53).
Discussion. Multiple antihypertensive use is associated with an increased risk of mortality over a two-year follow-up in residents with diagnosed hypertension. This risk is greatest in residents with dementia and among those who are most frail.

497 Use of HMG-Co-A-reductase inhibitors (statins) and risk of fall-related hospital admissions in residents of aged care facilities: a case-control study

Kate N Wang[1,3], J Simon Bell[1,2], Julia FM Gilmartin-Thomas[2], Edwin CK Tan[1], Michael J Dooley[3], Jenni Ilomäki[1,2]. Centre for Medicine Use and Safety[1], Monash University, Parkville, VIC, Australia; Department of Epidemiology and Preventive Medicine[2], Monash University, Melbourne, VIC, Australia; Pharmacy Department[3], Alfred Health, Melbourne, VIC, Australia

Introduction. Statins are widely prescribed in residential aged care facilities (RACFs) but have been associated with muscle-related adverse events. The rate of falls in RACFs is up to three times higher than in community settings.
Aims. To investigate the association between statins and fall-related hospital admissions among residents of RACFs.
Methods. The study sample included 336 RACF residents admitted to hospital after presenting to the Alfred Emergency and Trauma Centre between July 2013 and June 2015. Cases were residents with fall-related hospital admissions, and controls were residents admitted for infections. Cases and controls were matched 1:1 by age (±2 years), index date (± 6 months) and sex. Conditional logistic regression was used to compute adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for factors associated with fall-related hospital admissions. The risk of fall-related hospital admissions was compared among users of high versus low/moderate intensity statins. Stratified analyses were performed for residents with and without dementia.
Results. Overall, 32% of cases and 22% of controls used statins. After adjusting for clinically important covariates, statin users had over two times higher adjusted odds of a fall-related admission than non-users (adjusted OR=2.19, 95% CI 1.20-4.02). This association was significant among residents with dementia (adjusted OR=3.27, 95% CI 1.30-8.23) but not among those without dementia. High intensity statin users were not significantly more likely to be admitted for falls compared to low/moderate statin intensity users (adjusted OR=1.92, 95% CI 0.70-5.30).
Conclusion: Statins are associated with an increased risk of fall-related hospital admissions from RACFs, particularly among residents with dementia. However, there is minimal evidence for a dose-response relationship between statin intensity and fall-related hospital admissions.

498 Treatment Initiation for Type 2 Diabetes in Australia: Are the guidelines being followed?

Stephen Wood[1], J Simon Bell[1, 2], Dianna J Magliano[3], Jenni Ilomäki[1], Claire Keen[1]. Centre for Medicine Use and Safety, Monash University[1], Melbourne, VIC, Australia; School of Pharmacy and Medical Sciences, University of South Australia[2], Adelaide, SA, Australia; Baker Heart and Diabetes Institute[3], Melbourne, VIC, Australia.

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

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

Rong Hu[1], Daniel T Barratt[1], Janet K Coller[1], Benedetta C Sallustio[1,2], Andrew A Somogyi[1,3]. Disc of Pharmacology, Univ of Adelaide[1], Adelaide, SA; Dept of Clinical Pharmacology, Queen Elizabeth Hospl[2], Adelaide, SA; Dept of Clinical Pharmacology, Royal Adelaide Hospl[2], Adelaide, SA.

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

[1] LaRosa DF (2007) J Immunol 178:7503-9; [2] De Serres SA (2012) Clin J Am Soc Nephrol 7:1018-25; [3] Goldfarb-Rumyantzev AS (2010) Nephrol Dial Transplant 25:1039-47; [4] Chen L (2006) Am J Transplant 6:2282-91.

500 A narrative review of key stakeholder perspectives on the translation of pharmacogenomics into clinical practice

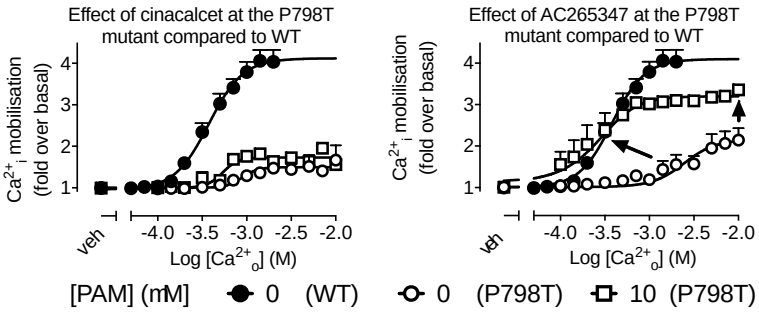
Priya Iyer, Adam La Caze & Christopher Freeman. School of Pharmacy, The University of Queensland, Brisbane, QLD, Australia.

Introduction. Since the completion of the Human Genome Project, there has been continued hope that our growing understanding of genetics will revolutionise the practice of medicine. The use of pharmacogenomic tests in clinical practice may assist in individualising drug treatment for patients. However, this hype is not yet a reality with the translation of pharmacogenomics into clinical practice still far from routine.
Aims. The aim of this study was to synthesise studies on clinicians, pharmacists, genetic counsellors, nurses and other non-medical prescribers, patients and the general public perspectives and attitudes towards pharmacogenomic testing.
Methods. A review of literature reporting stakeholder views on pharmacogenomic testing was conducted using PubMed, EMBASE, Scopus, PsycINFO, CINAHL and INFORMIT from inception to August 2018.
Results. Majority of the literature found studies conducted in the US with the surveys utilised as the main study design. There was an overlap across stakeholder perspectives regarding lack of knowledge about pharmacogenomic testing but general support of the technology. Views on issues related to privacy, cost and test result dissemination varied by stakeholder. Overall, clinicians and pharmacists envision a major role in the delivery and interpretation of pharmacogenomics testing but recognise their lack of adequate knowledge and experience with pharmacogenomic testing.
Discussion. As the clinical utility of pharmacogenomic testing for particular drugs becomes more apparent, key stakeholders will have an important role to play in the translation of this information. Health professionals' willingness and ability to participate in the provision of pharmacogenomic services will strongly influence appropriate use of these tests. Limited research has been conducted in Australia on key stakeholder perspectives regarding the translation of pharmacogenomics into clinical practice. The advancement of pharmacogenomics into clinical practice is inevitable and therefore acceptance and understanding by key stakeholders is essential. In Australia, pharmacogenomics testing is already available in practice. It is something health professionals are likely to engage with at some point in their career so it is important that they are adequately prepared for those situations.

501 Pharmacogenomic considerations for the treatment of neonatal severe hyperparathyroidism (NSHPT) and familial hypocalciuric hypercalcaemia (FHH)

Katie Leach[1], Le Vi Dinh[1], Aaron Debono[1], Jiayin Diao[1], Andrew Keller[1], Tracy Josephs[1], David Shackleford[1], Ben Capuano[1], and Karen Gregory[1]. Monash Institute of Pharmaceutical Sciences[1], Parkville, VIC, Australia

Introduction. Cinacalcet is a calcium sensing receptor (CaSR) positive allosteric modulator (PAM) used off-label to treat NSHPT and FHH, disorders caused by loss of function/expression mutations in the CaSR or its signalling partners. However, some patients are unresponsive to cinacalcet. AC265347 is a CaSR PAM that may be better at rescuing loss of function mutations because it is also an agonist. However, unlike cinacalcet, AC265347 cannot pharmacochaperone loss of expression mutants. This attests to the need for more effective CaSR PAMs/agonists that can rescue signalling and expression of mutant CaSRs.



Aims. To synthesise novel AC265347 analogues and compare cinacalcet, AC265347 and our novel analogues for their ability to rescue mutant CaSR signalling and expression.
Methods. CaSR PAMs were evaluated in HEK293 cells for their ability to rescue CaSR mutant signalling in Ca^{2+}_i mobilisation assays, and CaSR mutant expression using FACs analysis.
Results. AC265347 was effective at rescuing the signalling of mutants that were unresponsive to cinacalcet. However, like AC265347, none of our novel AC265347 derivatives rescued the expression of a loss of expression mutant, even though AC265347 and its analogues were all predicted to cross the plasma membrane and reach intracellularly-retained mutants.
Discussion. There remains a need to understand how PAMs pharmacochaperone CaSR mutants in order to identify novel PAMs that can rescue both the signalling and expression of CaSR mutations that cause NSHPT and FHH.

502 Aboriginal Australians have higher frequencies of variant alleles for drug efflux transporters and lower frequencies for uptake transporters compared to Europeans: implications for drug efficacy and toxicity.

Andrew A Somogyi, Daniel T Barratt. Discipline of Pharmacology, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia.

Introduction. Uptake and efflux drug transporters can be important determinants of drug efficacy and toxicity. Drug interactions and genetics affecting these transporters are becoming increasingly recognised as factors to be considered in drug prescribing. Nothing is known on whether the pharmacogenetics of these transporters in Aboriginal Australians is significantly different to Europeans.

Aims. To determine the frequencies of common SNPs in selected efflux (ABCB1: P-glycoprotein, ABCG2: BCRP) and uptake (SLC22A1: OCT1, SLCO1B1: OATP1B1) transporter genes which alter drug efficacy and toxicity.

Methods. Following ethics committees' approvals and informed consent, 148 Aboriginal Australians (AA) from Southern Australia and 173 European Caucasians (EC) provided a saliva sample using Oragene DNA Saliva Kits (DNA Genotek Inc. Canada). Samples were transported unrefrigerated to Adelaide for DNA isolation and genotyping by Affymetrix DMET Plus array for 5 *ABCB1*, 1 *ABCG2*, 1 *SLCO1B1* and 4 *SLC22A1* SNPs.

Results. For ABCB1, there were no differences in 61A>G and 1199G>A, but 1236C>T, 2677G>T/A and 3435C>T genotype differences were highly significant (P= 2 x 10^{-8} to 5 x 10^{-6}), with higher variant T allele frequencies in AA (Odds Ratios 2.2-2.8, P<0.0001). The most common ABCB1 haplotype in EC was AGCGC (40%), which was less common in AA (19%). For ABCG2, the 421C>T frequency was 14% in AA and 7% in EC (OR=2.1; P=0.006). In contrast, the SLCO1B1 521C>T frequency was 8% in AA and 17% in EC (OR=0.42 P=0.0008). The SLC22A1 **2* deletion was 12% in AA and 18% EC (P=0.03), and **4* was 1% and 5%, respectively (P=0.01), resulting in 69% of AA having 2 functional alleles versus 49% for EC.

Discussion. Aboriginal Australians have higher frequencies of variants in P-glycoprotein and BCRP efflux transporters making them more vulnerable to toxicity of drugs in selected tissues (e.g. brain). Conversely, lower frequencies of the uptake transporter variants would suggest better efficacy of metformin via OCT1 and less muscle pain with statins via OATP1B1.

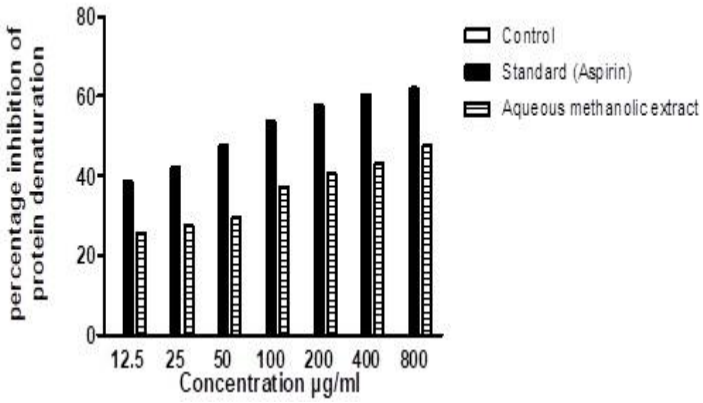
503 Evaluation of Anti Arthritic Activity of *Citrullus Colocynthis (L.) Schrad* by using *In-Vitro* Technique

Haseeb Ahsan[1*], Muhammad Naveed Mushtaq[2], Irfan Anjum[2]. Pharmacology, University of Sargodha [1], Sargodha, Pakistan; Pharmacology, university of Lahore [2], Lahore, Pakistan

Background and Objectives: *Citrullus colocynthis (L.) Schrad* has been used traditionally to treat rheumatism but pharmacological evidence of this effect has not been reported yet. The aim of the current study was to evaluate the effectiveness of *Citrullus colocynthis* (CC) by using *in vitro* technique.

Methods: Membrane stabilization and protein denaturation were performed for *in-vitro* study at concentration of 12.5-800 ug/ml.

Results: Our results showed dose dependent decrease in albumin denaturation and reasonable membrane stabilization. Significant effects were produced at a dose of 800 ug/ml.

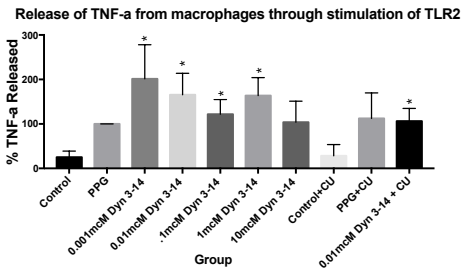


Conclusion: The present study has provided pharmacological evidence for the conventional use of *Citrullus colocynthis* in rheumatism. It becomes necessary to investigate the exact mode of action of phytochemicals present in *Citrullus colocynthis* (L.).

Figure 1. Effect of different concentrations of *C. colocynthis* on inhibition of bovine serum albumin

504 Dynorphin 3-14 Modulation of Peptidoglycan induced TNF-alpha release in macrophages

Margaret R A Perona[1], Peter J Cabot[1]. School of Pharmacy, University of Queensland[1], Woolloongabba, QLD, Australia.



Introduction. Endogenous Dynorphin 3-14 (DYN 3-14) is the primary non-opioid fragment metabolized from Dynorphin A in inflammation. Previous studies have shown DYN 3-14 to have activity at toll-like receptor (TLR) 4. TLRs are pattern-recognition receptors key to immune system activation and the inflammatory process (Rahiman et al, 2017). Given the impact on TLR4 it's possible that DYN 3-14 also has other effects on immunomodulatory processes and other TLR's.
Aims. Our aim was to determine the concentration-dependent relationship of DYN 3-14 on peptidoglycan (PPG) induced TNF-alpha release. Secondly, assess the selectivity of DYN 3-14 and PPG for TLR2/TLR1 through the use of the selective TLR2/TLR1 antagonist CUCPT22.
Methods. THP-1 cells were cultured in RPMI1640 medium and differentiated into macrophages for experimentation. 0.001mM, 0.01mM, 0.1mM, 1mM and 10mM concentrations of DYN 3-14 were added to wells in the presence of the previously optimized 0.1% PPG. 5mM CUCPT22 was added to wells with 0.1% PPG and +/- 0.01mM DYN 3-14. The two controls were RPMI1640 medium and 0.1% PPG. TNF-alpha in the supernatant was quantified using an alphalisa (Perkin Elmer™) on an Enspire-Alpha 2390 Multilabel Plate Reader.
Results. 0.001mM, 0.01mM, 0.1mM and 1mM DYN 3-14 caused a statistically significant increase in TNF-a release compared to PPG effects ($P<0.05$, test). CUCPT22 did not significantly reduce PPG evoked TNF-alpha release ($P>0.05$, test).
Discussion. Dynorphin 3-14 augmented TNF-alpha release caused by PPG. In addition, CUCPT22, a TLR2/TLR1 antagonist, did not significantly reduce the activity of PPG. However, the increases seen with 0.01mM DYN 3-14 were significantly reduced by CUCPT22, indicating the potential involvement of TLR1/2 in this response. It is possible that one or both of these substances are acting through different TLRs (i.e. the TLR2/TLR6 dimer) or other receptors involved in TNF-alpha release from macrophages. Indeed, TNF-alpha can be released through a multitude of complex pathways, so more research is needed to determine the mechanisms behind activity of both PPG and DYN 3-14.
Rahiman SSF et al (2017) Peptides 90:48-54

505 SOD off (or on?): mitotronics - powering inflammation and immunity

Tim Shaw[1,3], Andrew Peel[2,3]. Victorian Infectious Diseases Reference Laboratory, The Peter Doherty Institute[1], Melbourne, VIC, Australia; Scram Software[2], Melbourne, VIC. Australia; NucleopharmGT[3], Melbourne, VIC. Australia.

Introduction. Mitochondria, the evolutionarily ancient organelles that evolved from bacterial endosymbionts, are involved in a plethora of vital cellular functions related to bioenergetics, metabolism and apoptosis. Recent research has revealed that they also play a major role in inflammation and immunity and act as both a both a source of, and sink for, hydrogen peroxide, a primordial signaling molecule [1]. Hydrogen peroxide is generated enzymatically by superoxide dismutases, which act to rapidly detoxify otherwise potentially toxic superoxide, a byproduct of respiration and other metabolic activities. Mammalian genomes contain three different SOD genes which are differently regulated. Their protein products SODs 1, 2 and 3 are directed to the cytosol, mitochondrial matrix and extracellular space respectively. Charge transfer from superoxide can generate reactive oxygen species (ROS) which have important roles in cell signaling and defence.
Aims. To generate biologically rational models for the charge transfer reactions that link mitochondria to innate immunity and inflammatory responses and to devise testable electronic and mathematical analogues of these models.
Methods. A variety of resources that are freely accessible *via* the internet were used to identify and analyse regulatory elements that control the expression of SOD genes. Interactions were determined or predicted based on published experimental evidence.
Results and Discussion. The proximal promoters of the three SOD genes contain G-rich sequences which have the potential to form G-quadruplexes. These non-canonical structures can act as redox-sensitive switches [2], which under normal physiological conditions, appear to be on for SOD1 but off for SOD2 and SOD3, consistent with expression SOD1 being constitutive and with expression of SOD2 and SOD3 being inducible. Genetic-biochemical models for control of SOD gene expression and product interaction were developed and used to design testable mathematical and electronic analogues on which to base further work.

1. Munro D, Treberg JR (2017) J Exp Biol. 220:1170-1180.
2. Fleming AM *et al.* (2017) ACS Chem Biol. 12:2417-2426.

506 The use of non-steroidal anti-inflammatory drugs in an Australian elite athlete population.

Thomas Eason[1], Julie Cooke[1], Alison Shield[1] Faculty of Health, University of Canberra[1], Bruce, ACT, Australia.

Introduction. Non-steroidal anti-inflammatory medications (NSAIDs) are reported to be highly used by elite athletes, and their knowledge regarding the proper indications and side effects is often poor. Identifying areas where these athletes lack knowledge could lead to interventions to improve their health outcomes and minimize the risks associated with these medications.
Aims. The aim of this study was to determine how a representative sample of Australian elite athletes is using NSAIDs and their knowledge and perceptions of these medications.
Methods. An online survey with participant information was distributed to physically active adults for completion.
Results. A total of 379 participants completed the survey; 80 elite or professional athletes completed the survey, had utilized NSAID medication within the prior 6 months and were included in further analysis. The majority of participants (85%) used NSAIDs for the evidence-based indication of treating an acute injury, and 78% followed the instructions on the packaging regarding dosage. However, the athletes were unclear as to whether NSAIDs should be used prophylactically or to treat delayed onset muscle soreness (DOMS). They also lacked knowledge regarding side effects, with 30% suggesting liver damage as a side effect and 26% suggesting constipation, both of which are incorrect responses. Eleven percent of athletes reported having experienced an adverse reaction to NSAID use, although up to 30% admitted to knowing no side effects of NSAIDs. Only 52% perceive additional risk with using multiple NSAIDs concurrently.
Discussion. There was high use of NSAIDs among the participants, with most utilizing them for acute injury healing. Their perceptions and knowledge of side effects showed a general lack of knowledge regarding how these medications should be used. Improving their knowledge in these areas would reduce the risk of inappropriate use of these medications and reduce their risk of side effects.

507 SMRT is essential for phospholipase D dependent glucocorticoid insensitivity

Alastair G. Stewart[1], Yuxiu C. Xia[1], Trudi Harris[1], Shenna Y. Langenbach[1], Qianyu H. Chen[1], Meina Li[1]. [1]Lung Health Research Centre, Department of Pharmacology & Therapeutics, School of Biomedical Science, University of Melbourne, Parkville, Victoria 3010, Australia

Introduction. Our previous work has provided strong evidence for a role of phopsho-cofilin1 in glucocorticoid (GC) insensitivity induced by TGF-β1 in airway epithelia. Phospho-coflin1 has been well-documented as an activator of phospholipase D (PLD) [1, 2], raising the possibility that PLD activation may contribute to GC insensitivity.
Aims. To investigate the potential link between PLD activation and GC insensitivity; and to further investigate the underlying mechanism of PLD activation-impaired GC activity
Methods. Phospho-cofilin1 and PLD expression was assessed by immunohistochemistry (IHC) in human asthmatic cohort. *In vitro* GC activity in BEAS-2B cells was assessed by RT-qPCR and GRE-SEAP assays. The potential involvement of PLD in these settings was ascertained using pharmacological (VU0155069/FIPI) and genetic (siRNA) inhibition, as well as by the addition of exogenous PLD products ((lyso)phosphatidic acid). Data are presented as the mean ±SEM for *n* independent experiments.
Results. Severe, steroid-resistant asthmatic airway epithelium showed increased levels of immunoreactive phospho-cofilin1 and PLD1/2. Phospho-cofilin1 was implicated in the activation of phospholipase D (PLD). The PLD products (lyso)phosphatidic acid mimicked the TGF-β1-induced GC insensitivity in airway epithelia. TGF-β1 induction of the nuclear hormone receptor corepressor, SMRT (NCOR2), was dependent on cofilin1 and PLD activity. siRNA-mediated depletion of SMRT prevented GC insensitivity induced by TGF-β1.
Discussion. We show here for the first time that the products of PLD-mediated membrane phospholipid remodelling, lyso-phosphatidic acid and phosphatidic acid, induce GC insensitivity. This pathway for GC insensitivity offers several promising drug targets that potentially enable safer modulation of TGF-β1 in chronic inflammatory diseases than is afforded by global TGF-β1 inhibition.

[1] Han, L., *et al.* (2007). EMBO J *26*, 4189-4202.
[2] Han, X., *et al.* (2011). Mol Microbiol *81*, 860-880.

508 SULPHASALAZINE (SSZ, Salazopyrin), an enigmatic drug for chronic inflammation: some insights from antibacterial assays *in vitro*.

MW Whitehouse[1,2], W XI[2,3], P Peng[2,3], IE Cock[2]. Schools of Medicine[1] and Environment & Science[2], Griffith Univ, QLD, Australia. School of Pharmacy[3], Nanging Univ of Chinese Medicine, China.

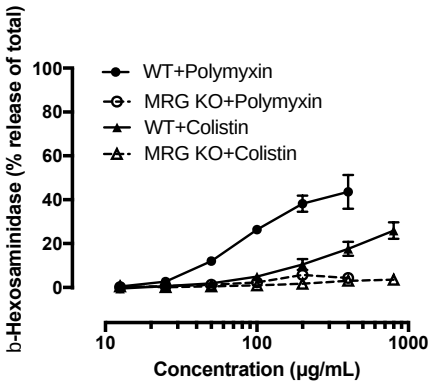
Introduction. SSZ was introduced in the 1940's (1) to treat bowel infections, then believed to be possible causes of rheumatoid arthritis (RA), ankylosing spondylitis (AS), and ulcerative colitis. Today it is valued as a drug for treating prodromal signs of RA.
Methods. SSZ and its two major metabolites, sulphapyridine (SP aka M&B693), an antibacterial and 5-aminosalicylate (5-AS), an antoxidant, were compared for their *in vitro* effects upon a) *Proteus* spp. that may cause RA, b) *Klebsiella pneumoniae* that may cause AS and c) *Acinetobacter baylyi* that may cause multiple sclerosis.
Results. SSZ was an effective (pro)-drug having significant anti-microbial activity against *Proteus mirabilis*, *P.vulgaris*, and *Klebsiella pneumoniae*. These three organisms were unresponsive to penicillin or erythromycin. *Proteus* spp. may contain an azoreductase (2) so might generate SP and 5-AS *in cellulo*. Synergistic effects were observed with tetrocycline and SSZ (*P.vulgaris*); tetracycline and 5-AS (*P.vulgaris*); chloramphenicol and 5-AS (*P. mirabilis*). *A.Baylyi* was more responsive to SSZ than to SP or 5-AS.
Conclusion. Antibiotic cocktails may be the way to maximise the benefits of SSZ, used as a delayed-release prodrug against pathogens in the lower gut that contain an azoreductase.

Swartz N (1942). J Int Med 110:577-598
Roxon JJ et al (1967) Food Chem Toxic. 5:645-656

509 Mechanism of human mast cell activation by polymyxins

Stephanie Zhang[1], Nithya Fernandopulle[1], James Ziogas[1] & Graham Mackay[1]. Department of Pharmacology and Therapeutics, University of Melbourne[1], Parkville, VIC, Australia.

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



Aims. To investigate the role of MRGPRX2 in the activation of human mast cells by two clinically used polymyxins, polymyxin B and colistin.
Methods. The FcεRI and MRGPRX2 expressing LAD2 human mast cell line was used to characterise the degranulation, calcium mobilization, cytokine release and cell viability following treatment with polymyxin B and colistin. CRISPR knock-down MRGPRX2 LAD2 cells were used to investigate the dependence of MRGPRX2 to the actions of the polymyxins.
Results. Polymyxin B and colistin caused degranulation, calcium mobilization and cytokine production, with no cytotoxicity, in LAD2 cells in a concentration-dependent manner. As shown in the graph, colistin was less active than polymyxin B in eliciting degranulation. In contrast, the two drugs had equivalent activity in calcium mobilization and cytokine production. Responses were ablated by greater than 90% in the MRGPRX2 knock-down cells.
Discussion. Polymyxin B and colistin activation of mast cells was shown to be mediated through MRGPRX2 and might contribute to patient hypersensitivity to these drugs. Better understanding the difference in mast cell degranulation induced by polymyxin B and colistin might enable more selective use of these drugs in clinical situation.

510 Toxicity of herbal phytochemicals: the role of cytochrome induction

Susan M Britza[1], Ian F Musgrave[1], Roger W Byard[2, 3]. Dept of Pharmacol, Univ of Adelaide[1], Adelaide, SA, Australia; Dept of Pathol, Forensic Science SA[2], Adelaide, SA, Australia; Dept of Pathol, Univ of Adelaide[3], Adelaide, SA, Australia.

Introduction. Complementary medicine use is increasing within Australia with a significant proportion being herbal medicines. While herbal medicines are widely considered safe, fatalities and severe adverse effects have been reported in Australia from their use. The growing use of multiple herbal preparations represents a poorly appreciated source of adverse reactions. A recent case of fatal hepatotoxicity was believed to be due to pharmacokinetic interactions between the *Psoralea corylifolia* toxic component, psoralen, a CYP3A4 inhibitor, astragaloside IV (AST-IV), from *Astragalus propinquus*, and *Atractylodes macrocephala*, atractylenolide I (ATR-I). Toxicity pathways of psoralen are unknown, though may follow similar pathways to known toxic chemical coumarin, thus CYP3A4 inhibition would result in increased toxicity. With over 50% of prescription medicines metabolized by CYP3A4 enzymes, and many inhibiting these enzymes, investigating the effect of CYP3A4 enzyme modulation on herbal toxicity is important.
Aims. To investigate the effect of CYP3A4 induction on toxicity of the key phytochemicals listed above.
Methods. Psoralen, its derivative 8-MOP, AST-IV and ATR-I were tested for toxicity in liver (HepG2) and kidney (BHK-21) cell models both untreated and pre-treated with CYP3A4 inducer, rifampicin. Paracetamol was used as a positive control for toxicity. Cell viability assessed using MTT colorimetric assays.
Results. Paracetamol in untreated HepG2 cells showed toxicity at 50mM, with toxicity increasing with rifampicin, indicating the ability of rifampicin to modulate toxicity ($p<0.05$). Significant toxicity to 8-MOP was observed at 0.6mM-1mM in untreated HepG2 cells ($p=0.0016$), and 0.2mM-1mM in rifampicin treated cells ($p=0.0004$). 8-MOP was toxic at all concentration in BHK-21 cells both untreated ($p<0.0001$) and rifampicin treated ($p=0.0053$). Psoralen was toxic in HepG2 cells at 0.6mM-1mM untreated and treated ($p=0.0184$; $p=0.01$), and BHK-21 at 0.4mM-1mM in untreated and treated ($p=00016$; $p=0.0015$). AST-IV and ATR-I were not toxic in either cell line, untreated or treated ($p>0.05$).
Discussion. Herb-herb interactions are poorly understood, with the metabolic pathways of active phytochemicals present in herbs remaining largely unknown. The results demonstrated that induction of CYP3A4 enzymes does not increase toxicity of psoralen, suggesting that psoralen breakdown mechanisms may not be identical to coumarin. Future experiments will be used to confirm this by inhibition of CYP3A4.

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

Lydia Conti[1,3], John Mach[1,2,3]. Victoria Cogger[3,4], Catriona McKenzie[3], David Le Couteur[3,4], & Sarah N Hilmer[1,2,3]. Kolling Institute, Royal North Shore Hospital and University of Sydney, Sydney, NSW, Australia[1]. Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia[3]. The ANZAC Research Institute, Sydney, NSW, Australia[4].

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

512 Evaluation of an early 20th century Afghan herbalist's preparations

Rachael Farrington[1], Ian Musgrave[1], Christine Nash[2], Meghan Coghlan[3], Roger Byard[1,2]. School of Medicines, The University of Adelaide, Adelaide, SA, Australia[1]; Forensic Science SA, Adelaide, SA, Australia[2]; Trace and Environmental DNA laboratory, Department of Environment and Agriculture, Curtin University, Bentley, WA, Australia.

Introduction. Mahomet Allum was a flamboyant philanthropist and herbalist who worked in South Australia in the early part of last century, whose herbal therapies generated some controversy at the time. Two of his preparations have survived to the present day, a general tonic and a treatment for liver and kidney dysfunction.
Aims. Given the frequent use of pharmaceutical drugs in "tonics" at the time toxicological analysis was undertaken at Forensic Science SA to determine if these historical "herbal" preparations contained contemporary drugs.
Methods. Liquid chromatography/quadrupole-time-of-flight mass-spectrometer (LC-QTOF MS), liquid-chromatography/ diode array detector (LC/UV) and gas chromatography/ nitrogen phosphorous- detector/mass-spectrometer (GC-NPD/MS) to look for common drugs. In addition DNA analysis was also undertaken at Curtin University to evaluate the types of plant products used to make these remedies.
Results. The general tonic contained genera from the Triticeae (wheat) family as well as Medicago family (includes alfalfa), possibly as fillers. Other genera found included *Utrica* (nettle) and *Passiflora* (passion flower). The preparation for liver and kidney disease also contained genera from the Medicago family as well as genera *Arctostaphylos* (bear berry) which traditionally has been used for the treatment of dysuria and bladder stones.
Discussion. No common drugs were found. Thus it appears that the two treatments prepared by Mahomet Allum contained only herbal substances consistent with Middle Eastern herbal traditions and not adulterant pharmaceutical agents. The herbals identified provide an insight into herbalist practices in the early 20th century.

513 Using single-ended transition state searching to mechanistically assess chemical skin sensitisation potential

Davy Guan[1], Slade Matthews[1]. Discipline of Pharmacology, The University of Sydney[1], Sydney, NSW Australia

Introduction. Skin sensitisation is a toxicological endpoint that is characterised by chemical interactions between the toxicant and predominantly nucleophilic protein moieties leading to the development of lifelong allergic contact dermatitis following dermal chemical exposure. In contrast to previous work finding chemical reactivity to be the key determinant in the solicitation of the immune response, the use of *in silico* methodologies to mechanistically study chemical reactivity from first principles have been limited due to the high computational costs, low throughput, and limited chemical applicability. In comparison to previous transition state search techniques, the recent single-ended growing string method does not require the product structure to reliably model reactions from the reactants to the transition states and products. This *in silico* method could enable efficient determination of thermodynamic reaction quantities across different reactive mechanisms which could be used to vastly improve data gap filling methodologies.
Aims. This study aims to determine the capability and feasibility of the single-ended growing string method for modelling chemical reactions associated to the development of skin sensitisation.
Methods. Six small molecules varying in EC3 sensitisation potential with either nucleophilic substitution (propiolactone), Michael addition (3,4-Dihydroxy-3-cyclobutene-1,2-dione (DHCBD), benzoquinone, acrolein), or acylating agent (formaldehyde, iodoacetamide) mechanistic domains were selected to computationally react with methanethiol. The single-ended transitional state search method identified transition structures which enabled quantification of the activation energy for each mechanistic reaction. All structures were optimised with the MMFF94 forcefield with transition state search calculations conducted at the default B3LYP/6-31G* level of theory.
Results. Weak or non-sensitising chemicals (iodoacetamide, acrolein, isopropanol control) did not react with methanethiol substantially enough to produce transition states that could quantify the activation energy. Moderate to strong sensitisers (formaldehyde, propiolactone, DHCBD) feature positive activation energy values ranging over 200 kJ/mol, while the extreme sensitiser (benzoquinone) found a negative activation energy value of -85 kJ/mol.
Discussion. Activation energy values generally followed a negative correlation with sensitisation potential, agnostic of distinct skin sensitisation mechanistic domains. Further development of this method could enable the toxicological characterisation of chemicals that have not been as well studied, such as metallic systems in skin sensitisation.

514 Using deep learning to improve chemical representation in skin sensitisation Quantitative Structure Activity Relationship models

Davy Guan[1], Slade Matthews[1]. Discipline of Pharmacology, The University of Sydney[1], Sydney, NSW Australia

Introduction. Quantitative Structure Activity Relationship (QSAR) models utilise experimental data to mathematically associate a computational chemical representation to biological endpoints. Key endpoints with limited data availability such as skin sensitisation have not been well-characterised with global QSAR models. Sequence to sequence (Seq2Seq) deep learning models aim to construct a compressed representation of the input data, presently SMILES strings, which can then be mapped to output text sequences to reconstruct the original input. This representation retains pertinent features and is used for skin sensitisation QSAR models.
Aims. We aim to compare QSAR model performance in low data domains with Morgan fingerprints (MorganFP) or Seq2Seq representations.
Methods. 729,000 SMILES strings were extracted from the DSSTOX database for toxicological Seq2Seq model construction with 10% held out for validation. 297 Local Lymph Node Assay (LLNA), 130 KeratinoSens, and 99 h-CLAT chemical assay results from SkinSensDB composed the skin sensitisation QSAR modelling datasets, after 35 molecules were held out for external validation. NaiveBayes and RandomForest machine learning algorithms modelled Seq2Seq or MorganFP subsets to assay outcomes and were assessed with 10x10-fold cross validation.
Results. MorganFP QSAR models found better training performance than Seq2Seq QSAR models for KeratinoSens (0.92 vs 0.76 auROC), LLNA (0.91 vs 0.83 auROC), and h-CLAT (0.91 vs 0.90 auROC). However, Seq2Seq QSAR models feature greater external validation performance than MorganFP models for KeratinoSens (0.68 vs 0.58 auROC), LLNA (0.66 vs 0.58 auROC), and h-CLAT (0.582 vs 0.514 auROC).
Discussion. Seq2Seq QSAR models were more resistant to overfitting issues with small datasets that lead to QSAR unreliability, resulting in improved external validation performance for skin sensitisation assays.

515 Strain-specific Ames mutagenicity modelling using multitask deep learning

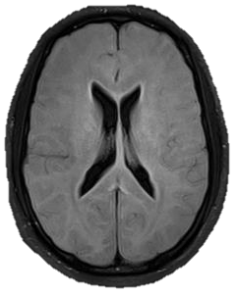
Raymond Lui[1], Davy Guan[1], Slade Matthews[1]. Pharmacoinformatics Lab., Discpl. Of Pharmacol., School of Med. Sci., Uni. of Sydney[1], Camperdown, NSW, Australia.

Introduction. The Ames test is a widely adopted *in vitro* assay that assesses the potential for chemicals to cause frameshift mutations or base-pair substitutions in the DNA of different strains of *Salmonella typhimurium* bacteria. Current *in silico* Ames QSAR models predict only a single positive/negative mutagenic outcome for an input chemical, which does not capitalise on related mechanistic information between strain activities.
Aims. Herein, we implement and evaluate a state-of-the-art multitask neural network to simultaneously model multiple *S. typhimurium* strain activities, compared to traditional 'single-task' QSAR architectures.
Methods. A cheminformatic approach was employed using datasets of chemicals tested on the TA97, TA98, TA1537, and TA1538 strains gleaned from the ISSSTY database. The data was curated by removing duplicate, metal-containing, and inorganic molecules, stripping salts and solvents, then neutralizing and recalculating atomic coordinates. We compare the predictivity of a multitask neural network modelling all strains, versus single random forest models for each strain. Model performance was measured by ROC AUC, a function of true positive rate against false positive rate.
Results. To date, we have successfully modelled four *S. typhimurium* strains (TA97, TA98, TA1537, TA1538). Mean ROC AUC on a held-out test set by the multitask neural network was 0.811. Mean test ROC AUC by the single-strain random forest models was 0.801.
Discussion. Predictions made by the multitask neural network (MT-NN) were marginally better than traditional random forest models, demonstrating potential for the MT-NN to share common chemical information to produce more generalisable predictions. Addition of more strain data into the MT-NN could incorporate more relevant mechanistic information to elucidate frameshift or substitution mechanisms of potential chemical mutagens.

516 Comparing molecular featurisation strategies for logP prediction

Raymond Lui[1], Davy Guan[1], Slade Matthews[1]. Pharmacoinformatics Lab., Discpl. Of Pharmacol., School of Med. Sci., Uni. of Sydney[1], Camperdown, NSW, Australia.

Introduction. There is a wide selection of chemical representations that generate molecular features, which can be correlated with physicochemical properties to develop Quantitative Structure-Property Relationships (QSPRs). The octanol/water partition coefficient, logP, is a toxicologically significant property which dictates membrane traversal and hydrophobic target interactions. There are few studies that compare the different features available to develop logP QSPR models.



Aims. We investigate the functionality of three chemical representations, encompassing global properties to specific functional groups, in predicting logP: physicochemical descriptors, structural keys, and circular fingerprints.

Methods. 13,343 chemicals were gleaned from the literature and featurised using the three aforementioned representations. We investigated their predictive performance in multilinear regression, support vector machine, and random forest algorithms using 707 held-out chemicals, as measured by root mean square error (RMSE). Real-world applicability of our best performing model was evaluated using an original dataset of regulatory-orientated molecules.

Results. Models using physicochemical descriptors predicted logP with the best accuracy regardless of algorithm used (1.11-1.29 RMSE). Our best model (1.11 RMSE) performed better than the established XLOGP method (1.31 RMSE) on the held-out chemicals. Model performance was comparable to XLOGP on the original regulatory-orientated dataset.

Discussion. Circular fingerprints and structural keys, which represent specific functional groups and molecular fragments, may better identify structural similarities within local chemical spaces. Conversely, where there is a lack of structural commonality between molecules, chemical similarities may be found instead through whole-molecule features as represented by physicochemical descriptors. Tailoring molecular feature representations to the chemical space could be one avenue in refining logP QSPR models to better predict unique, structurally complex toxicants.

517 A fatal case of toxic leukoencephalopathy from organic solvent abuse

Joanne Patel[1,2], Catherine J Lucas[1,2], Jessica Stabler[1], Allan Cala[1], Zahrul Ismadi[3], Ian Whyte[2,4], Jeanette Lechner-Scott[1,2], Jennifer H Martin[1,2]. John Hunter Hospital[1], Newcastle, NSW; University of Newcastle[2], NSW; NSW Health Pathology[3], Newcastle, NSW; Calvary Mater Newcastle[4], NSW.

Introduction. A 26 year old male presented with a four day history of ataxia, confusion and headache. An initial lumbar puncture and computed tomography (CT) scan of the brain revealed no abnormalities. Polypharmacy was initially thought to be the cause of his presentation, with multiple prescribed psychoactive drugs and many online purchased medications.

Results. . The patient deteriorated over several days as an inpatient, with magnetic resonance imaging (MRI) demonstrating high signal on diffusion and low signal on ADC map within all the white matter tracts throughout the brain and brainstem, consistent with a toxic leukoencephalopathy. Family discussion revealed the presence of numerous chemicals in the home, including organic solvents. The patient's diary detailed months of organic solvent use for experimental and recreational purpose. Chemical pathology testing was complicated by the presence of volatile substances from specimen collection bottles. Cerebral oedema secondary to toxic leukoencephalopathy caused increasing intracranial pressure. Despite insertion of an extra ventricular drain, the patient died from the neurological damage caused by organic solvent exposure.

Discussion. Inhalant abuse can result in serious medical consequences and death. Acute organic solvent exposure can cause headache, dizziness, incoordination, and mental status alterations. Chronic abuse leads to persistent neurological impairment, dementia and cerebral atrophy on MRI. In this case, post mortem neuropathology showed diffuse chemical leukoencephalopathy and areas of infarct. Acute on chronic exposure to high concentration organic solvents, including toluene, caused a fatal leukoencephalopathy.

Filley CM. Toluene abuse and white matter – a model of toxic leukoencephalopathy. Psychiatr Clin N Am 2013;36:293–302

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

Zhinoos Taidi[1], Cassandra Liang[1], Kylie Mansfield[2], Lu Liu[1]. School of Medical Sciences, UNSW Sydney[1], Sydney, NSW, Australia; School of Medicine, University of Wollongong[2], Wollongong, NSW, Australia

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

Lixin Zhang[1], Irit Markus[1], Mark Muhlmann, Francis Lam[2], Lu Liu[1] 1. School of Medical Sciences, UNSW Sydney, NSW, Australia, 2. Sydney Colorectal Associate, Prince of Wales Hospital, UNSW Sydney, NSW, Australia

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