400 Trends in anticoagulant use among people with dementia in Australia

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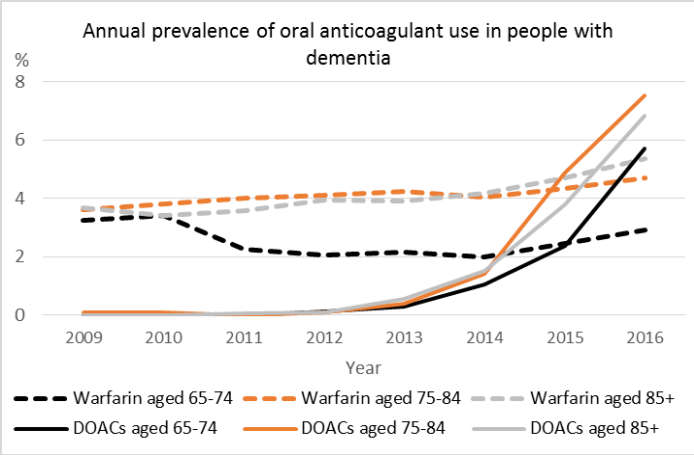
Introduction. People with dementia are less likely to use anticoagulants for the prevention of thromboembolic events due to perceived increased bleeding risk. It is unclear to what extent the introduction of the direct oral anticoagulants (DOACs) has impacted the overall prevalence of anticoagulant use.

Aims. To investigate the trends in anticoagulant use in people with dementia in Australia between 2009 and 2016.

Methods. We analysed a random 10% sample of Australian Pharmaceutical Benefits Scheme individual-level dispensing data. People with dementia were identified as recipients of acetylcholinesterase inhibitors or memantine and categorised according to age 65-74 years, 75-84 years and ≥85 years.

Results. The annual number of people with dementia increased from 5,709 in 2009 to 8,937 in 2016. The overall prevalence of warfarin use increased from 3.6% to 4.7% and DOAC use from 0.04% to 7.0%. The pattern of anticoagulant use was similar in sensitivity analyses excluding under co-payment medications or restricting the cohort to concession card holders. Age-specific trends in annual prevalence are presented in the Figure.



Discussion. The overall prevalence of anticoagulant use in people with dementia has increased sharply since the introduction of DOACs. This may mean more people with dementia receive appropriate treatment. However, there is a need for further research in the benefits and risks of anticoagulant use in people with dementia.

401 Mapping medication burden, prescribing and dispensing patterns within community dwelling elderly clients of community pharmacies

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Introduction. The coexistence of multiple illnesses in the elderly is common, and may lead to the use of multiple medicines. In turn, this can be associated with a patient visiting multiple prescribers, non-adherence and a plethora of negative consequences. Currently, there is a lack of knowledge surrounding the needs of older Australians residing independently within the community.

Aims. To quantify and describe: 1) Current patterns of medication load and presence of polypharmacy and, in particular, prevalence and variety of analgesics and any reported adverse events. 2) Prescribing and dispensing patterns for medications. 3) Each client's care team, how healthcare services are coordinated and his/her understanding of their regular medications.

Methods. Participants were recruited from three metropolitan community pharmacies in Adelaide, South Australia between June and August 2017. The study involved two stages – interviewing community dwelling older Australians and reviewing dispensing histories of those 65 years and over. Data analysis was conducted using descriptive statistics.

Results. Forty-five face-to-face interviews were conducted. Participants were taking 7.45 medicines on average with 76% using five or more regular medicines. Two hundred and twenty-three dispensing histories were collected. The average number of medicines taken by each participant was 8.27 with 86% of participants taking five or more regular medicines.

Discussion. A significant proportion of older Australians living in community dwellings were exposed to polypharmacy. Themes including lack of collaboration between healthcare professionals, the need for increased communication between prescribers and a requirement for increased education about medicines for patients were highlighted. ¶

402 Use of medicines with sedative or anticholinergic properties and medicine-induced deterioration in older people: an intermediary pathway to frailty

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Introduction. Medicines with sedative or anticholinergic properties have been associated with frailty but the intermediary pathways by which they contribute to frailty are less commonly studied.
Aims. To study the association between use of medicines with sedative or anticholinergic properties and i) medicine-induced deterioration (physical function, cognition or appetite), and ii) frailty.
Methods. The study population consisted of persons aged ≥65 years (n=2087) enrolled in the Australian Longitudinal Study of Ageing (ALSA). Physical function was measured using hand grip strength, walking speed, chair stands, activities of daily living (ADL) and instrumental activities of daily living (IADL). Cognitive function was measured using the Mini Mental State Examination (MMSE), while appetite was measured using the Center for Epidemiologic Studies Depression (CES-D) question 2, "I did not feel like eating; my appetite was poor". Frailty was measured using the frailty index.
Results. Almost half of the population were using medicines with sedative or anticholinergic properties (n=954, 45.7%). After adjusting for confounders, use of medicines with sedative or anticholinergic properties was associated with slower walking speed (p<0.001), poorer performance on chair stands (p=0.017) and poorer IADL score (p<0.001). There was no significant association between medicine use and cognitive function. Use of medicines with anticholinergic properties was associated with poorer appetite (p<0.001). Participants who used medicines with sedative or anticholinergic properties were significantly more likely to be frail compared with non-users (p<0.001).
Discussion. We described the pathways by which use of medicines with sedative or anticholinergic properties contribute to frailty, either directly or via an intermediary pathway. Preventing medicine-induced deterioration is important to reduce risk of frailty and subsequent adverse events such as falls and fractures.

403 'Real-world' haemorrhagic rates for antithrombotics using a self-controlled case series design

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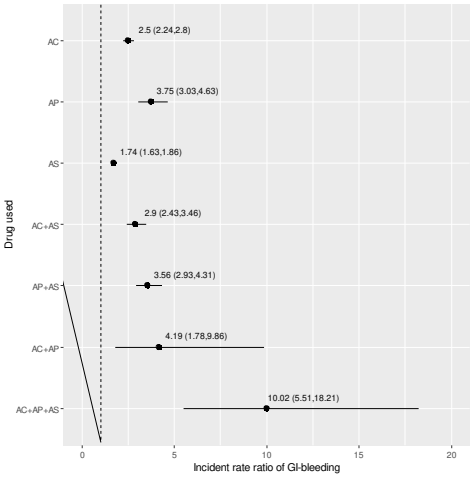
Introduction: Population level evidence for the safety of using antithrombotics in older people within the multi-morbidity is limited.
Aims: The overarching aim of this study was to examine the major gastrointestinal (GI) bleeding risks associated with antiplatelets, anticoagulants either as monotherapy, dual antiplatelet therapy (DAPT) or triple therapy (TT) under the context of confounding due to multi-morbidity.
Methods: Self-controlled case-series (SCCS) design and conditional Poisson regression (CPR) were used in this investigation. We identified 3378 individuals aged 65 and above, who had been diagnosed for the first time with GI-bleeding event, between 01/01/2005 and 31/12/2014. SCCS design controls for time-invariant confounding variables was used to estimate the increased risk of GI-bleeding due to DAPT, TT or the monotherapies, as incident rate ratios (IRR). Multivariable conditional Poisson regression was used to estimate the adjusted IRR.



Results: Amongst the 3378 individuals in the cohort, 78% (n = 2624) had their first-time GI-bleeding with antithrombotic exposures. Antiplatelet monotherapy (adjusted IRR = 3.75, 95% CI = [3.03, 4.63]) and DAPT (adjusted IRR = 4.19, 95% CI = [1.78, 9.86]) were associated with a higher GI-bleeding risk compared to anticoagulant and aspirin monotherapies. The risk of GI-bleeding was highest with TT use compared with anticoagulant and antiplatelet dual therapy use and the monotherapies (adjusted IRR = 10.02, 95% CI = [5.51, 18.21]).
Conclusions: The GI bleeding risk was higher in individuals using TT compared to anticoagulant and antiplatelet dual therapy as well as the monotherapies. The findings inform real world risk assessment posed by antithrombotics in older people.¶

404 Health professionals' and researchers' opinions on conducting clinical deprescribing trials

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Introduction. Clinical deprescribing trials can be conducted to produce favourable health outcomes in people taking potentially harmful medications. At present, there are no guidelines on conducting deprescribing studies.
Aims. To determine the perspectives, attitudes, interests, barriers, and enablers in relation to conducting clinical deprescribing trials among health professionals and researchers.
Methods. An anonymous survey was developed, reviewed and piloted by all investigators for content validity. Experts were contacted to inform the questionnaire content, which explored the purposes, enablers, and barriers of conducting deprescribing trials. The survey was distributed to members of national and international: deprescribing, pharmacological, and pharmacy organisations; and to researchers published in deprescribing.
Results. The survey was completed by 96 participants from June-August 2017. Participants indicated the main rationale for conducting deprescribing trials is to assess the efficacy of interventions to optimise clinical centred outcomes (79.2%). Common barriers to conducting deprescribing trials were forming relationships and maintaining communication with other health professionals involved in the deprescribing process. This barrier commonly affected the: effective completion of trials (32.0%); recruitment of potential patients (31.0%); and overall conduction of trials (17.1%). The most common reported enabler was the belief of health professionals treating trial patients that deprescribing was beneficial (24.4%). Classical randomised controlled trials were considered the most appropriate method for conducting deprescribing trials (93.2%) vs. crossover trials (45.2%). 60.0% of participants indicated a legal, regulatory, and good practice framework required developing, but only 38.9% stated that the CONSORT list needed to be updated to encompass deprescribing trials.
Discussion. Preliminary findings indicate recognition of the need for high quality randomised controlled deprescribing trials and the importance of engagement of treating clinicians in trials of these complex multidisciplinary interventions. Furthermore, the findings of this survey could inform a future clinical deprescribing trial framework, which participants indicated was required.¶

405 Prevalence of potentially inappropriate medication use in older inpatients with and without cognitive impairment: a systematic review

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Introduction. Older people with cognitive impairment are high users of acute care services in Australia and internationally. Potentially inappropriate medication (PIM) use may be associated with adverse outcomes, including hospital re-admission, functional disability and mortality.
Aims. This systematic review aims to quantify and compare the prevalence of PIMs in older inpatients with and without cognitive impairment.
Methods. A systematic search for observational studies was performed in Embase, Medline/PubMed, PsycINFO, International Pharmaceutical Abstracts, Scopus and Informit. Articles published in English during the period January 2007–June 2017 that reported the prevalence of PIMs in hospital inpatients ≥65 years were included. PIMs were defined as exposure to polypharmacy (multiple medication use) or using implicit or explicit tools, such as the Beers criteria and *Screening Tool of Older Person's Prescriptions* (STOPP). Two reviewers independently assessed the articles for eligibility and extracted the data.
Results. 47 articles were included. The prevalence of PIMs defined by polypharmacy exposure (n=15) ranged from 53.2% to 89.8% when cognitive impairment was reported, and 24.0% to 97.1% when unreported. In studies employing explicit and implicit tools (n=35), the prevalence of PIMs in where cognitive impairment was reported ranged from 20.6% to 80.5% using the Beers criteria, and 39.3% to 88.5% using STOPP. When cognitive status was unreported, the prevalence of PIMs ranged from 7.0% to 79.2% using the Beers criteria, and 20.0% to 63.4% using STOPP.
Discussion. Current published evidence suggests a substantial variation in the prevalence of PIMs in older inpatients with and without cognitive impairment. Future studies should investigate the impact of PIM use on patient-centred outcomes to inform enhanced acute care services and pharmacist interventions to reduce inappropriate prescribing.

406 Exercise and Weight Loss Supplements: Understanding the Risks

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Introduction. Oral exercise and weight loss supplements (e.g. pre-work out, post-workout, fatburners, protein supplements) are increasingly used in Australia despite uncertain benefits and mounting evidence of harm. There is limited Australian data on the demographics of users, types of supplements used, characteristics of use and adverse outcomes.
Aims. To describe exercise and weight loss supplement use and outcomes among a sample of regular users.
Methods. Adults with a recent history of supplement use in the last five years were recruited via social media using targeted snowballing and invited to complete a 51-item questionnaire about the type, frequency, duration and outcomes of supplement use.
Results. Of 423 respondents (58% female, mean age 28 years), 375 had used supplements in the past year with 234 (63%) using one or more daily. Adverse reactions were reported by 28% (96/347) including: neurological, cardiac, abdominal and skin reactions. Around 50% (150/316) were not aware of any risks associated with supplement use. Of the 120 who were prescription medicine users, only 27% discussed possible interactions with their doctor or pharmacist (33/120). Common risk behaviours included: using more than recommended dose (37% 117/316); using a supplement with unfamiliar ingredients (72%, 229/316); and continuing to use a supplement after an adverse reaction (30%, 28/95). Most users looked for information about supplement use on the internet 77% (239/310), and only 15% (48/310) consulted their doctor or pharmacist.
Discussion. Exercise and weight loss supplement use involves substantial health risks, but many users will not discuss supplement use or seek reliable evidence based advice qualified health service providers, and may continue supplement use while experiencing adverse reactions.

407 SGLT2 inhibitors and diabetic ketoacidosis - review of PI's and comparison with Endocrinology position statement

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Introduction. Sodium glucose-cotransporter 2 (SGLT2) inhibitors are a new drug class for type 2 diabetes mellitus. They are available as single ingredient products, or as a combination product together with metformin. Case reports of diabetic ketoacidosis (DKA) in association with their use have emerged in clinical practice. In mid-2016 the American Association of Clinical Endocrinologists (AACE) and the American College of Endocrinology (ACE) issued a position statement on SGLT-2 inhibitors and DKA.
Aims. To review and compare Australian Product Information for SGLT-2 inhibitors (single and combination) regarding information on DKA, and also compare with information in the AACE/ACE position statement.
Methods. Text analysis for key information regarding DKA including frequency, risk factors, clinical presentation, diagnostic issues, management, risk mitigation strategies and specific advice in relation to surgery.
Results. All PI's had revision dates after the AACE/ACE position statement. All PI's listed DKA, in the precautions and adverse events sections. All identified usual presenting DKA symptoms, but that blood sugar levels may be lower than expected. Risk factors and precipitants were identified, although there was variation in relation to identification of surgery as a precipitant. There was variation in relation to information on disease severity, from no information, to identification that the outcome may be fatal. No PI's included information on diagnostic difficulties or specific recommendations listed in the position statement. Management information varied, regarding urgency, need for hospitalisation and specific treatments. Specific advice in relation to management around surgery was generally lacking for single products, although recommendations to withhold before and after surgery were present for combination products due to presence of metformin.
Discussion. There is variation between Product Information statements of SGLT2 inhibitors regarding DKA, and useful clinical information from the AACE/ACE position statement is not fully represented. Risk mitigation could be improved with further modification of Product Information.¶

408 Health literacy and uptake of osteoporosis medications in a population-based sample of Australian women

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Introduction. Lower health literacy has been associated with poorer medication uptake and adherence in some chronic conditions, however, associations between health literacy and uptake of osteoporosis medications are currently unknown.
Aims. To investigate associations between health literacy and anti-fracture medication uptake in osteoporotic women.
Methods. Data were collected for women participating in the 15yr follow-up of the Geelong Osteoporosis Study (GOS), a population-based cohort in south-eastern Australia. Health literacy was ascertained using the Health Literacy Questionnaire (HLQ), a multi-dimensional tool that generates scores across nine scales. Bone mineral density (BMD) was measured by dual x-ray absorptiometry (DXA) (Lunar DPX-L) and osteoporosis was defined as a BMD T-score ≤2.5 at the hip and/or spine, or BMD in the osteopenic range (T-score -1 to -2.5) combined with any adult (aged ≥20yr) fracture. Self-reported current medications were classified using MIMS codes, with medications in category 6G 'Agent affecting calcium and bone metabolism' indicating osteoporosis treatment. Analysis of Variance (ANOVA) and Cohen's d effect sizes (ES [95%CI]) (categorised; Small >0.2-0.5, Moderate >0.5-0.8, Large >0.8) were calculated for differences in mean HLQ scale scores between participants with osteoporosis who did vs. did not self-report medication use.
Results. In our women, 134 (21.6%) had osteoporosis and 14 (10.5%) of those women were taking medication. Small and moderate ES observed indicated that women taking medication had lower HLQ scores in three scales; 'Navigating the healthcare system', 'Ability to find good health information' and 'Understanding health information' (ES 0.36 [0.25, 0.79], 0.41 [0.29, 0.87] and 0.64 [0.54, 1.03], respectively). No significant differences for any scales were observed using ANOVA; however, a trend was observed for the scale 'Understanding health information (p=0.09).
Discussion. These results suggest that women who may be less confident in their own ability to find and understand health information may be more likely to follow recommendations from their healthcare provider, and therefore take up prescribed medications more readily¶

409 SGLT-2 inhibitors and diabetic ketoacidosis - review of CMI and comparison with Endocrinology position statement

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Introduction. Sodium glucose-cotransporter 2 (SGLT-2) inhibitors are a new drug class for type 2 diabetes mellitus, available as single ingredient, or combination product with metformin. Reports of diabetic ketoacidosis (DKA) with their use have emerged in clinical practice. In mid-2016 the American Association of Clinical Endocrinologists (AACE) and the American College of Endocrinology (ACE) issued a position statement on SGLT-2 inhibitors and DKA.
Aims. To review the AACE/ACE position statement on SGLT-2 inhibitor associated DKA and compare with Consumer Medicines Information (CMI) for SGLT-2 inhibitors regarding information on DKA.
Method. Text analysis for key information regarding DKA including frequency, risk factors, clinical presentation and severity, risk mitigation strategies, management and specific advice in relation to surgery.
Results. The AACE/ACE position statement did not include any reference to patient education/consumer information regarding risk of diabetic ketoacidosis with SGLT-2 inhibitors. All CMI's had revision dates after the AACE/ACE position statement. All CMI's advised DKA was a contraindication for SGLT-2 inhibitors. All CMI's listed DKA, in the precautions and adverse events sections and reported incidence as rare. There was minimal or no documentation about risk factors that might precipitate DKA. All identified usual presenting DKA symptoms and disease severity as either serious or severe. Management information varied, regarding urgency and need for hospitalisation. Only one CMI suggested cessation of SGLT-2 inhibitor if symptoms of DKA occur. Advice in relation to management around surgery was generally lacking.
Discussion. There is an absence of consideration for patient education regarding SGLT-2 inhibitor associated DKA in the AACE/ACE position statement. The CMI statements of SGLT-2 inhibitors do not include information regarding risk factors and management of SGLT-2 inhibitor associated DKA that is present in the AACE/ACE position statement. CMI advice in relation to use of SGLT-2 inhibitors in the surgical setting is extremely limited. Risk mitigation could be improved with modification of CMI's. Patient education regarding risk of DKA with SGLT-2 inhibitors may have significant resource implications given use in the type 2 diabetes population where DKA is not generally expected.¶

410 Evaluation of bortezomib use in Queensland public hospitals for the treatment of multiple myeloma

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Introduction. Bortezomib (Velcade®) has been demonstrated to improve survival in patients with symptomatic multiple myeloma (MM). One phase III trial (VISTA) supported the PBS subsidy of bortezomib use as a first line treatment in non-transplant eligible MM patients. Bortezomib is expensive (approximately $6,000 per cycle) so we need to ascertain the health outcomes and costs in 'real world' patients.
Aims. We aim to examine the overall survival (OS) of patients using bortezomib and compare the results with trial data.
Methods. We retrospectively audited bortezomib use in non-transplant eligible MM patients in three Queensland public hospitals (October 2012 - December 2016). We retrieved data on patient characteristics, chemotherapy treatments, and survival outcomes from the oncology information system (CHARM®). We obtained pathology and clinical information from the pathology system (Auslab®) and medical chart audits at each site.
Results. We audited 75 patients who were treated with either CVD (bortezomib, cyclophosphamide, dexamethasone) or VMP (bortezomib, melphalan, prednisolone) regimens. The median age of the patients was 75 years, which was higher than that in the VISTA trial (71 years, n=344). The median cumulative dose of bortezomib was 19.14mg/msq, which was lower than the dose of VMP in the VISTA trial (38.5 mg/msq). The median OS was 40.7 months (VISTA trial 56.4 months) and median progression free survival (PFS) was 17.7 months (VISTA 21.75 months).
Discussion. MM patients in Queensland public hospitals were exposed to lower bortezomib doses, and achieved lower survival outcomes compared to key trial. This could be attributed to treatment and patient characteristics.

Abbreviation: meter squared – msq

411 What do women want to know about menopausal symptoms management: An Australian medicines call centre analysis

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Introduction. Management of menopausal symptoms has been shrouded in controversy over the last 15 years, with the evidence on safety and efficacy of hormonal treatments swinging back and forth. Despite much information on hormone therapy (HT) and complementary medicines (CM) for menopausal symptoms; women remain confused and uncertain.
Aims. To explore women's questions to an Australian medicines call centre regarding HT and CM.
Methods. We conducted a mixed method retrospective study on routinely collected data from an Australian national medicines call centre National Prescribing Service (NPS) *Medicines Line*. We quantitatively analysed data (September 2002 – 30 June 2010) for the demographic characteristics of callers, patients, motivations to help-seek, and enquiry types. We thematically analysed the callers' questions and derived key themes.
Results. We extracted 970 menopausal therapies (MTx) related calls (0.8% of calls). Most calls were made by women (97%) to seek information for themselves (95.3%). The top three enquiry types related to side-effects (23.1%), risks vs. benefits (16.4%), and interaction (14.9%). Most calls were prompted by inadequate information (38.5%), looking for second opinion (24.3%), and worrying symptoms (20.1%). There were no major changes in enquiry types and motivations to help-seek over time. Key question themes were: clarifying whether MTx caused/exacerbated symptoms; seeking advice for symptom management; and seeking reassurance to use or withdraw treatment. Concerns about the impact of MTx (especially HT) on underlying conditions focused on breast cancer, gynaecological, and cardiovascular conditions. In contrast, CM-related calls focused on efficacy and interactions.
Discussion. Women differ in their menopausal experiences and which medicines might be needed. Despite change in evidence to favour HT for up to five years for symptom management in perimenopause, women's concerns were fairly stable over time. These study elucidates women's information needs to enable the development of more directed and relevant information.

412 Patterns of oral anticoagulant use in people with and without dementia: A systematic review

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Introduction. People with dementia are equally likely to experience stroke than those without, however people with dementia are less likely to receive warfarin. Direct oral anticoagulants (DOACs) may be an alternative to warfarin for people with dementia, however the safety profile has not been rigorously established.
Aims. To compare the prevalence of oral anticoagulant use, in people with and without dementia across all healthcare settings and indications for oral anticoagulation.
Methods. A search of the literature was undertaken using MEDLINE, EMBASE and CINAHL from 2000 until July 2017. Studies were included if they reported original research demonstrating cross-sectional assessment of oral anticoagulant use for people with and without dementia. Two independent reviewers extracted data from included studies. Prevalence estimates for oral anticoagulant use among people with and without dementia were calculated from data of included papers. A meta-analysis was performed using unadjusted odds ratio (OR) and 95% confidence interval (CI). Data were pooled using a random effects model and heterogeneity was explored using I_2 statistics.
Results. 3625 articles were retrieved Full texts of 56 articles were reviewed, 21 were included in the final review. No studies reported prevalence for DOAC use. Stroke prevention in atrial fibrillation was the main indication reported. The prevalence of warfarin use ranged from 8% to 64% in people with dementia and from 7% to 76% in people without dementia (OR (95% CI), 0.50 (0.40-0.63) compared with those without dementia).
Discussion. Results indicate people with dementia receive oral anticoagulation less frequently than people without, despite equal or increased risk of stroke for people with dementia. No studies reported prevalence of DOAC use in people with dementia. Findings indicate underutilisation of oral anticoagulation in people with dementia. Further work is required to understand the reasons for under-use and the impact of the introduction of DOACs on oral anticoagulant prevalence in people with dementia. ¶

413 Consumer information gaps and concerns regarding opioid analgesics and anxiolytic/hypnotic/sedative medicines

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Introduction. Opioid analgesics (OA) are often prescribed to manage chronic pain; while anxiolytic, hypnotic and sedative (AHS) medicines are prescribed for insomnia and anxiety. These central nervous system depressants can produce physical and psychological dependence, resulting in tolerance, dose escalation, and misuse. These risks are of concern to patients, primary health care providers, and the wider public and media.
Aims. To identify consumer information gaps and concerns regarding use of OA and AHS medicines to enhance the utility of information made available to consumers.
Methods. We conducted a retrospective, mixed-method study of consumers' OA and AHS-related calls to the National Prescribing Service (NPS) *Medicines Line* (Sep 2002-30 Jun 2010). We analysed call characteristics and conducted a thematic analysis of question narratives for most common enquiry types when compared with rest of calls (ROC).
Results. Of 125,951 calls, 6,853 (5.4%) involved OA and 7,789 (6.8%) AHS. The mean age of OA callers and patients were 49.7 and 48.2 years, respectively. The mean age of AHS callers and patients was slightly older (50.8 and 49.7 years). While female callers predominated for both medicine classes, there were proportionately more male callers for AHS and OA medicines than other medicines. The two main motivations to help seek for both medicine classes were inadequate information (OA 44.1%; AHS 41.2%) and seeking a second opinion (OA 24%; AHS 24.2%). Side effects and interactions were the most common enquiry types but questions involving withdrawal or abuse were over three times more frequent for OA and AHS calls versus ROC (OA 12.6% versus ROC 2.7% and AHS 9.1 versus ROC 2.9%). The question themes were similar for both medicine classes: seeking additional information (e.g. risk of harm associated with misuse); therapeutic strategies (e.g. how to safely withdraw); seeking reassurance (e.g. drug will not cause addiction) and dose clarification.
Discussion. Consumers have many concerns about abuse and withdrawal of OA and AHS medicines, which may be under-recognised by healthcare providers. Developing user friendly, targeted information to address these concerns would contribute to safer and more effective use of medicines. ¶

414 The Medication Regimen Simplification Guide for Residential Aged CarE (MRS GRACE): a novel tool to optimise medication regimens for residents of aged care facilities.

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Introduction. Residents of aged care facilities use increasingly complex medication regimens. Reducing unnecessary medication regimen complexity (e.g. by consolidating number of administration times or using alternative formulations) may benefit staff administering medications and residents taking medications.
Aims. To develop and validate an implicit tool to facilitate medication regimen simplification in aged care facilities.
Methods. A purposively-selected multidisciplinary expert panel used modified nominal group technique to identify and prioritise factors important in determining whether a medication regimen can be simplified. The five prioritised factors were formulated as questions, pilot-tested using non-identifiable medication charts and refined by panel members. The final tool was validated by two clinical pharmacists who independently applied the tool to medication charts for a random sample of 50 residents to identify opportunities for medication regimen simplification. Inter-rater agreement was calculated using Cohen's kappa.
Results. The Medication Regimen Simplification Guide for Residential Aged Care (MRS GRACE) was developed as an implicit tool and accompanying explanatory statement. The tool comprises five questions related to resident and facility related factors, drug interactions, and formulation. Using MRS GRACE, two pharmacists independently simplified medication regimens for 29/50 and 30/50 residents (Cohen's kappa=0.38, 95%CI 0.12-0.64), respectively. Simplification was possible for all residents with five or more administration times. Changing an administration time comprised 75% of the two pharmacists' recommendations.
Discussion. By applying MRS GRACE, two clinical pharmacists independently simplified two-thirds of residents' medication regimens with fair agreement. MRS GRACE is a promising new tool to guide medication regimen simplification in aged care facilities.¶

415 What are the predictors of persistent prescription opioid analgesic use for non-cancer pain in Australia?

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Introduction. Long-term opioid analgesic use for chronic non-cancer pain is associated with uncertain clinical benefits but clear harms.
Aims. To identify patterns of opioid analgesic use and determined predictors of persistent opioid use among people without cancer.
Methods. A population-based cohort study of Australians initiating prescription opioids from July 2013 to December 2015 was conducted using data from a random 10% sample of people who accessed medicines through Australia's Pharmaceutical Benefits Scheme. A 12-month look-back period was used to define opioid initiation, exclude people with cancer, and determine comorbidities. Persistent use over 12-months since initiation was identified through group-based trajectory modelling. Odds ratios (OR) and 95% confidence intervals (CIs) for predictors of opioid persistence were estimated using logistic regression.
Results. The cohort consisted of 126,903 people who had opioids dispensed in ≥2 months during the 12-month follow up. A total of 11,323 (8.9%) persistent opioid users were identified. Predictors of persistence included initiation with transdermal opioids (OR 3.2, 95% CI 3.0-3.4), or with oral morphine equivalents (OME) ≥ 750 mg (OR 2.8, 95% CI 2.6-3.1), having depression (OR 1.3, 95% CI 1.3-1.4), or psychotic illness (OR 1.9, 95% CI 1.7-2.0). Previous dispensing of paracetamol (OR 1.7, 95% CI 1.6-1.8), pregabalin (OR 1.6, 95% CI 1.5-1.8) and benzodiazepines (OR 1.3, 95% CI 1.2-1.4) predicted persistence. Compared to people aged 18-44 years, those ≥75 years were 2.4 (95% CI 2.2-2.6) times more likely to be persistent users.
Discussion. Mental health comorbidities, older age, initiation with transdermal opioids and higher OMEs strongly predicted persistent opioid use among people without cancer. This information may help prescribers target monitoring and early intervention efforts in order to prevent opioid-related harms.

416 Anovulatory infertility in Australia: A retrospective analysis of medicine use and health outcomes

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Introduction. Anovulation is when the ovaries do not release an oocytes during the menstrual cycle. It is a relatively common cause of infertility, accounting for about 25% of all cases. There are four different medicines which are subsidised under PBS for use in Anovulatory Infertility. The first line drug for inducing ovulation is Clomiphene citrate (CC), and the second line drugs are Follitropin alfa, Follitropin beta and Human chorionic gonadotrophin (HCG). CC is available as tablet, whereas all other medicines are available in the form of Injections and are taken via sc route.
Aims. To analyze the subsidized use, cost and reported adverse events of drugs used to induce ovulation in Anovulatory Infertility patients in Australia, following their inclusion on the Pharmaceutical Benefits Scheme (PBS).
Methods. Pharmacoepidemiological and Cost analysis of dispensed prescriptions from Medicare Australia. Adverse event data were obtained from the Therapeutic Goods Administration. Medicine use was measured by the defined daily dose (DDD) per 1000 population per day for each calendar year. Adverse events were counted by organ class system.
Results. There was significant increase in the use of second line drugs compared to first line therapy. The average percentage increase in the utilisation of three available strengths of Follitropin alfa (300 IU, 450 IU and 900 IU) and Follitropin beta (300 IU, 600 IU and 900 IU) was 728% (2004 to 2016, 0.0005 to 0.0051 DDD/1000 population/day) and 189% (2002 to 2016, 0.0008 to 0.0025 DDD/1000 population/day) respectively. Between 1992 and 2016, dispensing of HCG (1500 IU) in Australia increased 477% from 0.008 to 0.047 DDD/1000 population/day. Whereas First line drug (Clomiphene citrate) showed drastic decline (90%) in the usage from 1992 to 2016 (0.06 to 0.006 DDD/1000 population/day). The major reported adverse events were reproductive system and breast disorders, skin and subcutaneous tissue disorders, nervous disorders, eye disorders, and gastrointestinal disorders.
Discussion. The rising trend of gonadotrophins and significant decrease in the use of Clomiphene citrate over the years is a matter of concern and is pointing towards clinically inappropriate prescribing of ovulation induction agents for treating anovulatory infertility.

417 New drug formulation for combating antibiotic resistance

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Introduction. Bacterial biofilms are associated with 80–90% of infections. Bacteria in biofilms show significant resistance to antimicrobials and host immune defenses, compared with planktonic bacterial cells. Consequently, biofilm infections present many challenges including chronic inflammation, faulty wound healing, antimicrobial resistance, and the spread of infections.
Aims. To develop a novel 3-in-1 nanostructure-based formulation technology capable of storing nitric oxide (NO), which can provoke dispersal of biofilms into an antibiotic susceptible planktonic form, together with the aminoglycoside gentamicin and reactive oxygen species, capable of killing the bacteria.
Methods. In this study, we combined in one formulation a NO donor, reactive oxygen species and gentamicin. In this approach, the NO donor was directly obtained by reaction of gentamicin with NO gas to yield gentamicin-NONOate complex. By engineering the nanoparticles, a simultaneous and sustainable release of gentamicin, light induced reactive oxygen species and NO was obtained. All released agents acted synergistically on biofilms.
Results. The gentamicin-NONOate nanoparticles were found to effectively disperse biofilms of the model organism P. aeruginosa. At the NO concentrations of 10 μM, the viability of both biofilm and planktonic cells decreased by more than 90%. In contrast, gentamicin, reactive oxygen species and NO donor alone showed a lower efficiency against biofilm and planktonic cells.
Discussion. Combined and simultaneous delivery of NO, ROS and gentamicin is highly innovative concept that would allow eradicating the biofilm and also potentially overcome multidrug resistance. Encapsulated within nanostructures the three therapeutic agents are likely to have enhanced pharmacodynamic properties for systemic or local treatments. Furthermore, these compounds might be useful when applied as surface coating for the inhibition and prevention of biofilm formation on clinical surfaces or implants. The formulation is also very attractive for topical treatment with low risk of systemic side effects compared to parenteral or oral drug administration.

418 Angiotensin II receptor type 1 transactivation of EGFR via TRIO-dependent mechanisms.

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Introduction. The transactivation of receptor tyrosine kinases by G protein-coupled receptors is now a well-established paradigm. Of particular interest is the transactivation of epidermal growth factor receptor (EGFR) by the angiotensin II type 1 (AT_1) receptor, which has been shown to be crucial for AT_1 receptor-mediated growth effects, and involved cardiovascular pathologies. Although this transactivation has been described for many years, the mechanisms underpinning it are yet to be fully elucidated. Recently, a potential intermediate of this process was identified when it was discovered that the multidomain-containing kinase called TRIO was involved in the AngII/AT_1 receptor-mediated transactivation of EGFR (George et al, 2013).
Aims. To investigate the mechanism by which TRIO acts as an intermediate in AngII/AT_1 receptor-mediated EGFR transactivation.
Methods. To investigate this process, a variety of bioluminescence resonance energy transfer (BRET) protein-protein proximity assays were used.
Results. Upon AngII-induced activation of the AT_1 receptor, TRIO is trafficked around the cell through several cellular compartments. It also interacts with a variety of signalling and regulatory proteins. Many of these effects were specific to AngII-induced activation of the AT_1 receptor as they were not observed upon EGF-induced activation of the EGFR.
Discussion. Our data have demonstrated several AngII/AT_1 receptor-mediated effects on TRIO that appear to be involved in regulation of EGFR transactivation.

George et al (2013) J Cell Sci 126: 5377–5390. ¶

419 Smad2 linker region: a central integrating point for GPCR mediated transactivation of tyrosine and serine/ threonine kinase receptors.

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Introduction. G protein coupled receptors (GPCRs) can transactivate protein tyrosine kinase receptors (PTKR) and serine/threonine kinase receptors (S/TKR). GPCR transactivation of PTKR is approximately equally important as the transactivation of S/TKR with 209 and 177 genes regulated respectively, via either signalling pathway [1]. The two transactivation dependent signalling pathways share in over 65% of differentially expressed genes. The biochemical mechanisms between the two transactivation pathways are distinct [2].
Aims. To assess transcription factor Smad2 as a common integrating point for thrombin transactivation of PTKR and S/TKR with the downstream target as the expression of genes involved in the initiation and elongation of GAG chains on lipid-binding proteoglycans.
Methods. GAG synthesizing gene expression was measured and quantified by real time-PCR. Smad2 linker region phosphorylation was detected and quantified by western blotting.
Results. Thrombin phosphorylation of the serine residues in Smad2L are regulated by serine/threonine kinases. The differential action of these kinases regulate thrombin mediated expression of two genes that drive elongation of the GAG chain. Phosphorylation of the threonine residue in Smad2L is associated with the initiation of GAG chain synthesis.
Discussion. These findings highlight a specific signalling paradigm for GPCR mediated transactivation dependent pathways in the context of GAG initiation and elongation. Thus Smad2L integrates GPCR mediated transactivation of PTKR and S/TKR which can be therapeutically targeted to treat other pathophysiological conditions.

1.Kamato, D., et al., RNA sequencing to determine the contribution of kinase receptor transactivation to G protein coupled receptor signalling in vascular smooth muscle cells. PLoS One, 2017. **12**(7): p. e0180842.
2.Kamato, D., et al., The expansion of GPCR transactivation-dependent signalling to include serine/threonine kinase receptors represents a new cell signalling frontier. Cell Mol Life Sci, 2015. **72**(4): p. 799-808.¶

420 Reversal of age related pseudocapillarization using direct actin & lipid raft disruptor drugs on *in vitro* liver sinusoidal endothelial cells

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Introduction: The liver is a key driver in lipid metabolism & insulin handling, functions imperative to the prevention of metabolic disorders. An age-related change that occurs in the liver is loss of transcellular pores, called fenestrations, within liver sinusoidal endothelial cells (LSEC), in a process called pseudocapillarization. Fenestrations act as ultra-filters allowing an exchange of lipoproteins & insulin; this is impaired by fenestration loss in old age contributing to postprandial hypertriglyceridemia & insulin resistance. Regulation of fenestrations is promoted via changes in LSEC plasma membranes. Lipid rafts are bound to the actin cytoskeleton forming a complementary structure across LSEC membranes. Both actin & lipid rafts can be modified by chemical agents.
Aim: This study aimed to investigate the actions of Cytochalasin D (CytoD), an actin disrupting agent, 7-ketocholesterol (7-KC), a lipid raft reducing agent, & a potential drug of interest (Drug A) to promoting re-fenestration in old (18m, n=3) & young mice (4m, n=3).
Methods: Mice LSECs were treated for 30 min with a single agent and prepared for scanning electron microscopy. Images of LSEC fenestrations were analyzed to determine their diameter & frequency, & calculate cell porosity.
Results: Both young & old LSEC showed an increase in porosity & fenestration frequency following treatment with CytoD (0.5µg/mL), 7-KC (4.5µM, 9µM) & Drug A. Fenestration diameter was also increased after 7-KC treatment. Age-related reductions in fenestrations were observed between young & old controls.
Discussion: This study has shown that actin & lipid raft modifying drugs can increase fenestrations. Both Drug A & CytoD showed re-fenestration while maintaining cellular architecture in young & old mice. These did not induce plasma membrane modifications, which were seen after 7-KC treatment. The novel finding that the porosity & number fenestrations are increased by Drug A, which potentially modulates lipid rafts & the actin cytoskeleton, further research is underway to understand this mechanism.

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421 The rational design of self-adjuvanting subunit vaccines by site-specific conjugation of protein antigens with Toll like receptor ligands

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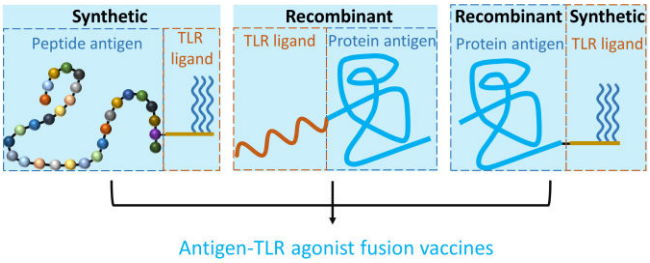

Introduction. Simultaneous delivery of antigens and TLR agonists to antigen presenting cells ensures the colocalization of both molecules to the same endosome or phagosome, within the same APC, thereby enhancing the antigen presentation and processing efficiency. A number of strategies have been developed to fulfill the goal of codelivery, with enzyme-mediated ligation receiving significant attention due to their ability to simply and site-specifically modify proteins; and maintain the native 3-dimentional structure of protein antigens that is most critical in order to elicit protective immune responses. A number of enzymes have been successfully used for ligation reactions with proteins, with *Staphylococcus aureus* sortase A (SrtAsa) the most thoroughly characterised and commonly used.
Aims. To develop a platform technology to enable the efficient and simple site-specific conjugation of TLR agonists onto folded recombinant antigens using a semisynthetic-ligation approach under native conditions.
Methods. Expression and purification of polytope antigens and SrtAsa proteins. Synthesis of a lipid adjuvant peptide (TLR2/6 agonist). Immunization and challenge studies to investigate the vaccine efficacy against invasive disease.
Results. Reaction conditions were screened, optimized and confirmed for maximizing the ligation yield. Lipid adjuvant peptides were successfully conjugated onto polytope antigens with a high yield. Conjugate vaccines confer protection against lethal challenge in mice.
Discussion. The amount of SrtAsa required for conjugation reactions is significantly decreased due to introduction of a SrtAsa mutant. This platform technology provides high yield of protein antigen-lipid adjuvant conjugate vaccines that have the capacity to generate more efficient, potent, and protective immune responses when compared to their formulation with alum.

422 The novel fatty acid epoxide analogue CTU targets the mitochondrion and depletes cardiolipin to promote killing of MDA-MB-231 breast cancer cells

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Introduction. The atypical phospholipid cardiolipin plays an important regulatory role in apoptosis by modulating the release of cytochrome c from the mitochondrial membrane. We have prepared a metabolically stable fatty acid epoxide bioisostere (termed CTU) that targets the mitochondrion and activates endoplasmic reticulum stress in MDA-MB-231 breast cancer cells leading to decreased cell viability (Choucair et al, ASCEPT 2016).
Aims. This study was undertaken to evaluate the role of the mitochondrion in CTU-mediated cancer cell killing.
Methods. In MDA-MB-231 cells, cardiolipin/phosphatidylglycerol was estimated using a commercial kit. Cell viability was assessed by ATP formation, measurement of caspase-3/7 activity and annexin V/7AAD staining. Gene profiling was undertaken by real-time RT-PCR, and altered protein expression was assessed by Western immunoblotting.
Results. Addition of CTU to MDA-MB-231 cells significantly decreased the cellular content of cardiolipin and its precursor phosphatidylglycerol at 24 h. Mitochondrial cytochrome c release was increased in cells treated with CTU at 24 h but not at 6 h. However, the expression of pro-apoptotic mitochondrial membrane permeabilizing proteins of the Bcl-2 family, Bax and Bak, was decreased at 6 and 24 h. Neither the Ca^{2+} chelator BAPTA-AM nor the mitochondrial permeability transition pore inhibitor cyclosporin A altered the CTU-mediated decrease in ATP formation. Co-supplementation with the monounsaturated fatty acid oleic acid, which is essential for cardiolipin maintenance, prevented the CTU-mediated depletion of cardiolipin/phosphatidylglycerol, upregulation of endoplasmic reticulum stress genes, mitochondrial cytochrome c release, caspase-3/7 activation and annexin V/7AAD staining.
Discussion. The novel fatty acid bioisostere CTU has emerged as the first in a new class of agents with activity against cancer cells produced by targeting of the tumor cell mitochondrion and cardiolipin depletion. CTU-mediated apoptosis in MDA-MB-231 cells is independent of Bax and Bak and the mitochondrial permeability transition pore.

Choucair H et al (2016) ASCEPT 2016.¶

423 Anti-proliferative activity of novel ω-3 epoxy fatty acid analogues in MDA-MB-231 triple negative human breast cancer cells

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Introduction. Many experimental studies have shown that ω-3 polyunsaturated fatty acids reduce the risk of certain cancers. We recently synthesised a metabolically stable analogue of ω-3 epoxy eicosapentaenoic acid termed CTU that inhibits proliferation and activates apoptosis in highly metastatic MDA-MB-231 breast cancer cells (Murray et al, 2017). In this study, further CTU analogues were synthesised and tested for their anti-proliferative activity.
Methods. New CTU analogues termed TR16, CP19, CP21 and CP22 were produced by modifying the nature of the aromatic system in CTU. The viability of MDA-MB-231 cells was evaluated by ATP formation, cell cycle distribution was determined by flow cytometry, and immunoblotting was used to evaluate the expression of cyclin regulatory proteins.
Results. CP22 and CP21 were more effective than CTU and CP19 in decreasing ATP production in MDA-MB-231 cells compared to control (43±5.2%, 56±8.8%, 65±13% and 76±12.7%, respectively; 10 µM, 24 h); however, TR16 was inactive. Flow cytometry analysis showed a significant increase in the cell proportion in S phase and G2/M phase with CP22 (45.3 ± 5.2% and 21.1 ± 7%) and CP21 (27.9 ± 8% and 26.1 ± 2.7%) treatments relative to control ($P<0.05$). On the other hand, compared to control, a decrease in the cell population in G0/G1 phase was also noted with CP22 and, to a lesser extent, CP21, CP19 and CTU. Consistent with findings from flow cytometry, treatment of MDA-MB-231 cells with CP22, CP21, CP19 and CTU (10 µM, 24 h) produced decreases in cyclin D1 (to 6-fold, 5.9-fold, 3.2-fold and 3.7-fold of respective control) and CDK4 (to 6.4-fold, 2.9-fold, 2-fold and 1.9-fold of respective control) immunoreactive protein expression. In contrast, cyclin D1 and CDK4 expression were unaffected by TR16.
Discussion. CP22, CP21 and CP19 were more effective than CTU in impairing energy metabolism in MDA-MB-231 breast cancer cells and disrupting the cell cycle in S phase and G2/M phase. Additionally, the expression of cyclin D1 and CDK4 proteins was strongly down-regulated by CP22, CP21, CP19 and CTU, which may contribute to their anti-proliferative actions. These properties are promising for the development of novel anti-cancer therapeutics.

Murray M et al (2017) Biochem Pharmacol , 139:117.¶

424 Assessment of taste 1 receptor allosteric ligands for activity at metabotropic glutamate receptors

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Introduction. The Class C G protein-coupled receptors (GPCRs) include eight metabotropic glutamate receptor (mGlu) subtypes, Calcium-sensing receptors (CaSR) and taste 1 receptors. Class C GPCRs recognise a diverse array of ligands from ions (Mg^{2+}, Ca^{2+}, Gd^{3+}) to small molecules and proteins. Class C GPCRs, in particular $mGlu_2$ and $mGlu_5$, are attractive targets for a number of psychiatric and neurological disorders, for which the current therapeutic treatments are suboptimal and can often lead to side effects due to non-selectivity. Therefore, there is a great need to identify novel mechanisms of receptor activation. We hypothesised that taste 1 receptor ligands would have allosteric interaction with other Class C GPCRs.
Aims. We tested the hypothesis that taste 1 receptor allosteric ligands can also bind to and modulate activity of $mGlu_2$ and $mGlu_5$.
Methods. Functional effects of sweet proteins (thaumatin and monellin) and small molecules (NHDC, cyclamate and lactisole) were tested in HEK293A cells stably expressing $mGlu_2$ or $mGlu_5$ in LANCE cAMP accumulation and intracellular calcium (iCa^{2+}) mobilisation assays. Binding assays using the radiolabelled $mGlu_5$ allosteric ligand [^{3}H]mPEPy were employed in HEK293A-$mGlu_5$ cells to determine affinity.
Results. Monellin caused robust iCa^{2+} mobilisation in HEK293A-$mGlu_5$ cells. These effects were not evident in non-transfected HEK293A cells. Thaumatin and synthetic sweetener NHDC had no effect at either receptor. Lactisole and cyclamate, which interact with the 7 transmembrane spanning domain of taste 1 receptors, did not appreciably displace [^{3}H]mPEPy binding to $mGlu_5$.
Discussion. These results show that monellin is an allosteric agonist at the mGlu5 receptor. Sweet proteins extracted from plants have been shown to activate Class C G protein-coupled taste receptors through binding at the cysteine-rich domain, therefore, it is possible that monellin also recognises this cysteine-rich domain in $mGlu_5$. Future work will aim to identify the monellin binding site, which may lead to development of a novel class of $mGlu_5$ ligands with therapeutic potential.
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425 Small molecule inhibitors of Amyloid β and α Synuclein (αSA53T) protein aggregation

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Introduction. Amyloid β (Aβ) and α Synuclein (αS) protein aggregation into amyloid fibrils is associated with the pathology of various neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) etc.
Aims. To understand and compare the effects of a diverse set of natural polyphenol compounds (honokiol, punicalagin, myricetin, luteolin, transilitin and semi-synthetic flavone 2-D08) and synthetic compounds selected through virtual screening, (dibenzyl imidazolidine and triazole acetamide derivatives) on Aβ and αS protein aggregation and neurotoxicity.
Methods. Thioflavin T fluoroscopic assay and transmission electron microscopy (TEM) were used to study inhibition of aggregation. Viability of Phaeochromocytoma (PC12) cells after exposure to either amyloidogenic protein or a combination of protein and aggregation inhibitors was measured by MTT assay. Molecular docking was used to understand the protein and small molecules interaction. For αS protein, its aggregation prone mutant αSA53T was expressed using *E. coli* BL21(DE3) cell line containing human αSA53T gene inserted into a pRSETB vector and purified following Volles and Landsbury method and size exclusion chromatography[1].
Results. Each of the polyphenols and two synthetic imidazolidine compounds demonstrated significant inhibition of both Aβ and αS protein aggregation. They also exhibited significant neuroprotection when cells were exposed to Aβ or prefibrilised αS. The predicted good binding from molecular docking was correlated with inhibition of both amyloidogenic protein aggregation.
Discussion. Together, these findings highlight the anti-aggregatory properties of a structurally diverse set of compounds, of both natural and synthetic origin, against pathological misfolded Aβ and αSA53T proteins. Such compounds could further inform the development of disease-modifying drugs against AD and PD.

Volles, P.T. (2007) Journal of Molecular Biology, 366: 1510-1522

426 Bias in fluorescence-based voltage-gated sodium channel assays

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Introduction. Voltage-gated sodium channels (Na_Vs) are key therapeutic targets for pain, epilepsy and cardiac arrhythmias, therefore high-throughput fluorescence-based assays are used to screen and characterise novel Na_V modulators. However, results obtained from fluorescence-based assays do not always correlate well with results obtained from conventional patch-clamp electrophysiology.
Aims. Systematically assess the effects of different Na_V channel modulators using fluorescence-based assays and patch-clamp electrophysiology to identify assay bias.
Methods. HEK293 or CHO cells heterologously expressing human $Na_V1.1$–1.8 (SB Drug Discovery; ChanTest) were used for functional assays. Changes in fluorescence were assessed using a FLIPR^{TETRA} (Molecular Devices), with commercial dyes that detect changes in membrane potential (FLIPR membrane potential red, Molecular Devices) or intracellular sodium ion influx (Asante NaTRIUM Green-2 AM, Abcam) used. Electrophysiology parameters were assessed using an automated whole-cell patch-clamp platform (QPatch-16, Sophion Bioscience).
Results. Fluorescence-based assays were able to detect Na_V channel activators and inhibitors with different binding sites and mechanisms of action. The EC_{50} values obtained from fluorescence-based assays for activators generally correlated well with EC_{50} values obtained from conventional patch-clamp, however the most robust responses were obtained from activators that caused persistent and/or tail currents (eg. veratridine, deltamethrin). The IC_{50} values obtained from fluorescence-based assays correlated well with conventional patch-clamp for pore blockers (eg. tetrodotoxin) but not for gating modifiers (eg. µ-theraphotoxin-Pn3a).
Discussion. While the endogenous activator of Na_V channels is voltage, fluorescence-based assays rely on using Na_V channel modulators to activate the channels, with the alkaloid veratridine (for $Na_V1.1$-1.7) or the pyrethroid deltamethrin (for $Na_V1.8$) commonly used. These Na_V channel activators likely stabilise different channel conformations or result in competitive binding, causing fluorescence-based assays to exhibit bias towards detection of pore blockers over gating modifiers. ¶

427 Nrf2 activators in medicinal plants of the Australian Aboriginal Dharawal people

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Introduction. Nrf2 (nuclear erythroid 2-related factor 2) is a transcription factor which binds to the antioxidant response element (ARE) to regulate the expression of cytoprotective genes involved in detoxification, glutathione production and mitochondrial protection. Simultaneously failure of Nrf2 regulation can also exacerbate the production of pro-inflammatory markers via regulation of nuclear factor (NF)-κB due to oxidative stress (Bryan et al, 2013). Previously we have determined anti-inflammatory activity in the medicinal plants (Akhtar MA et al, 2016) of the Australian Aboriginal Dharawal people (collected from the Australian Botanic Gardens in Mount Annan); and currently we are investigating these plants for the presence of Nrf2 activators.
Aims. To identify whether extracts of medicinal plants of the Australian Aboriginal Dharawal people can activate Nrf2-mediated transcription.
Method. AREc32 cells (stably transfected with Nrf2 reporter gene (luciferase)) were activated with different concentration of ethanolic extracts of 15 selected plants in a dose dependent manner. After 24h of activation, the cell lysates were assayed for luciferase activity.
Results. Among 15 selected plants, *Pimelea linifolia* exhibited a 6-fold increase in Nrf2 activation, followed by *Acacia falcata* and *Hakea salicifolia* showing a 2-fold increase in comparison to non-activated cells.
Discussion. *Pimelea linifolia, Acacia falcata and Hakea salicifolia* extracts have shown a dose-dependent Nrf2 activation. Our future work will focus on isolation and structural identification of the active phytochemical constituents from these plants.

1. Bryan KH et al (2013) Biochem Pharmacol 85 :(6) 705-717.
2. Akhtar MA et al (2016) Evid Based Complement Alternat Med. 2016:1-8.

428 Phosphatase and tensin homolog (PTEN) silencing suppresses Ca^{2+} responses in MDA-MB-231 breast cancer cells.

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Introduction. Phosphatase and tensin homolog (PTEN) is a gene that is mutated in many cancers and the loss of functional PTEN is associated with the activation of pathways that may promote proliferation, metastasis and loss of apoptotic sensitivity. Ca^{2+} signalling is a key regulator of events important in tumour progression (Monteith et al, 2017). Ca^{2+} signalling events may be altered as a consequence of PTEN loss, but this has not been fully explored in breast cancer cells (Bittremieux et al, 2016).
Aim. To assess the effects of PTEN loss on Akt phosphorylation and Ca^{2+} signalling in triple-negative MDA-MB-231 breast cancer cells stably expressing the GCaMP6m genetically-encoded Ca^{2+} indicator (GCaMP6m-MDA-MB-231).
Methods. MDA-MB-231 cells were treated with non-targeting (NT) or PTEN siRNA. Silencing efficiency was assessed using qPCR. Akt phosphorylation was assessed using immunoblotting with a phosphospecific antibody. For assessment of cytosolic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) responses, fluorescence changes in response to Ca^{2+}-mobilising agents (ATP, trypsin and cyclopiazonic acid (CPA)) were assessed using a Fluorescence Imaging Plate Reader (FLIPR).
Results. siPTEN reduced PTEN mRNA levels and significantly increased levels of pAkt. Silencing of PTEN supressed increases in $[Ca^{2+}]_{CYT}$ elicited by the purinergic receptor activator adenosine triphosphate (ATP) (16.5% reduction at 100 µM) and the protease activated receptor trypsin (17.02% reduction at 100 nM). The loss of PTEN did not significantly alter $[Ca^{2+}]_{CYT}$ increases induced by the sarco/endoplasmic reticulum Ca^{2+}-ATPase inhibitor CPA.
Discussion. These studies suggest PTEN loss in triple negative breast cancer cells results in the suppression of some Ca^{2+} signalling events. This may be via a reduction in inositol 1,4,5-trisphosphate (IP_3) responses either through pAkt or other effects on IP_3 receptors (IP3Rs). These effects may lead to suppression of responses to apoptotic stimuli.

Monteith GR et al (2017) Nat Rev Cancer. 17:367-380.
Bittremieux M et al (2016) Biochim Biophys Acta. 1863:1364-78.¶

429 Comparison of analgesic and constipation profile of two G-protein biased endomorphin-2 analogue after intracerebroventricular administration in rats

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Introduction. Strong opioid analgesics are the mainstay for the clinical management of moderate to severe nociceptive pain. The analgesic effect of opioids are mainly mediated by the G-protein pathway of mu opioid receptor (MOPr). Opioid-related adverse effects such as constipation are thought to be mediated by the β-arrestin2 signalling pathway.
Aims. The aim of the present study was to evaluate and compare the analgesic and constipation profile of CYX-5 and CYX-6. These compounds are endomorphin 2 analogues. These compounds are G-protein biased MOPr agonists, delta opioid receptor (DOPr) antagonists and they lack β-arrestin2 recruitment.
Methods. Prior to experimentation, approval was obtained from the UQ Animal Ethics Committee. In anaesthetised male Sprague Dawley rats, an intracerebroventricular (i.c.v.) guide cannula was stereotaxically implanted. Five to 7 days later, each rat received a single i.c.v. bolus dose of either CYX-5 (3, 10, 20 nmol), CYX-6 (3, 10, 20, 30 nmol), morphine (100 nmol) or vehicle. Antinociception was assessed using the warm water tail flick test (52.5±0.5°C) and constipation was assessed using castor oil-induced diarrhoea and charcoal meal gut motility tests.
Results. Intracerebroventricular CYX-6 is ~ 5 times more potent than morphine in producing analgesia. The ED_{50} (95% CI) of i.c.v. CYX-6 for evoking antinociception in rats was estimated at 9.2 (6.8 to 12.6) nmol by nonlinear regression. CYX-5 was less effective in evoking analgesia. However, unlike morphine, CYX-6 did not alter stool consistency or gut motility even at higher doses. CYX-5 also did not affect the gut motility in rats.
Discussion. These results demonstrate that analgesia can be evoked without producing opioid-related gastrointestinal adverse effects. This in vivo profile of CYX-5 and CYX-6 suggests that highly selective MOPr agonists with G-protein bias may have benefit in dissociating analgesia from gastrointestinal adverse effects.

430 Structure-based virtual screening for the rapid discovery of selective butyrylcholinesterase inhibitors

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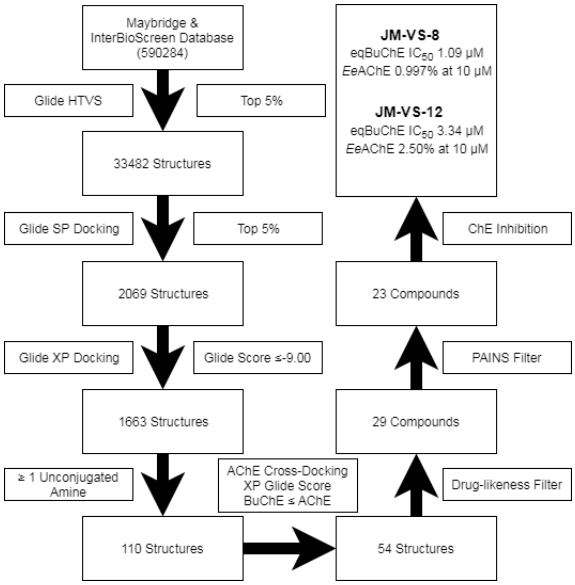
Introduction. Alzheimer's disease (AD) is characterized by the progressive loss of cholinergic neurotransmission in the brain, and is symptomatically treated using acetylcholinesterase (AChE) inhibitor drugs. However, short-term benefit and high incidence of side effects limit the efficacy of these drugs. Recently, selective inhibitors of butyrylcholinesterase (BuChE) have been suggested as an alternative with superior efficacy and side effect profiles compared to AChE inhibitors, particularly in late-stage AD.



Methods. We used structure-based virtual screening (SBVS) to rapidly search two libraries containing over 590284 structures for inhibitors of BuChE. Additional *in silico* filtering was also employed to prioritize drug-likeness, selectivity for BuChE over AChE, and remove promiscuous inhibitors. The 23 compounds resulting from this workflow were screened *in vitro* for inhibition of eqBuChE and *Ee*AChE. Analogues of the top hits were also screened to examine structure-activity relationships.

Results. From the initial 590284 input structures, the top 1663 compounds after SBVS went through additional filtering to yield 23 final hits. Of these, two compounds inhibited BuChE with low-micromolar IC_{50} values and showed significant selectivity for BuChE over AChE. Analogues of these hits, combined with virtual docking models, shed light on the structure-activity relationships.

Discussion. These results highlight the usefulness of SBVS as a tool for rapid drug discovery in AD, and provide two selective BuChE inhibitors which form a basis for the development of symptomatic treatments for late-stage AD.¶

431 Natural product honokiol reduces $A\beta_{42}$-induced toxicity in *Caenorhabditis elegans*, $A\beta_{42}$ fibrillation, cholinesterase activity, DPPH radicals, and chelates iron(II)

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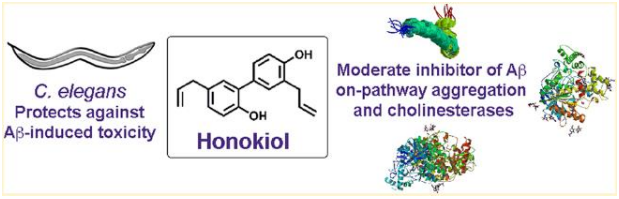
Introduction. Honokiol is a neuroprotective natural product which has been proposed as a treatment for central nervous system disorders such as Alzheimer's disease (AD). There are many factors which contribute to the development of AD, including the progressive death of cholinergic neurons in the brain, accumulation and fibrillation of amyloid beta peptide (Aβ); and toxicity resulting from metal ions and oxidative stress.



Methods. We used transgenic *Caenorhabditis elegans* expressing $A\beta_{42}$ as an *in vivo* model for assessing the effect of honokiol against Aβ-induced toxicity. Additionally, we evaluated the *in vitro* ability of honokiol to inhibit $A\beta_{42}$ oligomerization and fibrillation; inhibit acetylcholinesterase and butyrylcholinesterase; scavenge 2,2- diphenyl-1-picrylhydrazyl (DPPH) radicals; and chelate iron(II).

Results. Honokiol proved similar to resveratrol and (−)-epigallocatechin gallate (EGCG) at delaying $A\beta_{42}$-induced paralysis in *C. elegans*. However, honokiol has superior chemically stability relative to the highly unstable EGCG. We also showed that honokiol possesses moderate-to-weak activity to inhibit $A\beta_{42}$ aggregation and cholinesterase, scavenge DPPH radicals, and chelate iron(II).

Discussion. Considering these results, along with its drug-likeness and brain availability, honokiol may be a candidate for drug development and that the synthesis of analogues to further improve these properties should be considered.

432 Development and optimisation of a FLIPR high-throughput cAMP assay to screen for $G_{\alpha i}$ mediated GPCR modulators

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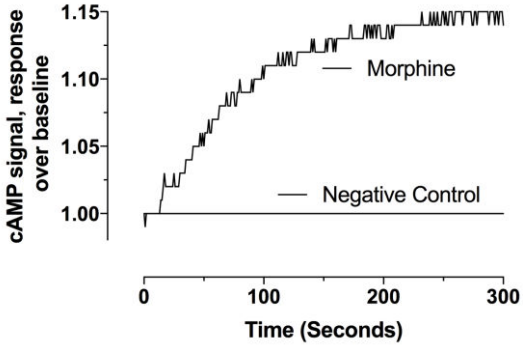
Introduction. A number of cAMP assays are available to screen Gαi G protein-coupled receptor (GPCR) ligands, however they often entail multiple steps, require forskolin to activate cAMP production, lack cAMP kinetics data and can be labour intensive. We aimed to establish a high throughput assay for quick identification of $G_{\alpha i}$ GPCR modulators.

Methods. Human embryonic kidney cells stably expressing different opioid receptors (μ,κ or δ) were transfected with a fluorescent cAMP sensor (downward cADDIS cAMP sensor, Montana Molecular) and a mutant $G_{\alpha s}$ subunit. Cells were plated on a 384 well plastic bottom plate and allowed to adhere overnight. Opioid ligands were added to the wells by the automated FLIPR pipettor head and fluorescence measured and results.



Results. Activation of the opioid receptor resulted in a quick and sustained production of cAMP demonstrated by a dose-dependent increase in fluorescence, reaching maximal fluorescence after 3 minutes. While the dynamic range of the assay was relatively small, ligands displayed accurate and reproducible EC_{50} values and equivalent to other commercially available kits.

Discussion. The FLIPR cAMP assay provides a quick, simple method to determine activity of compounds at Gαi GPCRs and could be used for high-throughput screening of ligands.

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433 Development of a BRET based assay for AT_1R-EGFR transactivation: evidence for functional heteromers

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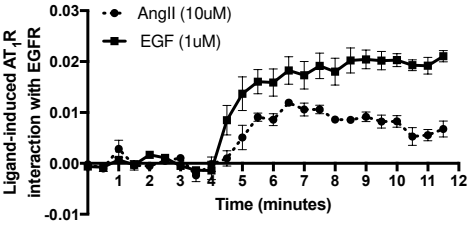
Introduction. The Renin Angiotensin System (RAS) acts via the type 1 angiotensin receptor (AT_1R) to control cardiovascular physiology and pathophysiology. The actions of AT_1R in cardiac growth and remodelling involve a capacity to "transactivate" signalling pathways downstream of the Epidermal Growth Factor Receptors (EGFRs), but demonstrating this EGFR transactivation directly, in live cells, in real time has been challenging.



Aims. Identify and characterise the molecular, temporal and spatial aspects of AT_1R-EGFR transactivation

Methods. Bioluminescence Resonance Energy Transfer (BRET), GPCR-HIT assays and Bimolecular Fluorescence Complementation (BIFC) were used to functionally characterise the molecular basis of EGFR transactivation and AT_1R-EGFR complex formation.

Results. AngII and EGF stimulation resulted in recruitment of Grb2 to the EGFR, indicating EGFR transactivation. Moreover, BIFC revealed AT_1R and EGFR exist as heteromers at the membrane with GPCR-HIT data showing ligand stimulation further enhances AT_1R-EGFR complex formation.

Discussion. BRET is a valid tool to characterise transactivation in living cells. Data suggests that following AngII & EGF treatment a close complex forms between the two receptors, thus facilitating transactivation. The underlying mechanism driving AT_1R-EGFR complex formation forms part of ongoing investigations.

434 Consequences of pharmacological inhibition of store-operated calcium entry on calcium signalling in MDA-MB-468 breast cancer cells.

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Introduction. Store-operated calcium entry (SOCE), describes the process whereby there is an influx of calcium ions (Ca^{2+}) after intracellular Ca^{2+} stores are depleted. A remodelling of the molecular components of SOCE is evident in breast cancers of the poor prognosis basal molecular subtype (McAndrew et al, 2011). However, pharmacological studies of this pathway in breast cancer cells have often used non-specific SOCE inhibitors, non-physiological mechanisms of calcium store depletion and just one basal breast cancer cell line - MDA-MB-231 (Yang et al, 2009).
Aims. To assess the effects of the selective SOCE inhibitors YM-58483 and Synta66 on calcium influx mediated by the Ca^{2+} store pump inhibitor cyclopiazonic acid (CPA), the purinergic receptor activator adenosine triphosphate (ATP), the protease-activated receptor-2 (PAR-2) activator trypsin and epidermal growth factor (EGF) in MDA-MB-468 basal breast cancer cells in the presence of extracellular Ca^{2+}.
Methods. MDA-MB-468 cells were loaded with the Ca^{2+} sensitive indicator Fluo-4 and cytosolic free Ca^{2+} levels ($[Ca^{2+}]_{CYT}$) were assessed during treatment with CPA, ATP, trypsin and EGF in the absence or presence of YM-58483 or Synta66 using a Fluorescence Imaging Plate Reader (FLIPR).
Results. CPA, ATP, trypsin and EGF exhibited $[Ca^{2+}]_{CYT}$ transients with different degrees of sustained Ca^{2+} influx. The effects of Synta66 and YM-58483 were greatest for CPA and ATP mediated Ca^{2+} influx. Sustained Ca^{2+} influx after stimulation was reduced by 35.1 and 35.5% for CPA (10 µM) and 52.4 and 48.6% for ATP (10 µM) by Synta66 and YM-58483, respectively.
Discussion. These studies define a role for SOCE as a consequence of activation in the regulation of sustained Ca^{2+} influx in MDA-MB-468 basal breast cancer cells.

McAndrew D et al (2011) Mol Cancer Ther. 10:448-60
Yang S et al (2009) Cancer Cell. 15:124-34¶

435 The sweet taste receptor: a novel target for drug discovery?

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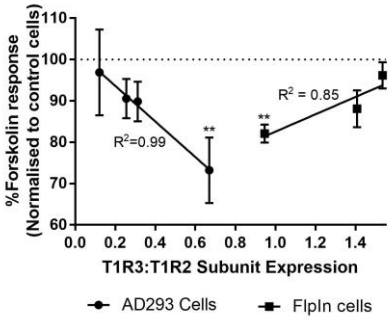

Introduction. Sweet taste receptors are expressed in many tissues throughout the body, and are implicated in obesity and diabetes. The canonical receptor is a heterodimer consisting of subunits T1R2 and T1R3 in a 1:1 ratio. However, in the pancreas and adipose tissue, the expression of these subunits has been shown to be unequal. It is essential to understand if this altered expression profile leads to changes in receptor function, so that this receptor may be harnessed as a novel drug target in the treatment of diabetes and obesity.
Aims. To examine the impact of altering subunit expression on receptor signalling and surface trafficking.
Methods. Heterologous expression systems were generated using either sequentially transfected AD293 cells, or the FlpIn system. Subunit expression was quantified by RT-PCR. Signalling through the Gi pathway was measured as a reduction in % forskolin response determined by cAMP assay using the BRET CAMYEL sensor. Surface trafficking was determined by biotinylation pull-down experiments.
Results. Subunit expression closest to 1:1 lead to the greatest functional responses to aspartame, as shown in the figure above. Expression of both sweet taste receptor subunits was found to be predominantly intracellular, and was not improved by 1:1 expression of both subunits.
Discussion. Unequal expression of the two sweet taste receptor subunits lead to an alteration in signalling profile – in this study, a reduction in Gi signalling. This suggests that the sweet taste receptor may either be non-functional, or signals through alternative pathways in tissues where there is unequal expression of subunits. Surprisingly, surface expression did not appear to correlate with functional response. More research is therefore needed to understand tissue-specific signalling profiles, to enable the development of the sweet taste receptor as a novel drug target.

436 Assessment of calcium responses induced by the transient receptor potential cation channel subfamily V member 4 (TRPV4) activator GSK1016790A in MDA-MB-468 breast cancer cells using automated epifluorescence microscopy.

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Introduction. The transient receptor potential cation channel subfamily V member 4 (TRPV4) is elevated in the basal molecular subtype of breast cancer (Peters et al, 2017). These breast cancers have poor prognosis and significantly overlap with the triple negative breast cancers. TRPV4 appears to contribute to the migration potential of breast cancer cells (Lee et al, 2017). However, the consequences of pharmacological activation of TRPV4 using the TRPV4 activator GSK1016790A have not been fully explored, particularly in the context of single cell Ca^{2+} imaging.
Aims. To assess temporal and spatial changes in cytoplasmic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) induced by the TRPV4 activator GSK1016790A in MDA-MB-468 basal breast cancer cells.
Methods. MDA-MB-468 cells were plated onto 96-well microplates and loaded with the Ca^{2+} sensitive indicator Fluo-4 or Fura-2. Fluorescence changes induced by 0, 1 or 100 nM of GSK1016790A were detected using an automated epifluorescence microscope (ImageXpress). Image segmentation analysis was used to assess changes in $[Ca^{2+}]_{CYT}$ as assessed by Fluo-4, and ratiometric imaging was used to assess relative levels of $[Ca^{2+}]_{CYT}$ in Fura-2 loaded MDA-MB-468 breast cancer cells.
Results. MDA-MB-468 breast cancer cells exhibited spontaneous $[Ca^{2+}]_{CYT}$ oscillations. GSK1016790A at 100 nM induced pronounced, rapid and sustained increases in $[Ca^{2+}]_{CYT}$ in MDA-MB-468 breast cancer cells. Pronounced single cell heterogeneity was observed in $[Ca^{2+}]_{CYT}$ changes.
Discussion. These studies provide further evidence that MDA-MB-468 cells express functional TRPV4 channels and suggest that there may be significant heterogeneity in MDA-MB-468 breast cancer cell responses to TRPV4 activation.

Peters AA et al (2017) Oncogene (in press)
Lee WH et al (2017) Oncogenesis. 6:e338.¶

437 PAR_1 and PAR_2 open TRPV4 with conserved signalling pathways

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Introduction. We have previously shown that the pro-inflammatory G-protein coupled receptor, protease-activated receptor 2 (PAR_2) signals to and opens TRPV4 channels in HEK293 cells (Poole et al., 2013). We identified molecules which transduce signals from PAR_2 to TRPV4 using siRNA inhibition and identified signalling molecules which include heterotrimeric G-proteins, phospholipases and protein kinases (Darby et al., unpublished). In this study, we investigated whether any of the identified siRNA targets also transduce signals from PAR_1 to mediate TRPV4 opening.
Aims. To determine if PAR_1 and PAR_2-dependent opening of TRPV4 in HEK293 cells shares signalling mechanisms.
Methods. Parental HEK293 cells and HEK293 cells stably expressing human TRPV4 were transfected with Dharmacon SMARTpool siRNAs and each well was subsequently assayed for PAR_1-dependent opening of TRPV4 using a fura-2am fluorescence ratiometric intracellular calcium ($[Ca^{2+}]_i$) assay. Cells were injected with PAR_1 activating peptide (TFFLR-NH_2, 50 µM) followed by the selective TRPV4 agonist (GSK101067A, 30 nM), 85 s later. The area under curves from 50 – 90 s was compared using one-way ANOVA with Sidak's post hoc t-test.
Results. In parental HEK293 cells, PAR_1 activation transiently increased $[Ca^{2+}]_i$ (area = 12 ± 3). Functional expression of human TRPV4 caused a sustained increase of $[Ca^{2+}]_i$ (area = 43 ± 6) which was abolished by the TRPV4 antagonist (HC067047, 1 µM) (area = 9 ± 4). siRNA knockdown of $G\alpha_{13}$ and $G\gamma_8$ significantly ($p < 0.05$) inhibited PAR_1-dependent opening of TRPV4 reducing area by 52 ± 8% and 39 ± 7% respectively. Inositol-tetrakisphosphate 1-kinase (ITPK1), mitogen-activated protein kinase 13 (MAPK13) and lysine deficient protein kinase 4 (WNK4) also reduced area by 44 ± 12%, 69 ± 9% and 39 ± 9% respectively. Phospholipase A_2 group 4 (PLA_2G4) reduced the area by 54 ± 12%.
Discussion. Activation of GPCRs results in simultaneous activation of parallel signalling pathways. Therefore, inhibition of TRPV4 opening by a specific siRNA pool is an indication that the target protein contributes to PAR_1-dependent opening of TRPV4. Like PAR_2 receptors, PAR_1 receptors were found to couple to TRPV4 through heterotrimeric G-protein subunits, $G\alpha_{13}$ and $G\gamma_8$, PLA_2G4, and kinases ITPK1, MAPK13 and WNK4 in HEK293 cells.

Poole et al. (2013) J Biol Chem, 288:5790-5802¶

438 Neuronal calcium sensor-1 (NCS-1) in the regulation of calcium homeostasis and cell death in MDA-MB-231 basal breast cancer cells

Alice HL Bong[1], John J. Bassett[1], Sarah J Roberts-Thomson[1], Michael J. G. Milevskiy[2], Gregory R. Monteith[1,3]. School of Pharmacy, The University of Queensland[1], Brisbane, QLD, Australia; The Walter and Eliza Hall Institute of Medical Research[2], Melbourne, VIC, Australia; Mater Research, The University of Queensland[3], Brisbane, QLD, Australia.

Background: Altered calcium (Ca^{2+}) signalling in cancer cells may promote cancer hallmarks such as resistance to apoptosis. Proteins regulating these signals represent attractive therapeutic targets. Neuronal calcium sensor-1 (NCS-1) is associated with tumour aggression and poor prognosis in breast cancer patients. However, the characterisation of NCS-1 in breast cancer molecular subtypes, the effects of NCS-1 silencing on intracellular Ca^{2+} homeostasis in breast cancer cells and on the cytotoxic effect of the anti-cancer drug doxorubicin, remain unexplored.
Aim: To assess the expression of NCS-1 in public breast cancer datasets and assess the consequences of silencing NCS-1 on intracellular Ca^{2+} signaling and sensitivity to doxorubicin in the MDA-MB-231 basal breast cancer cell line.
Methods: The expression of NCS-1 in patient breast tumours was stratified by PAM50 molecular subtype and assessed using breast cancer public datasets. MDA-MB-231 cells stably expressing the GCaMP6m Ca^{2+} sensor were transfected with non-targeting control or NCS-1 siRNA. The effects of NCS-1 silencing on cytosolic Ca^{2+} in response to Ca^{2+}-mobilising agonists (ATP, trypsin and cyclopiazonic acid (CPA)) and on constitutive Ca^{2+} influx were measured using a Fluorescent Imaging Plate Reader (FLIPR). The sensitivity to doxorubicin (24 h) following gene silencing of NCS-1 was determined by propidium iodide staining.
Results: NCS-1 was expressed higher in basal molecular subtype breast cancers. Silencing NCS-1 did not alter cytosolic Ca^{2+} changes induced by ATP, trypsin or CPA treatment. However, NCS-1 silencing suppressed constitutive Ca^{2+} influx. NCS-1 silencing also promoted MDA-MB-231 cell death in combination with doxorubicin (1 µM) treatment.
Discussion: These results implicate NCS-1 in basal breast cancer, a subtype with poor prognosis. Indirect modulators of endoplasmic reticulum Ca^{2+} levels such as NCS-1 may alter constitutive Ca^{2+} influx pathways and influence processes important in cancer such as sensitivity to anti-cancer agents.
Monteith GR et al (2017) Nat Rev Cancer. 17:367-380.
Moore LM et al (2017) Mol Cancer Res. 15(7); 942–952¶

439 Understanding the physiological role of endogenous allosteric modulators in the muscarinic acetylcholine receptors

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Introduction. Allosteric binding sites on G protein-coupled receptor (GPCR) can be targeted by synthetic or natural (endogenous) molecules (van der Westhuizen et al., 2015). However, the (patho)physiological role(s) of many endogenous allosteric modulators remain poorly understood. One interesting example is major basic protein (MBP), a highly basic peptide that acts as a negative allosteric modulator (NAM) of acetylcholine (ACh) at airway M_2 muscarinic acetylcholine receptors (mAChR; Jacoby et al., 1993). We hypothesized that, in addition to MBP, other endogenous basic peptides, including the antimicrobial, LL-37, involved in chemotaxis, maturation of immune cells and apoptosis (Kahlenberg et al., 2013) could also interact allosterically with the M_2 mAChRs and have major physiological impacts.
Aims. To characterise the pharmacological properties and the putative (patho)physiological roles of LL-37 at mAChRs.
Methods. Using IMR-32, a native cell line endogenously expressing human M_2 mAChRs and mouse tissues predominantly expressing mouse M_2 mAChRs, we performed [^{3}H]NMS radioligand binding and [^{35}S]GTPγS turnover as a functional measure of receptor activation, to assess the allosteric effect of LL-37.
Results. LL-37 mediated a concentration-dependent partial inhibition of the antagonist [^{3}H]NMS binding in IMR-32 cells and mouse cardiac tissues (pK_B=4.7±0.3 and 5.6±0.5, respectively), a hallmark of allostery. Additionally, LL-37 also negatively modulated ACh-mediated G protein activation in mouse hypothalamus preparations.
Discussion. Our results suggest that LL-37 is a NAM of antagonist binding and agonist function at the M_2 mAChR. The M_2 mAChRs are highly expressed on both neuronal and non-neuronal cells, including immune cells and epithelial cells, and are known to be involved in their survival outcome. In the context of inflammation and cancer, when LL-37 is highly expressed, the antagonism of M_2 mAChR activity by the peptide could therefore have unappreciated (patho)physiological consequences.
van der Westhuizen ET al. (2015) J Pharm Exp Ther 353(2):246-60.
Jacoby et al. (1993) J Clin Invest 91:1314-1318.
Kahlenberg et al. (2013) J immunol 191(10):4893-901. ¶

440 Experiences in defining Entrustable Professional Activities to drive the learning of undergraduate pharmacy students

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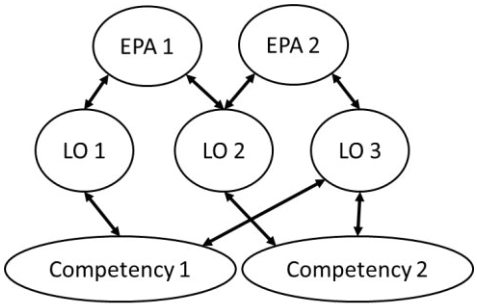
Introduction. Entrustable professional activities (EPAs) are discrete tasks or responsibilities that a trainee is entrusted to complete and document with appropriate supervision. EPAs (e.g. dispensing or treatment of a minor ailment) link directly to a work-based assessment framework and allow for a natural continuation of learning in pharmacy from an undergraduate student to an intern to a pharmacist. EPAs also map to learning outcomes, see figure, and therefore to professional competencies.



EPA = entrustable professional activity;
LO = learning outcome

Aims. To define entrustable professional activities appropriate for pharmacy undergraduate education.

Methods. A group of core and elective EPAs were identified using interviews with pharmacists, a survey of pharmacy services, and the pharmacy services stipulated by the Pharmaceutical Society and Pharmacy Council of New Zealand. For each EPA, one of five levels of attainment was assigned; where level 1 was "observation only" and level 5 "supervision provided by the student to more junior students".

Results. Nineteen core EPAs were defined. An example of a core EPA is dispensing which was assigned an attainment level of 4 (defined as "execution with post-hoc supervision"). Twenty-two elective EPAs were identified and were all assigned an attainment level of 1 or 2. An example is anticoagulation monitoring.

Discussion. EPAs are used extensively in medical education but not yet in pharmacy undergraduate programmes. They are a useful tool in the teaching and assessment of pharmacy services and professional competencies.

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441 Older people as university-based instructors to improve empathy and attitudes toward older people among first-year pharmacy students

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Introduction. There is increasing recognition of the need to provide comprehensive and holistic care for older people with multi-morbidity and polypharmacy (Mc Namara et al, 2017). Monash University began offering a 5-year vertically integrated MPharm program, in 2017, providing an opportunity to design a supported encounter with an older person for students. Professional Practice was one of three units of study taught within the first semester of the degree.

Aims. The objective of this education brief is to describe implementation of a workshop to improve first year pharmacy students' empathy and attitudes toward older people.

Methods. An eight item survey based on empathy and attitudes towards older people was developed. Students completed the survey instrument before and after the workshop. The 2-hour workshop required students to engage with older people as part of the Professional Practice course for first year undergraduate pharmacy students at Monash University, Melbourne, Australia.

Results. Engaging older consumers as university-based instructors for first year pharmacy students was associated with significant short-term improvements in three of the eight attitudinal items assessed. Following the workshop students' were more likely to report older people are pleasant to be with, more likely to understand what it feels like to have problems with aging, and less likely to believe older people become confused and less organised.

Discussion. A two-hour workshop involving older consumers as university-based instructors produced immediate improvements in self-reported attitudes towards older people. Engaging older people as university-based instructors for first year pharmacy students may be a successful strategy to develop positive attitudes, empathy and oral communication skills.

Mc Namara KP et al (2017) Age Ageing 46:291-9.

442 A digital portfolio: Learning gains and efficiencies for placements in new BPharm programme.

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Introduction. The implementation of a new BPharm curriculum at the School of Pharmacy University of Auckland in 2016, presented an opportunity for significant change to experiential learning placements: amount of dedicated placement time; range of placement sites; use of digital technology.
Aims. To describe the key education principles that have informed the development an ePortfolio used by BPharm students during placement modules and report on initial learning from the implementation.
Methods. The placement team and a learning designer worked in partnership to develop an ePortfolio to guide student learning during placement modules and to assess competency of essential skills, knowledge and behaviours. Feedback was sought from key staff, students and preceptors to enable ongoing review and refinement of ePortfolio design and development.
Results. Education principles of alignment, relevance, scaffolding, Millers's Prism of Professional Competence[1] and Pharmacy Council of New Zealand competence standards have been applied in the designing of the ePortfolio. The design process resulted in student owned ePortfolio and website that bring together a comprehensive set of resources to support students before, during and after their placement modules. It has been designed to enable students to develop transferrable skills to support their transition to the pharmacy profession. Use of digital technology, in particular ePortfolio, has afforded efficiencies in terms of administration, submission and assessments for the placement modules in the new BPharm programme.
Discussion. The ePortfolio is used throughout the entire BPharm programme providing students with a longitudinal record of their learning in one ePortfolio. It provides a unique opportunity for students to draw on learning from across the curriculum and to progressively develop individualised learning and skill development from year to year.

Miller GE. The assessment of clinical skills/ competence/ performance. Acad Med (1990);65:s63-s67.¶

443 Education for vancomycin – what works?

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Introduction. Dosing and monitoring guidelines are readily available for vancomycin. However, hospital audits consistently show suboptimal vancomycin therapy (Davis et al., 2013). Few studies have examined the types, strengths and weaknesses of educational resources used to support vancomycin prescribing.
Aims. To explore the opinions and experiences of Australian educators on the methods used to educate health professionals about vancomycin in order to identify the most effective approach to education.
Methods. Health professionals involved in delivering antibiotic education to clinical staff were approached via email and invited to participate in a semi-structured interview. Questions focused on the use of educational resources and methods for vancomycin dosing and monitoring practices. Interviews were transcribed verbatim and analysed independently by two researchers for emerging themes.
Results. Pharmacists (n=18) and Infectious Disease physicians (n=6) were interviewed. The most frequent mode of vancomycin education reported was an annual lecture during junior staff orientation. This was in contrast to what educators viewed to be ideal education (one-on-one, case-based, tailored learning). Educators reported that different methods were likely to be effective for different healthcare professionals (e.g. doctors vs. nurses). Access to online resources (such as vancomycin.com.au and Qstream) and dosing calculators were also seen to enhance vancomycin education. Time constraints were a major limitation to clinical education, with development of readily accessible and efficient educational strategies a priority.
Discussion. Effective education was reported to be multimodal, including strategies such as academic detailing and interactive, problem based learning using case studies.

Davis *et al.* (2013) Pharmacotherapy 33:1256–1263.

444 Creating a labelling standard for compounded medicines – a learning task requiring higher order thinking skills

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Introduction. Involving students in authentic learning tasks which require high order thinking skills such as synthesis, design, evaluation and creation has been shown to engage students in more active and productive learning. A novel learning task was developed and integrated into the 3rd Year Dispensing Unit of Study. Groups of Bachelor of Pharmacy students were required to synthesise information from legislation, professional standards and research findings in order to create a "labelling standard" for producing and evaluating labels for compounded medicines.
Aims. The aim of this study was to explore students' perceptions of the newly developed learning task.
Methods. Students' perceptions were explored using focus group discussions conducted during the semester following the learning task. Thematic content analysis was used to explore and organise the findings. The Consolidated Criteria for Reporting Qualitative research (COREQ) guided the conduct, analysis and reporting of the study.
Results. Three focus groups were conducted over two weeks involving 25 students (11% of cohort). Two main themes were extracted. The first theme was perceptions of learning style. Students conveyed deep self-reflections about the benefit of "thinking outside the box", rather than answering questions in "recycled assignments". Not all students recognised the benefit of learning this way and some expressed a distinct distaste for the need to be creative in a pharmacy degree. When criticising the learning task, students focussed on the practical challenges. They cited problems with resource availability, lecturer guidance, clarity of the grading rubric, timing in relation to other assessments and aversion to group work. The second theme pertained to students' perspectives about the impact of their learning on how they labelled compounded medicines. While not universally reported, students recounted having better insight into consumer perspectives, legislative and professional guidance. Students recognised the benefit of having a written labelling standard for dispensing tasks, although its relevance to practice was questioned.
Discussion. Some pharmacy students embrace and thrive on creativity and critical analysis, however many do not expect their degree to include learning tasks which require synthesising information from a variety of resources to solve practical problems. Overall, students valued the resulting "labelling standard" and their constructive comments have informed modifications prior to integration into the curriculum. ¶

445 Nursing students are more reliant on ongoing assessment scores to succeed in pharmacology than paramedic or optometry students

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Introduction. Ongoing assessment and examinations are often used to test different aspects of learning with examinations testing the assimilation of knowledge and ensuring that the students complete the work themselves. However, the proportional allocation of marks for ongoing assessment and examinations is often made on an arbitrary basis, and the consequences of this are not known.
Aim. The aim was to compare percentage marks and failure rates in ongoing assessment and examinations for the successful nursing, paramedic and optometry students completing a pharmacology unit in 2013/4/5.
Methods. In the unit, 40% of total marks were allocated to ongoing unsupervised assessment and 60% to examinations. The marks for each student who passed the ongoing assessment and exams were calculated as a percentage and compared by Students paired test. Students who achieved less than 50% in each component were considered to have failed the component; failure rates were compared by Odds ratio. In the Table below, significance is at *P < 0.05; the number of students is also indicated (n).
Results. Results for each year were similar. All student cohorts obtained significantly better marks in ongoing assessment than exams in the pharmacology unit. A higher percentage of nursing, than paramedic or optometry students, failed the ongoing assessment and exam components of the unit.

	Percentage Marks (number)		**Failure rates (percentage)**	
Student cohorts (2014)	Ongoing assessment	Exams	Ongoing assessment	Exams
Nursing	72.4 ± 0.5 (215)	57.7 ± 0.9 (215)*	18/215 (8.4%)	61/215 (28.4%)*
Paramedic	74.2± 1.1 (95)	66.1 ± 1.3 (95)*	1/95 (1.1%)	9/95 (9.5%)*
Optometry	84.4 ± 1.0 (50)	66.6 ± 1.8 (50)*	0/50 (0%)	2/50 (4%)

Discussion. The nursing students who passed the pharmacology unit were more reliant on scores obtained in ongoing unsupervised assessment. The nursing students, who passed the unit but not the examinations, may not have assimilated the necessary knowledge to continue in their courses. Additionally, some of the passing nursing students may have succeeded due to work done by others in ongoing assessment.¶

446 Do pharmacy students have different personal characteristics than other students?

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Introduction. The personal characteristics of pharmacists will help shape the future of the profession, especially in determining whether students as future pharmacists will embrace advanced pharmacy roles. We consider three frameworks. Are their achievement goals motivated by internal standards (either striving for mastery or avoiding failure) or their performance relative to others (striving to beat others or avoiding failure)? How do they score on the five-factor model of personality (openness, conscientiousness, extraversion, agreeableness, neuroticism)? Do they prefer to make decisions based on rational deliberative processes, or experiential intuitive processes?
Aims. To determine the differences in trait characteristics between students entering the Otago pharmacy programme and students studying other subjects; and between students who apply only for pharmacy, versus those who apply for multiple health professional courses.
Methods. All second year students at the University of Otago were invited to take an online questionnaire, containing measures of the 'big five' personality traits, the Achievement Goals Questionnaire – Revised, and the Rational-Experiential Inventory, along with demographic variables.
Results. 565 students (97 pharmacy, 465 non-pharmacy) completed the survey, from an estimated 3536 invited (16% response rate). Relative to non-pharmacy students, pharmacy students were more motivated by achieving mastery, $p = .001$, $d = -.31$ [-0.53, -0.09] , but were lower on rational decision-making, $p = .019$), $d = 0.26$ [0.04, 0.48] and also experiential decision-making, $p = .03$, $d = 0.24$ [95% CI 0.02, 0.46]. Pharmacy students were slightly higher on Agreeableness, $p = .041$, $d = -0.23$ [-0.45, -0.01]. Students who applied for multiple health professional courses were more highly motivated by avoiding failure against their internal standards ('mastery avoidance') than students choosing pharmacy, $p = .003$, $d = 0.54$ [0.12, 0.97].
Discussion. Overall, pharmacy students had relatively similar characteristics to non-pharmacy students. However, there were some promising characteristics that may predict engagement with advanced pharmacy roles, including a higher focus on mastery, and higher agreeableness. Our future work will determine whether these are predictive of future role engagement. ¶

447 Student engagement in learning: learning space matters

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Introduction. Course delivery in biomedical sciences at The University of Sydney relies on a mix of traditional, transmission-style (lecture), active learning, and laboratory-based activities. Research developing the active learning classroom (ALC) has sought to replace the outdated traditional model (TM) of education by changing pedagogy, room design and instructor/student interaction. Outcomes from many studies demonstrate greater student engagement in learning in active learning settings. Nonetheless, much of the literature fails to adequately define or operationalize proximal measures of engagement, and often relies heavily on subjective self-report of student experiences.
Aims. We aimed to develop a tool for student engagement in learning and to use this tool to evaluate student engagement across a range of learning spaces and learning activities.
Methods. Cognitive and social psychology were utilized to develop a model of engagement to predict behavioural phenotypes. The predictions were applied to both ALC and TM classrooms (n=50) to measure "on-target" and "off-target" student behaviours using observational coding. Participants were students aged 18-25 enrolled in biomedical science courses. A total of 21,826, (ALC=10,647, TM=11,509) behaviours were recorded and analysed across ALCs and TM settings.
Results. Analysis showed greater frequency of on-target behaviours, suggesting higher levels of engagement, in ALCs relative to TMs. Further trends in student behaviours from certain instructor interventions, environmental features, class scheduling and types of activity were found.
Discussion. Our findings inform physical, instructional, and social design decisions in biomedical curricula, and effective use of learning spaces.

448 Pilot study of a clinical pharmacology exam for medical students prior to hospital internship in Newcastle

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Introduction. There is clear evidence that suboptimal prescribing is common, with errors attributable to a complex mixture of antecedent and contextual factors. New doctors are responsible for a large proportion of prescribing and are often underprepared and inadequately supported.
Aim. To evaluate the Prescribing Skills Assessment (PSA) test, adapted from the UK version for the Australian setting, as a summative tool for assessing medical student's ability to prescribe appropriately and safely.
Methods. Final-year medical students in the Joint Medical Program of the University of Newcastle and University of New England were invited to undertake the PSA - a two hour, computer-based, limited open book, pass/fail assessment of the skills, judgement and supporting knowledge related to prescribing medicines, based on eight competencies identified by the General Medical Council (including writing new prescriptions, reviewing existing prescriptions, calculating drug doses, identifying and avoiding both adverse drug reactions and medication errors and amending prescribing to suit individual patient circumstances). The content of each item is based on prescribing scenarios commonly encountered by junior doctors. Prior to the PSA students were able to complete example questions in order to familiarise themselves with the test format.
Results. Mean candidate score exceeded the pass mark by > 10%. Although the majority of candidates agreed that “the assessment was an appropriate test of the prescribing skills expected of a medical student upon graduation”, few candidates agreed that “my course prepared me for the content of the questions in this assessment”.
Discussion. The results of this pilot will be used to develop the PSA as a summative test of prescribing for use in future years of the Medical program. It will also stimulate improved education by helping to identify areas of teaching within the Medical program that require further development. Ultimately the use of the PSA will help improve the competence of junior doctors to prescribe, which will lead to better patient outcomes.

Dornan T, Ashcroft D, Heathfield H et al (2009) EQUIP study. London. General Medical Council.¶

449 Evaluation of a new integrated Master of Pharmacy curriculum

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Introduction. A new integrated Master of Pharmacy 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.
Aims. This study aimed to examine the effectiveness of the new integrated Master of Pharmacy curriculum.
Methods. Unit of Study surveys (USS) collected feedback on the student experience at the unit of study level. Its content is aligned with items/scales of the national course-level survey, the SES. There are ten quantitative items and two open response items.
Results. USS results improved for the Master of Pharmacy overall compared to the previous discipline based approach. The combined USS mean score for first year, was 4.05 for core items 1-6 and 4.09 for overall items 1-10.
Discussion. The new integrated Master of Pharmacy curriculum demonstrated favourable results compared to the previous discipline based curriculum.

450 Development of a program wide pharmaceutical compounding strategy using the scaffold learning approach to improve student learning outcomes

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Introduction. In our review of the Dosage Form Design courses, we realized that our Pharmacy students were not confident in undertaking extemporaneous compounding in Dosage Form Design 3 (DFD 3), a course taught in third year of the program in which expectations of the written and physical skills were high and tasks were assessed stringently. While students had spent time in the laboratory undertaking practical classes and aspects of Pharmacy practice in prior years, they struggled to combine the two skills.
Aims. The data and student feedback was pointing to two main problems. Hence we aimed to address the two main concerns by increasing students' prior exposure: 1. to compounding techniques. 2. to filling in batch sheets which form the record keeping component in the practical sessions.
Methods. Collectively, we decided to introduce the activity to students in a structured way, first familiarising them with the basic concepts in the first two years to develop the necessary laboratory skills and confidence required to perform the task individually in its entirety in the later years of the Pharmacy program. This scaffolding approach to the task was developed, extending throughout the four years of the Pharmacy program.
Results. Since changes in 2015, DFD3 has consistently had over 98% pass rate for the practical component, with more than 75 % of student attaining distinction and above for their practical component. In the third and fourth year practical classes, students exhibit more confidence, resilience and more independence. Lecturers have noticed that since the implementation of the changes, students have increasingly become independent learners.
Discussion. The scaffolding approach whereby we tailored our instruction by providing support incrementally improving a learners' ability to build on prior knowledge, as this was seen as an important learning approach for addressing this issue. By providing this support and development across the Pharmacy program, students have achieved a significantly enhanced ability and associated outcomes in pharmaceutical compounding. ¶

451 Gamification to enhance learning of difficult concept in Pharmacology

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Introduction. It is often challenging to engage students in learning difficult concepts in pharmacology as well as other pharmaceutical biology disciplines. Gamification, serious games and simulations are gaining popularity as new approaches to teaching and learning in higher education. Gamification has the potential in enhancing motivation and engagement and is used in pharmacology teaching.
Aims. To explore the use of gamification and game-based learning in pharmacology teaching with the aim to transform students' perceptions from 'pharmacology equals an extensive amount to know and remember' to 'pharmacology equals an interesting and essential subject that enhances competency in both clinical pharmacology and the prescription of medications'
Methods. A series of pharmacology games, including Drug Review Bingo, Speedy Drug Challenge block game, Pharmacology crossword puzzle and Speed Drug Dating were developed and used in Pharmacology teaching in the second and third year of the Bachelor of Pharmacy course. Collective and accumulative qualitative feedback were obtained through the University evaluation process, eVALUate, from 2012 to 2017. Students' perception on the benefits, favourite game(s) and areas for improvement were analysed using NViVo analysis.
Results. Based on the qualitative comments by students through eVALUate, Drug Review Bingo and the Speedy Drug Challenge Block game used as revision for each module of the pharmacology units were the two favourite tools perceived to enhance students learning. The usefulness of the Speed Drug Dating varied depending on the collaborative nature of the class in preparation of tasks before the activities. In classes where students came prepared, all comments indicated that it had been beneficial in fostering deep learning.
Discussion. Overall students commented that the various games were engaging, fun and improved their ability to understand more complex content of pharmacology and foster internalisation of knowledge allowing long term memory to occur more effortlessly.

452 Improving student engagement: utilising a wet pain practical

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Introduction. Practical classes provide a valuable technique based experience, which enhances the development of laboratory skills, reinforces lecture content and supports the development of critical reasoning. Students informally surveyed and on course CATEI indicated that they preferred 'wet practicals' to computer based and scenario based practicals.
Aims. To develop a new 'wet practical' conducive to clinical reasoning to replace a scenario based pain practical.
Methods. A practical which allowed the students to simply measure a pain response was developed. A pre-lab including a video demonstrating the procedure as well as a series of question that tested the student's comprehension was made available at the start of the class. In groups of 3, students were assigned as a subject, tester, or record keeper. Von Frey bristles were utilised to provide both a 'touch' and 'pain' response on the ventral side of the forearm. Control responses were obtained and tallied using a square grid, where students count the number of positive scores for each grid (maximum of 25). Two EMLA (eutectic mixture of local anaesthetics) patches (lignocaine and prilocaine) were applied on the opposite forearm for 30 and 60 minutes respectively. Scores were tallied in the same way as for controls and class data were collated (PHAR3251, practical manual).
Results. The outcome of this approach has been an observed improvement in comprehension of underlying theory (improved exam results). Students must successfully engage with the pre-lab and answer all questions correctly before they can proceed with the class. The class successfully combines the topical administration of drugs and the inhibition of pain in a wet practical where pain measurement is notoriously difficult to achieve. Student's comments (CATEI 2016) Best features of the course were: 'Labs support lectures well. Use of clinical examples are great.'; 'The labs allowed us to see how drugs worked first hand'. Students were in agreement with the question 'the course was effective for developing my thinking skills e.g. critical analysis, problem solving' (L&T agree 100% CATEI 2016).
Discussion. Providing the students with a wet practical, linked to their lecture component, where they can obtain 'hands on' experience has improved engagement and participation in practical learning. Improved their theoretical competence and allowed them to directly experience some of the difficulties/issues encountered when measuring pain. ¶

453 Demonstrating the ability to prescribe medicines: a multi-professional view

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Introduction. An increasing number of Australian health professions have gained authority to prescribe medicines. Preparing students to competently prescribe medicines is challenging yet critical; as is the demonstration of ongoing fitness to prescribe.
Aim. To explore the opinion of a multi-professional cohort of clinicians, educators and regulators regarding key issues central to the development of a safe and effective prescribing workforce.
Method. An anonymous survey was available for completion using two formats: as part of a workshop conducted during a national conference and via email using an on-line format. Data generated from both sources were subsequently amalgamated and analysed.
Results. A total of 71 responses were received from a cohort who described their primary role as: practising clinician (31%), education and training (30%), accreditation and standards oversight (14%), policy and governance (21%). A further 8% were involved in professional development. Respondents represented a total of nine professions, including both established prescribing professions and those that currently do not prescribe medicines.
Respondents overwhelmingly agreed there is a need for a consistent approach to the demonstration of student prescribing competence, both within (92% agreed) and across (83% agreed) prescribing professions. A number of methods were proposed to provide an indication of prescribing ability. Direct observation of performance was frequently chosen as an effective method to demonstrate the ability to undertake many aspects of the prescribing process in the student context. The demonstration of ongoing fitness to prescribe was considered important by over 90% of respondents. A combination of assessment methods was considered most useful in this setting.
Discussion. The survey suggests that there is agreement between health professions that clear demonstration of prescribing ability is important, both at the time of initial achievement of prescribing authority and in an ongoing capacity. A number of methods were considered useful to assist with this process. Further development of appropriate competence assessment processes may positively impact the development and maintenance of a safe and effective prescribing workforce.

454 Does attending lectures matter when lecture recordings are available? Results for a preliminary study of nursing students in pharmacology

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Introduction. Before the introduction of technology into teaching, it was assumed by many teachers that grades were related to lecture attendance; students who attended classes more frequently, obtained better grades. However, there have been no studies of the effects of lecture attendance on academic outcomes for nursing students in pharmacology. Also, the introduction of lecture recordings may have led to reductions in lecture attendance and/or better results for non-attending students.
Aims. To determine the effect of lecture attendance on academic outcomes in bioscience for nursing students provided with access to lecture recordings. The study was undertaken in 2013/4.
Methods. Prior to the start of lectures on gastrointestinal bioscience and/or microbiology anti-infectives, attending students provided their ID numbers upon submission of a short quiz, as part of another study. The academic outcomes for attending and non-attending students in the tutorial assessment (40%), examinations (60%), and final grade in pharmacology were compared by Student's unpaired t-test with *P < 0.05 (Table); the number of students is also indicated (n).
Results. The uptake of lecture recordings and lecture attendance was higher at the start than end of semester. Only a third of the nursing students attended the gastrointestinal and microbiology lectures late in the semester. Attending students obtained better outcomes in the tutorial assessment, examinations and final grade.

	Tutorial assessment - % mark		**Examinations - % mark**		**Grade**	
Year	Non-attending	Attending	Non-attending	Attending	Non-attending	Attending
2013	77.2 ± 0.7 (392)	80.6 ± 1.5 (50)	59.1 ± 0.7 (392)	66.0 ± 1.8 (50)*	4.8 ± 0.1 (392)	5.3 ± 0.1 (50)*
2014	75.9 ± 0.7 (308)	80.2 ± 1.9 (23)	57.4 ± 0.8 (308)	64.4 ± 2.9 (23)*	4.7 ± 0.1 (308)	5.3 ± 0.2 (23)*
Combined	76.6 ± 0.5 (700)	80.5 ± 1.2 (73)*	58.1 ± 0.5 (700)	65.3 ± 1.6 (73)*	4.8 ± 0.0 (700)	5.3 ± 0.1 (73)*

Discussion. This preliminary study suggests that nursing students attending lectures had better academic outcomes in pharmacology than those that did not attend lectures, and that it is still important to provide face-to-face lectures for these students.¶

455 Implementation of a consistent and structured approach to small class workshops: A case study in pharmacy education at Monash University.

Nilushi Karunaratne[1]. Faculty of Pharmacy and Pharmaceutical Science, Monash University[1], Melbourne, VIC, Australia.

Introduction. Small class teaching forms an essential part of the pharmacy education at Monash University. Traditionally, tutorials and workshops are delivered in conventional classroom settings, with variable timetable scheduling and rotating tutors. In addition, setup of teams for group activities is variable from session to session and variability also exists in the development of workshop materials and in their presentation. One of the key features of Monash University's new Bachelor of Pharmacy (Honours)/Master of Pharmacy degree (also known as the Vertical Integrated Master's or VIM) is the implementation of a consistent and structured approach to small class teaching.
Methods. Units within the VIM were structured to allow workshops to take place weekly on the same day, time, and location. A pool of experienced facilitators were sourced for each unit and underwent further training to effectively facilitate specifically within the VIM, and were rostered such that each small class engaged with the same facilitator throughout the semester. Within workshops, students were allocated into teams of 4-5 and these teams remained consistent throughout the semester. Workshops took place in newly designed technology-enhanced learning spaces featuring collaborative pods with whiteboard table surfaces, mobile computers on wheels (MCOWs) being accessible to each pod, and a master room allowing AV control of surrounding learning spaces enabled by a 'patch-in' system. Workshop materials were developed in line with a 'best practice' template which included having Monash branded PowerPoint presentation slides to visually guide students, a 'running time sheet' to aid facilitators to effectively manage a workshop session, and time dedicated for 'closing of the loop' at the conclusion of each workshop to tackle common misconceptions and to link concepts covered within the session to the profession of pharmacy.
Results. The combination of these improvements and best practices have been applied to over 90 workshop sessions across 2 semesters in the new VIM degree in 2017.
Discussion. Designing effective learning environments, providing students consistency in terms of timetabling, facilitator rostering and student grouping, together with improving instructional modalities has the potential to positively impact student-centered learning.

456 Practicing pharmacists' preferences for skills taught in an undergraduate pharmacy program

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Background: Pharmacy is a rapidly evolving profession and a broad range of skills is required for practicing pharmacy. As such, schools of pharmacy need to keep pace with changes in the profession to ensure that the skills that are being taught are in line with practice. The objective of this study was to determine practicing pharmacists' preferences for skills taught in an undergraduate pharmacy program.
Methods: A comprehensive search of the published and grey literature (including pharmacy web pages) resulted in the identification of 16 unique skills that were presented to practicing pharmacists in a Best Worst Scaling (BWS) choice experiment. The experiment was accompanied by The Change Readiness Questionnaire (CRQ) and questions to collect demographic and practice information from respondents. The Pharmaceutical Society of NZ sent a web-based link to the survey to all registered pharmacists and followed up two weeks later with a reminder. Standard descriptive analyses were used to characterize the sample and paired conditional logit was used to analyze the BWS choice data to generate the random utility values for each skill.
Results: At least some of the survey was answered by 388 pharmacists (10% response rate). Respondent demographics were similar to those of all registered pharmacists in NZ (67% female, mostly B.Pharm. educated, urban based, working in independent community pharmacies, and credentialed to provide various services). The most preferred skills to be taught were comprehensive disease management, medicine therapy assessment, medicine use review and dispensing; whereas, the least preferred skills were specialty compounding, independent prescribing, physical examination to assess and monitor drug therapies, and injections. Stratifying preferences by those within and outside the different domains of the CRQ (resourcefulness, optimism, adventurousness, drive, confidence, and tolerance for ambiguity) changed the magnitude but not the order of preferences for the skills.
Discussion: Practicing pharmacists provided distinct preferences for 16 skills that can be taught in a pharmacy undergraduate program. These skills encompass both traditional and advanced practices.¶

456.1 Developing a new unit in a new curriculum

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Introduction. As of 2017, the Faculty of Pharmacy and Pharmaceutical Sciences at Monash University introduced a new Bachelor of Pharmacy (Honours)/Master of Pharmacy degree. The new degree seeks to equip graduates with the necessary skills and knowledge to lead practice in the ever-changing world of healthcare and medicine.
Aims. To develop a new, foundational, double credit point unit (How the Body Works).
Methods. The way in which the unit was to be delivered differed significantly from previous iterations of the unit. Firstly, the unit was a double credit point unit. Secondly the unit was delivered using a very structured approach: the 'DEAR' model. Briefly, on a weekly basis, for every 4 hours of pre-learning **D**iscovery (the information was presented in Moodle books, including revision questions), there were 4 hours of integrated lectures (students **E**xplored the discovery material using questions / scenarios posed by staff) and 4 hours of workshops (where students **A**pplied the information from discovery and the integrated lectures. Finally students were asked to continuously **R**eflect on their learning. An important aspect of the new unit was the focus on skill development. In How the Body works we focussed on communication and teamwork.
Results. As a team, we developed and delivered a dynamic unit incorporating the new teaching approach. Staff reported that students were better communicators and team players by the end of the unit. Exam and unit results were noticeably higher (~20%) than the previous year.
Given the new teaching approach, it could be anticipated that students would initially struggle with the concept of having to be prepared before class so that the integrated lectures and workshops were meaningful. This was also true of students who had transferred from the old course or another course and were therefore used to the 'old' style of teaching. It was not surprising that the overall unit evaluation result was lower than other years (~3.5/5 vs ~4.5/5). Students provided meaningful feedback by identifying areas which could be improved.
Discussion. Utilising a different teaching approach, we developed a new unit as part of the new Pharmacy curriculum which focuses on skill development. Qualitative data suggests that the students were noticeably better communicators and team players by the end of the unit. Exam results also demonstrated that the students performed comparably better than last year. Feedback obtained from staff and students will be used to further develop the unit.

457 Bleeding-related admissions in patients with atrial fibrillation receiving antithrombotic therapy: results from the Tasmanian Atrial Fibrillation (TAF) study.

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Introduction. Limited data are available from the Australian setting regarding bleeding in patients with atrial fibrillation (AF) receiving antithrombotic therapy.

Aims. We aimed to investigate the incidence of hospital admissions due to bleeding and factors associated with bleeding in patients with AF who received antithrombotic therapy.

Methods. A retrospective cohort study was conducted involving all patients with AF admitted to the Royal Hobart Hospital, Tasmania, Australia, between January 2011 and July 2015. Bleeding rates were calculated per 100 patient-years (PY) of follow-up, and multivariable modelling was used to identify predictors of bleeding.

Results. Of 2202 patients receiving antithrombotic therapy, 113 presented to the hospital with a major or minor bleeding event. These patients were older, had higher stoke and bleeding risk scores, and were more often treated with warfarin and multiple antithrombotic therapies than patients who did not experience bleeding. The combined incidence of major and minor bleeding was significantly higher in warfarin- versus DOAC- and antiplatelet-treated patients (4.1 vs 3.0 vs 1.2 per 100 PY, respectively; p = 0.002). Similarly, the rate of major bleeding was higher in patients who received warfarin than in the DOAC and antiplatelet cohorts (2.4 vs 0.4 vs 0.6 per 100 PY, respectively; p = 0.001). In multivariate analysis, increasing age, prior bleeding, warfarin, and multiple antithrombotic therapy were independently associated with bleeding.

Discussion. The overall rate of bleeding in this cohort was low relative to similar observational studies. The rate of major bleeding was higher in patients prescribed warfarin compared to DOACs, with a similar rate of major bleeding for DOACs and antiplatelet agents. Our findings suggest potential to strategies to reduce bleeding include using DOACs in preference to warfarin, and avoiding multiple antithrombotic therapy in patients with AF.

458 Epicatechin's cardiovascular protective effects are mediated via opioid receptors and nitric oxide

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Introduction. Cardiovascular disease is the leading cause of mortality globally. Epicatechin has previously been shown to improve vascular responses and possess cardioprotective properties. However the mechanisms underpinning these cardiotropic outcomes are yet to be fully identified.
Aims. The aim of this study was to determine epicatechin's mechanism of action in the cardiovasculature.
Methods. The effects of epicatechin on isolated rat conduit and resistance arteries were investigated on resting tension and precontracted vessels both in the presence and in the absence of various antagonists. Changes in cardiac electrophysiology were assessed by microelectrode recordings from isolated left ventricular papillary muscles in the presence and absence of various antagonists.
Results. At resting tension, epicatechin alone did not affect the vasoreactivity of either conduit or resistance vessels. In noradrenaline pre-contracted thoracic aortic arteries and potassium chloride pre-contracted mesenteric vessels, epicatechin (10^{-9}M – 10^{-4}M) induced significant vasorelaxation. The addition of naloxone (10^{-5}M), N^{G}-nitro-L-arginine methyl ester (10^{-5}M), 4-aminopyridine (5mM) and verapamil (10^{-5}M) attenuated epicatechin-mediated vasorelaxation. No change in epicatechin-mediated vasorelaxation was observed with the addition of atropine (10^{-5}M). Epicatechin significantly improved cardiac electrophysiology by reducing the resting membrane potential, action potential amplitude and force of contraction that was mitigated following the addition of naloxone (10^{-5}M). Epicatechin significantly decreased the action potential duration at 20%, 50% and 90% of repolarisation and time to 90% relaxation of force that was unchanged following the addition of naloxone (10^{-5}M).
Discussion. These findings suggest epicatechin's vascular responses and cardioprotective effects are mediated through the opioid receptors, nitric oxide, potassium channel and calcium channel activation and highlight the importance of the endothelium/nitric oxide in epicatechin mediated vasorelaxation.¶

459 Exploiting E3-ubiquitin ligase mediated protein degradation pathways as new therapeutic target strategies for cardiovascular disease and beyond

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Despite the longstanding success of the statins in controlling lipid levels, there is still significant residual cardiovascular risk in high-risk patients. Statins function by inhibiting cholesterol synthesis, which is subject to extensive feedback regulation. Another cholesterol related pathway considered for therapeutic targeting is upregulation of cholesterol export from cells via the ABC lipid transporters ABCA1 and ABCG1, which are essential for maintenance of cholesterol balance in macrophages and cells such as neurons and insulin-secreting beta cells. Cholesterol-filled macrophages are a first critical step in the development of atherosclerosis, and transcriptional upregulation of ABC transporter mediated cholesterol export has been exploited therapeutically with the development of LXR ligands. These have so far failed to reach the clinic due to side effects and new avenues to upregulate these transporters are of interest.
Here we describe how E3-ubiquitin ligase-mediated ABC transporter degradation may be exploited in order to upregulate these important lipid pumps. E3 ligases are enzymes that form the rate-limiting step in the protein ubiquitination cascade, which tags proteins with ubiquitin and sends them off for degradation. These ligases are becoming increasingly of interest as therapeutic targets in the cancer field. Using nano-liquid chromatography mass spectrometry, we identified three E3 ligases associated with human ABCG1, namely HUWE1, NEDD4-1 and HECTD1. siRNA silencing of HUWE1 and NEDD4-1 increased ABCG1 protein levels and cholesterol export activity from cells, while overexpression of these ligases showed the opposite effect in cells expressing ABCG1 and macrophages in culture (1). siRNA silencing of HECTD1 stabilised ABCA1 protein and increased cholesterol export activity in human macrophages (2). We are currently investigating whether these E3 ligases utilize co-factors in order to characterize these pathways in depth. These observations highlight a new role for E3 ligases in the regulation of cholesterol homeostasis, which may provide new avenues for therapeutic targeting in future.

Aleidi et al (2015) J Biol Chem 290:24604-13
Aleidi et al (under review)¶

460 Nattokinase: A promising alternative in prevention and treatment of cardiovascular diseases

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Introduction. Nattokinase (NK), the most active ingredient of Natto, possesses a variety of favourable cardiovascular effects and the consumption of Natto has been linked to the reduction of cardiovascular disease (CVD) mortality. However, the effect of NK on atherosclerosis in human has never been studied.

Aims. This study was designed to evaluate the efficacy of oral NK in the reduction of common carotid artery intimal medial thickness (CCA-IMT) and carotid artery plaque size, lowering blood lipids, and to explore the underlying mechanism of action of NK. We further explored NK's potential as an alternative treatment to Statin (ST) in the clinic.

Methods. All enrolled patients were randomly assigned to one of two groups, NK and ST. 26 weeks' treatment applied to both NK Group (NK were given at a daily dose of 6000 FU) and ST Group (treated with statin-simvastatin 20 mg daily). CCA-IMT, carotid plaque size and blood lipid of the patients were measured before and after treatment.

Results. A total of 90 patients were enrolled in the study and 81 patients (NK=43, ST=38) completed the study. Following the treatments for 26 weeks, there was a significant reduction in CCA-IMT and carotid plaque size in both groups compared with the baseline before treatment. The carotid plaque size and CCA-IMT reduced from 0.25±0.12cm^2 to 0.16±0.10cm^2 and from 1.15±0.12mm to 1.02±0.11mm, respectively. The reduction in the NK group was significantly more profound (P<0.01, 36.6% reduction in plaque size in NK group versus 11.5% change in ST group). Both treatments reduced total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG). While the reduction in both groups was shown to be statistically significant (P<0.01), the reduction of TC, LDL-C and TG in ST group was significantly greater (P<0.05). In addition, NK significantly increased the level of high-density lipoprotein cholesterol (HDL-C) (P<0.05), in contrast, HDL-C in the ST group did not change. The lipid lowering effect observed in the NK group was not corrected to the reduction of CCA-IMT and carotid artery plaque size.

Discussion. Our findings from this pioneer clinical study suggests that daily NK supplementation is an effective way to manage the progression of atherosclerosis and potentially may be a better alternative to statins which are commonly used to reduce atherosclerosis and further to prevent cardiovascular attack and stroke in patients. The mechanism underlying the reduction of carotid atherosclerosis by NK may be independent from its lipid-lowering effect, which is different from that of statins. Together with the long history of natto consumption in Asia and its favourable effects on CVD, the findings further support that oral NK administration can be used as an alternative in the prevention and treatment of CVD. ¶

461 Gaps in anticoagulation knowledge in patients with atrial fibrillation

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Introduction. Knowledge regarding oral anticoagulant (OAC) therapy can influence treatment outcomes in patients with atrial fibrillation (AF). The relationships between anticoagulant knowledge, treatment expectations, convenience and satisfaction, health information overload and medication adherence have not been adequately studied in patients with AF.

Aims. To measure the level of anticoagulation knowledge in patients with AF taking OACs, investigate the association between patient-related factors and anticoagulation knowledge, and compare these results in patients taking warfarin and direct acting oral anticoagulant (DOACs).

Methods. Participants were recruited for an online survey via Facebook. Survey components included the Anticoagulation Knowledge Tool, the Perception of Anticoagulant Treatment Questionnaires (assessing treatment expectations, convenience and satisfaction), a modified Cancer Information Overload scale and the Morisky Medication Adherence Scale. Treatment groups were compared and predictors of OAC knowledge were identified.

Results. Participants taking warfarin had a higher knowledge score compared to those taking DOACs (n =386, 73.4 ± 13.2 vs 65.7 ± 13.7, p<0.001). Advancing age, type of OAC, health information overload and ease of OAC use (treatment expectation) were significant predictors of knowledge. Treatment expectations, including the belief that OAC treatment would cause bleeding side effects, varied significantly between participants taking warfarin and DOACs (4 vs 3, p = 0.011).

Discussion. The study identified knowledge gaps in patients taking OACs, and these deficiencies appear to be greater in participants taking DOACs. Knowledge assessment should be integrated within patient counselling sessions to help identify and resolve knowledge deficits. The relationship between these patient-related factors and treatment outcomes is an important area for further research.

462 Phosphoinositide 3-kinase p110 gene delivery limits cardiac remodelling and inflammation in a pre-clinical model of type 2 diabetes

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Introduction. Diabetic cardiomyopathy in both type 1 (T1D) and type 2 diabetes (T2D) is characterised by cardiac inflammation, remodelling and dysfunction, with diastolic dysfunction preceding systolic dysfunction. Phosphoinositide 3-kinase (PI3K)-p110α is cardioprotective in type 1 diabetes but its effectiveness in the more prevalent T2D is unknown.
Aim. To test the hypothesis that PI3K gene therapy rescues diabetic cardiomyopathy in a preclinical model of T2D.
Method. T2D was induced in 6wk-old male mice by low dose streptozotocin (55mg/kg/day i.p. for 3 days) combined with high-fat diet for 24wks. After 18wks of diabetes, diastolic dysfunction was confirmed by echocardiography. A single i.v. injection of recombinant adeno-associated virus (rAAV6)-caPI3K ($2x10^{11}$vg) or null vector was then administered, and mice were followed for a further 8wks (n=8-12/group).
Results. Diabetes induced increases in cardiac inflammatory markers tumor necrosis factor-α and NF-κB, which was not observed in rAAV6-caPI3K-treated T2D mice (↓53±11%, ↓15±6% vs null-treated-T2D, respectively; both P<0.05). Cardiac interstitial and perivascular fibrosis induced by T2D were also significantly reduced (to baseline levels) in rAAV6-caPI3K-treated T2D mice (↓67±16%, ↓49±17% vs null-treated-T2D, respectively; both P<0.05). rAAV6-caPI3K also reduced expression of cardiac pro-fibrotic genes in T2D, including connective tissue growth factor, transforming growth factor-β and tissue inhibitor of metalloproteinase-2 (reduced by 49±16%, 43±10% and 45±13% vs null-treated-T2D, respectively, all P<0.05). These cardioprotective actions of PI3K gene therapy were accompanied by improvements in LV diastolic (isovolumic relaxation time, ↓12±5% vs null-treated-T2D and e'/a', ↑44±10% vs null-treated-T2D; both P<0.05) and systolic function (fractional shortening: ↑31±8% vs null-treated-T2D, P<0.05).
Conclusion. This study is the first to demonstrate that PI3K gene delivery rescues T2D cardiomyopathy and limits the associated cardiac remodelling and inflammation.¶

463 Targeting Annexin-A1 to treat diabetic cardiomyopathy

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Introduction: Inflammation plays an important role in the progression of many diabetic complications, including diabetic cardiomyopathy.
Aim: To test the hypothesis that the anti-inflammatory protein Annexin-A1 mimetic, Compound17b (Cmpd17b) attenuates markers of diabetic cardiomyopathy in Type 1 Diabetes (T1D).
Methods: Diabetes was induced in 6wk-old C57/Bl6 male mice using streptozotocin (55mg/kg i.p. /day, 5 days). After 8 weeks, diabetic mice were randomly allocated to receive either Cmpd17b (50mg/kg/day i.p) or its vehicle for 8 weeks, prior to echocardiography and tissue collection in anaesthetized mice using ketamine/xylazine (60/6mg/kg i.p.).
Results: As shown in the table, diabetic mice exhibited significant hyperglycaemia and diabetic cardiomyopathy. Cmpd17b tended to limit hypertrophic β-myosin heavy chain gene expression (p<0.05) and cardiac fibrosis (p<0.05) in diabetic mice.
Conclusion: Annexin-A1 mimetics such as Cmpd17b may be potential interventions for the treatment of diabetic cardiomyopathy.

Results (mean±SEM)	Non-diabetic mice	Diabetic + Vehicle mice	Diabetic + Cmpd17b mice
n	10	9	11
Body weight (g)	30.2 ± 0.7*	22.9 ±1.2	22.1 ± 0.8
Blood Glucose (mM)	8.7 ± 0.4*	31.7 ± 0.7	32.1± 0.8
LV β-myosin heavy chain expression(fold)	1.0 ± 0.2	6.6 ± 1.6*	3.8 ±0.7#
LV CD68 expression (fold)	1.0 ± 0.2	0.5 ± 0.1*	0.3 ± 0.05
LV Pro-collagen 3 expression (fold)	1.0 ± 0.1	0.9 ± 0.2	0.4 ± 0.1#
LV Total Collagen (%)	0.20 ± 0.04	0.60 ± 0.20*	0.30 ± 0.10#
LV Function: E/A	1.9 ± 0.2	1.4 ± 0.1*	1.6 ± 0.1

*p<0.05 versus non diabetic sham, #p<0.05 versus diabetic vehicle One way ANOVA followed by *post hoc* Newman-Keuls test.

464 β-Ile^5-Angiotensin II as a novel treatment for cardiac fibrosis

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Introduction. Excessive collagen accumulation results in organ fibrosis culminating in end-organ failure, for which new treatments are needed. A beta-isoleucine substitution to angiotensin II (β-Ile^5-Ang II) exhibits high selectivity for type 2 receptors (AT_2R) which counter-regulates pathophysiological effects induced by type 1 receptors (AT_1R).
Aims. To determine the anti-fibrotic effects of β-Ile^5-Ang II in vitro and in experimental models of fibrosis.
Methods. Human cardiac fibroblasts (HCF; ScienCell) were treated with recombinant human transforming growth factor beta-1 (TGF-β1) (5ng/ml) ± β-Ile^5-Ang II (10-1000 nM) and incubated for 72 hours. Fibrosis and collagen turnover were assessed by Western blotting using extracted protein. Male FVB/N mice were subjected to an 8-week model of high salt (HS; 5% diet)-induced cardiac fibrosis. From weeks 5-8, mice (n=5-8/group) were treated with β-Ile^5-Ang II (75pmol/kg/min) ± PD123319 (AT2R antagonist; 1mg/kg/day) or A779 (MasR antagonist; 1mg/kg/day) via osmotic pumps. Cardiac inflammation, fibrosis and collagen turnover were measured and compared with normal salt (NS)-fed mice.
Results. β-Ile^5-Ang II caused dose-dependent reductions in TGF-β1-stimulated collagen-I, alpha-smooth muscle actin (α-SMA; marker of myofibroblast differentiation) and tissue inhibitor of metalloproteinase (TIMP)-1 in HCF (all $P<0.05$, n=4-6). HS generally increased cardiac inflammation (F4/80 and phosphorylated-IkB levels) and cardiac fibrosis (left ventricular interstitial fibrosis measured by picrosirius red-staining), which was associated with increased myofibroblast differentiation (α-SMA) and TGF-β1 (all $p<0.05$ vs NS group). β-Ile^5-Ang II significantly reversed HS-induced cardiac inflammation (F4/80, phosphorylated-IkB) and cardiac fibrosis (picrosirius red, α-SMA and TGF-β1; all $p<0.05$ vs HS group). The anti-fibrotic and anti-inflammation effects caused by β-Ile^5-Ang II were abolished by PD133319 and A779 in combination.
Discussion. The novel peptide β-Ile^5-Ang II exerts anti-fibrotic and anti-inflammatory effects via the stimulation of both AT_2R and MasR; associated with inhibiting collagen production and enhancing collagen degradation. ¶

465 Functional regulation of bitter taste receptors (T2Rs) by 2-adrenergic and M2 muscarinic acetylcholine receptor

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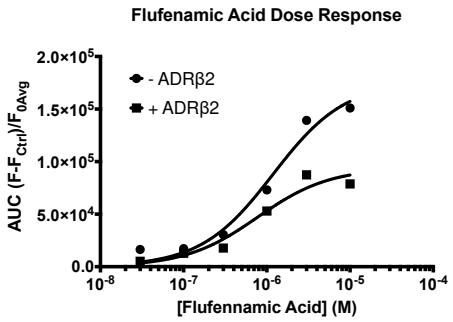
Introduction. G protein-coupled receptors (GPCRs) are key mediators of cardiac physiology and targeted for therapeutics. The ectopic expression of bitter taste receptors (T2Rs) in heart was first reported by the Thomas Laboratory. Stimulation of T2R14 in human right atrial tissue with bitter ligands produces a dramatic cardiodepressant effect, but it is not known whether the actions of T2R14 are modulated by other GPCRs related to cardiac contractility, principally the adrenergic and muscarinic receptors.
Aims. To determine the effect of co-expressing and activating the β2-adrenergic receptor and the M2 muscarinic receptor on the activation of the T2R14 bitter receptor.



Methods. AD293 cells were transfected with T2R14, chimeric G protein $G\alpha_{16/gust44}$, and the Ca^{2+} sensor GCaMP5. Ligand stimulated intracellular Ca^{2+} was measured by fluorescence imaging via an automated fluorometric plate reader. Fluorescently tagged T2Rs were used in confocal imaging studies, focusing on the expression and localisation of T2Rs.
Results. The co-expression of the β2-adrenergic receptor significantly reduced T2R signalling in response to flufenamic acid (see figure). Conversely, an increase in T2R function was observed when co-expressed with the cardiac parasympathetic regulator, M2 muscarinic acetylcholine receptor. These changes did not involve alterations in the expression and cellular localisation of T2R14. Pre-treatment with adrenergic/muscarinic ligands did not affect subsequent activation of the T2R14.
Discussion. Co-expression of T2Rs with the adrenergic and muscarinic receptors alters their responsiveness and efficacy to bitter ligands, leading to consequent effects on cardiomyocyte contractility. Ongoing investigations are probing the mechanism involved.

466 Gene therapy targeting the hexosamine biosynthesis pathway (HBP) attenuates markers of diabetic cardiomyopathy in a mouse model of type-2 diabetes (T2D)

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Introduction. Diabetic cardiomyopathy is characterized by left ventricular (LV) diastolic dysfunction, and cardiac remodelling. The nutrient-sensing HBP has been implicated diabetic cardiomyopathy. The final product of the HBP, 'O-GlcNAc' glycosylates proteins via the enzyme O-GlcNAc transferase (OGT), altering their function. O-GlcNAcylation is reversed by O-GlcNAcase (OGA). Sustained O-GlcNAcylation in diabetes impairs LV function.
Aim. To determine if rAAV6-human-OGA (hOGA) gene therapy improves diabetic cardiomyopathy in T2D mice *in vivo.*
Methods. 6-week-old male FVB/N mice were randomised into citrate vehicle control, or T2D induced by low-dose streptozotocin (STZ, 3x55mg/kg, i.p) and high-fat-diet (HFD, n=22). After 18-weeks untreated diabetes, rAAV6-hOGA or null-vector-rAAV6 ($2x10^{11}$ vector genomes, i.v.) was administered to diabetic mice (n=11/group). Citrate controls received null-vector (CIT-Null, n=5). Diastolic function was measured by Doppler echocardiography at 6, 24, and 32-weeks-of-age in mice under anaesthesia (Ketamine/Xylazine/Atropine, 60/6/0.6 mg/kg, i.p.) and blood glucose levels measured fortnightly. Eight-weeks after gene therapy, mice were euthanised and tissues collected.
Results. Blood glucose, HbA1c, bodyweight and fat mass of diabetic mice were elevated compared to citrate controls ($P<0.05$). Echocardiography indicated a reduced E/A ratio at 18-weeks diabetes ($P<0.05$), however this was not improved 8-weeks post hOGA-rAAV6. LV collagen deposition was increased in STZ-HFD-Null mice ($P<0.05$) and was attenuated by hOGA ($P=0.05$). LV CTGF expression in STZ-HFD-Null was elevated compared to CIT-Null. hOGA-rAAV6 reduced LV OGT expression ($P<0.0001$) and endogenous LV OGA expression. Augmented pan O-GlcNAcylation in T2D was attenuated by rAAV6-hOGA ($P<0.01$). No changes in cardiomyocyte hypertrophy or ROS-generation were evident.
Conclusions. hOGA-rAAV6 gene therapy reduces fibrosis and total O-GlcNAcylation associated with diabetic cardiomyopathy in T2D, but neither cardiomyocyte hypertrophy, ROS-generation, nor cardiovascular function *in vivo* were protected at this dose.¶

467 Blood pressure lowering post-stroke; a review of the evidence supporting recommendations of draft Clinical Guidelines for Stroke Management 2017

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Introduction. An 88-year-old normotensive woman collapsed with hypotension after initiation of guideline directed blood pressure lowering therapy (BPLT) following a haemorrhagic stroke. Hypotension is the most frequent precipitant for Medical Emergency Team calls within hospitals. When developing guidelines, selection of evidence from a defined clinical population is recommended and appropriate (National Institute for Health and Care Excellence). Updated draft Clinical Guideline for Stroke was available for public consultation in 2016.
Aim. To review the public consultation draft stroke guideline, particularly in relation to recommendation for blood pressure lowering post-stroke.
Method. Recommendations for blood pressure lowering post-stroke were identified. References were reviewed and classified according to study design, inclusion of post-stroke population and evidence for efficacy and safety.
Results. The draft stroke guideline recommended, "All stroke and TIA patients, regardless of baseline blood pressure, should have long term blood pressure lowering therapy initiated or intensified, unless contraindicated by symptomatic hypotension" with five supporting references. Two were intervention based (BPLT) meta-analysis, where a specific patient population was not defined, one was a post hoc analysis of a randomized controlled trial, one was an open label randomized trial that found no difference in outcome with varying blood pressure thresholds and another was a review and meta-analysis of blood pressure lowering post-stroke that included studies where blood pressures up to 160 mmHg were considered normal.
Discussion. The evidence base supporting recommendations in the draft stroke guideline included meta-analysis of intervention based studies (BPLT) rather than studies in post-stroke populations. Clinical relevance to post-stroke populations is uncertain. Robust evidence of benefit for BPLT post-stroke for all patients, irrespective of blood pressure was lacking. Guidelines should be based on evidence derived from appropriate clinical populations.

468 Resveratrol shows neuronal and vascular-protective effects in older, obese, streptozotocin-induced diabetic rats

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Introduction. Old, obese streptozotocin-induced (STZ) diabetic rats can provide a disease model of type 1 diabetes mellitus with some aspects of type 2 diabetes mellitus and metabolic syndrome. While the cardioprotective effects of resveratrol in young STZ rats are well-established, the effectiveness of this polyphenol antioxidant compound in maintaining cardiovascular health in old, obese STZ animals remains largely unknown.
Aims. The aim of this study was to determine whether resveratrol, when administered at a dose that can be reasonably obtained through supplementation, could prevent the development of cardiovascular complications in older, obese, diabetic rats.
Methods. Diabetes was induced in 6-month old, obese, male Wistar rats via a single intravenous dose of STZ (65 mg/kg). Randomly selected animals were administered resveratrol (2 mg/kg) via oral gavage daily for 8 weeks. Changes in body mass, blood glucose levels, food intake and water consumption were monitored as indicators of diabetes. Vascular reactivity, left ventricular function and tactile allodynia were assessed at the end of the treatment period.
Results. Resveratrol therapy significantly improved tactile allodynia as well as vascular contractile and relaxation functionality in diabetic rats ($P < 0{\cdot}05$). There was no significant effect of resveratrol on left ventricular compliance or heart rate. Similarly, plasma glucose concentrations, water consumption and body mass were not significantly affected by resveratrol administration in diabetic animals.
Discussion. Resveratrol-mediated improvements in vascular and nerve function in old, obese, diabetic rats were associated with its reported antioxidant effects. Resveratrol did not improve cardiac function nor mitigate the classic clinical symptoms of diabetes mellitus (i.e. hyperglycaemia, polydypsia and a failure to thrive). This suggests that supplementation with resveratrol at a dose achievable with commercially available supplements would not produce significant cardioprotective effects in individuals with diabetes mellitus.¶

469 The microRNA miR-124 inhibits vascular smooth muscle cell proliferation by targeting S100 calcium-binding protein A4 (S100A4)

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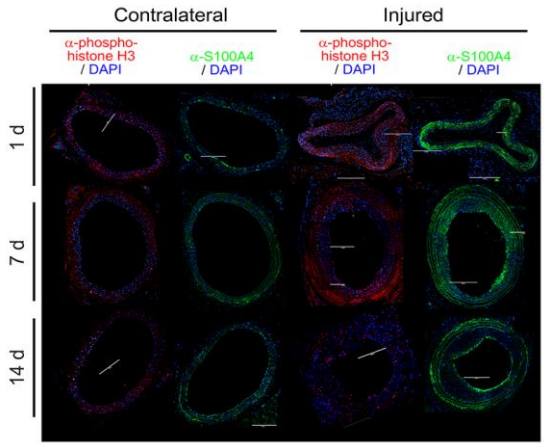
S100 calcium-binding protein A4 (S100A4) induces proliferation and migration of vascular smooth muscle cells (VSMCs). We aimed to find the microRNA regulating S100A4 expression. S100A4 transcripts were abruptly increased in the acute phase of carotid arterial injury at 1 day but gradually decreased at 7 and 14 days. Bioinformatics analysis revealed that *miR-124* targeted S100A4. VSMC survival was attenuated by *miR-124 mimic* but increased by *miR-124 inhibitor*. *miR-124* was decreased immediately after carotid arterial injury but dramatically increased at 7 and 14 days. *miR-124 inhibitor*-induced cell proliferation was blocked by *S100A4 siRNA*, whereas *miR-124*-induced cell death was recovered by S100A4. *miR-124* is a novel regulator of VSMC proliferation and may play a role in the development of neointimal proliferation.



Choe et al., FEBS Lett 591: 1041-52, 2017¶

470 The role of phospholipase A_2 in the cardiovascular effects of *Pseudechis australis* snake venom

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Introduction. Phospholipase A_2 enzymes (PLA_2s) are abundantly present in snake venoms and cause the anticoagulant and myotoxic effects of *Pseudechis australis* (*P. australis*) venom (Hart et al, 2014). LY315920 has been shown to be a potent inhibitor of PLA_2s (Schevitz et al, 1995) and thus provides a valuable tool to assess the role of these enzymes.
Aims. To assess the cardiovascular effects of *P. australis* snake venom in isolated cardiac and vascular tissues *in vitro* and to determine the role of PLA_2s in these effects.
Methods. In organ baths, the inotropic and chronotropic effects of the venom (0.01-30 µg/mL) were assessed in the rat isolated left and right atria, respectively, in the absence or presence of LY315920 (1.0 µM). Using myography, in U46619 pre-contracted rat small mesenteric arteries (221-400 µm i.d.) venom-induced (0.3 or 1.0 µg/mL) vasorelaxation was assessed in the absence or presence of LY315920 (1.0 µM).
Results. In left and right atria *P. australis* venom caused positive inotropic and chronotropic effects with similar EC_{50} values of 1.3±0.2 (n=14) and 1.2±0.2 µg/mL (n=12), respectively. LY315920 (1.0 µM) pre-treatment significantly inhibited both inotropic and chronotropic effects, right-shifting the curves. The resulting EC_{50} values were 11.5±0.9 µg/mL (n=9; P<0.001) in the left atria and 5.5±1.0 µg/mL (n=6; P<0.001) in the right atria. In the mesenteric arteries, *P. australis* venom (0.3 and 1.0 µg/mL) caused a transient relaxation with a decrease of pre-contractile tone of -68±7% (n=5) and -33±7% (n=15), respectively; the lower concentration of venom caused a significantly greater relaxation (P=0.017). LY315920 (1.0 µM) pre-treatment significantly inhibited the vasorelaxation caused by venom 0.3 µg/mL and 1.0 µg/mL by 87% (n=7; P=0.003) and 67% (n=9; P=0.035), respectively.
Discussion. This study suggests that PLA_2s in *P. australis* venom contribute significantly to the venom-induced inotropic and chronotropic effects, and vasorelaxation in mesenteric arteries. The venom concentration-independent vasorelaxant responses require further investigation.

Hart AJ et al (2014) Clin Toxicol 52:604-610
Schevitz RW et al (1995) Nat Struct Biol 2:458-465¶

471 Endotoxin tolerance-like response in human abdominal aortic aneurysm (AAA) macrophages

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Introduction. Macrophages are involved in the pathogenesis of AAA. Endocytosis of lipid rafts reduces the expression of Toll-like receptor 4 (TLR4) on macrophage membranes, reducing responsiveness to bacterial lipopolysaccharide (LPS) (Józefiwsju et al., 2017). Prior exposure of macrophages to LPS leads to attenuated cytokine production (eg Tumor necrosis factor-α; TNF-α), on subsequent exposure; a phenomenon called endotoxin tolerance.
Aim. To investigate whether human AAA macrophages exhibit endotoxin tolerance after LPS exposure.
Methods. Blood-derived macrophages were obtained from men with small (<5.5 cm) AAA (75±6 yr, n=19) and age-matched non-AAA male controls (72±5 yr, n=36). Cells were incubated in culture with 0.1 µg/mL LPS for 24 or 28h. Biomarkers of inflammation were determined by ELISA, and distribution of lipid rafts by confocal microscopy.
Results. The LPS-stimulated release of inflammatory biomarkers (8-isoprostane, TNF-α, interleukin-6) from macrophages was significantly lower in AAA compared to non-AAA participants. Protein expression of TLR4 was significantly reduced in AAA compared to non-AAA macrophage lysates. Lipid rafts were localised to the membrane of non-stimulated macrophages from n=3/3 control participants. Lipid raft internalisation was observed in LPS-stimulated macrophages from n=3/3 control, and in non-stimulated macrophages from n=5/10 AAA participants.
Discussion. This refractoriness of AAA macrophages to an LPS stimulus is reminiscent of endotoxin tolerance. Internalisation of lipid rafts in some AAA participants may contribute to the apparent endotoxin tolerance-like response. Reduced responsiveness to TLR4-activators may increase risk of infection and non-resolving inflammation.

Józefiwsju et al. (2017) Cellular Immunology 312:42-50¶

472 β_3-Adrenergic Receptors in the Rat Cremaster Muscle Artery

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Introduction. The β_3-adrenergic receptor (β_3-AR) was first isolated and cloned in 1989. There exists evidence of this receptor having a role in the cardiovascular system, as seen in various studies undertaken in the heart and vasculature. Its role in the microvasculature in particular however, remains elusive.
Aims. This project aims to determine β_3-AR expression and function in the rat cremaster muscle artery.
Methods. Cremaster muscle arterioles were isolated from adult male Sprague Dawley rats. Immunofluorescence and PCR techniques were used to assess β_3-AR expression. Functional studies were performed using pressure myography (70 mm Hg). Concentration-response curves were obtained using a variety of β-AR agonists and antagonists. The data was analyzed by means of two-way ANOVA, followed by Sidak's Multiple Comparisons Test, using GraphPad Prism v7.0.
Results. The β_3-AR agonists, CL-316,243 and mirabegron alone had no effect on basal myogenic tone of the arteries. Another β_3-AR agonist, SR-586,11A, caused a dilation of 5813% from baseline when administered alone (EC_{50} = -6.0 0.8, *n* = 5). In the presence of the β_1/β_2-AR antagonist, nadolol (10 µM), both CL-316,243 and mirabegron caused vasodilation (EC_{50} = -8.5 9.9, *n* = 5 and EC_{50} = -8.4 2.3, *n* = 3 respectively), which was blocked by the β_3-AR antagonist L-748,337 (1 µM). The potency of SR-586,11A was enhanced in the presence of nadolol (EC_{50} = -8.6 6.8, *n* = 3, *P* < 0.05) and responses to SR-586,11A were also prevented by L-748,337. Vasodilation induced by the non-selective β-AR agonist, isoprenaline was abolished by nadolol, but was not altered by L-748,337. CL-316,243 (1 µM) was shown to attenuate dilation induced by the endothelium dependent vasodilator, ACh (EC_{50} = -5.9 0.4, *n* = 5), compared to control (EC_{50} = -6.9 0.2, *n* = 5).
Conclusions. β_3-AR causing vasodilation are masked when the β_1-AR and β_2-AR are fully functional. β_3-AR may also inhibit endothelium-dependent vasodilation.¶

473 The association between short sleep duration and BMI in Australian Indigenous children

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Associations between short sleep duration and obesity and the relationship between obesity and chronic illness are well documented. Obese children are likely to become obese adults. To date there is a paucity of information regarding sleep duration and quality for Indigenous Australian people. It may be that poor quality, short sleep is contributing to the gap in health outcomes for Indigenous people compared with non-Indigenous Adults and Children. This study sought to investigate the possibility that poor sleep quality may be contributing to health outcomes for Indigenous children by exploring associations between sleep duration and BMI.
Methods: Participants: 1253 children aged 7 – 12 years in Wave 7 of the national Longitudinal Study of Indigenous Children survey. Interviewers asked primary carers about children's sleep times. Body mass index (BMI) was derived from measurements of children made by researchers.
Results: Regardless of age, relative socioeconomic disadvantage and level of remoteness, unhealthy weight was associated with less sleep duration than healthy weight for Indigenous children.
Conclusion: The relationship between short sleep duration and BMI in Indigenous children has important implications for their future health outcomes. Both overweight conditions and short sleep are established modifiable risk factors for metabolic dysfunction and other chronic illnesses prominent in the Indigenous population. It is important to consider strategies to optimise both for Indigenous children in an attempt to help "close the gap" in health outcomes and life expectancy between Indigenous and non-Indigenous people.

474 Vascular effects of Australian brownsnake venoms (*Pseudonaja* spp.): the role of secretory phospholipase A_2s

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Introduction. The World Health Organisation recognised snakebite management as a public health crisis and listed envenomation as a neglected tropical disease in 2017. While antivenoms exist for most venomous snake species, reports of their high cost, scarcity and ineffectiveness have grown considerably over the years. Many toxic effects of snake venoms may be caused by secretory phospholipase A_2s (sPLA$_2$s), therefore inhibition of this enzyme family may have therapeutic potential for the treatment of snakebite (Lewin et al, 2016).
Aims. The aims were to assess the role of sPLA$_2$s in the vascular effects of four different brownsnake venoms (*Pseudonaja* spp.) and to test the effectiveness of treatment with LY315920, a potent inhibitor of sPLA$_2$s.
Methods. Rat isolated small mesenteric arteries (i.d. 250–350 μm), mounted in myographs, were either pre-contracted with U46619 (for relaxation studies) or electrically-stimulated (for sympathetic nerve responses). Crude venom from *P. affinis*, *P. inframacula*, *P. mengdeni* or *P. textilis* was added (30 μg/ml). In separate experiments, arteries were pre-treated with LY315920 (1 μM) before additions of snake venoms.
Results. All venoms elicited significant relaxation from the pre-contractile tone (–63-93% tone, n=6-10; P<0.0001); only *P. mengdeni* and *P. textilis* venoms were inhibited by LY315920 (48±11%, n=9; P=0.0007, and 66±23%, n=7; P=0.017, of relaxation by venom alone, respectively). The venoms, with the exception of *P. mengdeni* (22±17%, n=6; P=0.12), inhibited sympathetic nerve stimulation by 38-56% compared with the control group. Only *P. inframacula* and *P. textilis* venoms were affected by LY315920 (inhibition 58±18%, n=9; P=0.0046 and 77±25%, n=6; P=0.011, respectively). LY315920 did not inhibit the relaxation or sympatholytic effects caused by *P. affinis* venom (P>0.05).
Discussion. These results suggest that sPLA$_2$s in the venom of these snake species (with the exception of *P. affinis*) from the same genus (*Pseudonaja*) contribute to vascular relaxation as well as inhibition of sympathetic nerve stimulation via pathways yet to identified. Furthermore, the treatment with LY315920 showed that a sPLA$_2$ inhibitor may offer an effective alternative in the treatment of snake envenomation.

Lewin et al (2016) Toxins 8:248. ¶

475 Delineating the signal transduction pathways by which relaxin mediates its anti-fibrotic actions in human cardiac fibroblasts.

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Introduction. The ovarian and cardiovascular hormone, relaxin, mediates its anti-fibrotic effects via Relaxin Family Peptide Receptor 1 (RXFP1)/angiotensin II type 2 receptor (AT_2R) heterodimers (Chow B et al., Kidney Int 2014, 86:75-85) and inhibition of TGF-β1 expression and activity. Currently, the extent to which relaxin signals through AT_2R-dependent mechanisms and the TGF-β1/NADPH oxidase (NOX) axis remains unknown.
Aims. To determine whether relaxin mediates its anti-fibrotic effects via modulation of 1) AT_2R-dependent phosphatase activity and/or 2) specific NOX isoforms that are expressed by primary human cardiac fibroblast (HCFs).
Methods. HCFs (fetal ventricular and atrial fibroblasts; ScienCell, USA) were screened by real-time and/or Western blotting for their ability to express the AT_2R-dependent tyrosine (SHP-1, SHP-2), serine/threonine (PP2A) and MAP kinase (MKP-1) phosphatases as well as NOX1, NOX2, NOX4 and NOX5. HCFs were also stimulated with TGF-β1 (5ng/ml) and treated with human recombinant relaxin (RLX; 16.8nM = 100ng/ml) or the AT_2R-agonist, Compound 21 (C21; 1μM), in the absence or presence of the PP2A inhibitor, okadaic acid (10nM) for 72 hrs. Known end-points of RLX activity: phosphorylated (p-)ERK1/2, p-nNOS, α-SMA (myofibroblast differentiation) and collagen I were then assessed by Western blotting. The effects of RLX (5 or 16.8nM) or C21 (1 or 5μM) on NOX4 mRNA and protein as well as amplex red-hydrogen peroxide (H_2O_2) levels after 72 hrs were also determined (all 3-4 separate times in duplicate).
Results. HCFs were found to express the PP2A and MKP-1 phosphatases, and predominantly expressed NOX4 mRNA and protein in comparison to NOX1, NOX2 and NOX5. TGF-β1 stimulation of HCFs significantly increased NOX4 mRNA and hydrogen peroxide levels (both p<0.05 vs unstimulated cells), while RLX or C21 normalised the TGF-β1-stimulated H_2O_2 levels (both p<0.05 vs TGF-β1) without affecting NOX4 expression. RLX or C21 also significantly increased p-ERK1/2 and p-nNOS, but decreased α-SMA and collagen I expression by HCFs; while the anti-fibrotic effects of either therapy were abrogated by co-administration of okadaic acid (all p<0.05 vs RLX or C21 alone).
Discussion. RLX appears to mediate its anti-fibrotic effects in HCFs via AT2R-dependent PP2A phosphatase activity but not via modulation of NOX4 expression, strengthening the finding that it can signal through RXFP1/AT_2R dimers.

476 Targeting IRAP: A Novel Treatment to Stabilize Existing Abdominal Aortic Aneurysms

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Introduction. Abdominal aortic aneurysm (AAA) is a degenerative disease with no pharmacological treatment available to prevent progression or risk of rupture. Preliminary evidence from our laboratory indicated inhibition/deficiency of the enzyme, insulin regulated aminopeptidase (IRAP) prevented AAA formation in angiotensin (Ang) II-infused mice, indicating IRAP may be a novel target in treatment of AAA.
Aim. To determine if the IRAP inhibitor, HFI-419 can stabilize established AAA in Ang II-infused apolipoprotein E deficient (ApoE KO) mice.
Methods. 12 week old male ApoE KO mice were infused with Ang II (1000ng/kg/min) for 6 weeks to induce AAA. Once presence of established AAA was confirmed mice were randomized to receive either vehicle or HFI-419 (500ng/kg/min; s.c.) from weeks 2-6. Ultrasound imaging (to measure aortic diameter and area) and systolic blood pressure (SBP; tail cuff method) measurements were performed fortnightly to track AAA development and SBP changes
Results. Two-week infusion of Ang II induced aneurysm formation in >90% of all mice. Co-infusion of HFI-419 with Ang II significantly reduced aneurysm area and diameter in the absence of any effect on SBP. Immunohistochemistry analyses confirmed increased expression of IRAP in proximal aorta and AAA sections taken from Ang II infused mice whilst IRAP inhibition tended to reduce IRAP expression. HFI-419 treatment attenuated elastin degradation which was correlated with reduced matrix metalloproteinase (MMP)-9 and macrophage expression in AAA sections.
Discussion. Inhibition of IRAP significantly reduced progression of established AAA, although underlying protective mechanisms are still under investigation. This study highlights the potential of inhibiting IRAP as a novel therapy for treatment of AAA
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477 Comparing the anti-fibrotic effects of emerging treatments: Serelaxin and the IRAP inhibitor, HFI-419 to a clinically-used ARB and ACE inhibitor in a high salt-induced mouse model of kidney disease.

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Introduction. Fibrosis is a hallmark of chronic kidney diseases and its inability to resolve causes severe organ dysfunction and end-organ failure. The limited anti-fibrotic efficacy of current therapies suggests a need for alternative treatments.
Aims. To compare the anti-fibrotic effects of serelaxin (human recombinant relaxin; RLX) and HFI-419 to the AT1 receptor blocker, Candesartan cilexetil (CAND) or ACE inhibitor, Perindopril (PERIN) in a murine high salt (HS) diet-induced model of kidney disease.
Methods. 8-10 week male C57Bl/6J mice were subjected to 8-weeks of HS (5% NaCl) diet-induced renal injury. From weeks 5-8, sub-groups (n=4-8) were treated with either vehicle, RLX (0.5mg/kg/day), HFI-419 (0.72mg/kg/d), CAND (2mg/kg/day) or PERIN (4mg/kg/d). Each drug dose used had previously demonstrated anti-fibrotic efficacy in other experimental models. Mice maintained on a normal salt (NS) diet (0.5% NaCl) for 8-weeks were used as controls. Various measures of renal inflammation and fibrosis as well as plasma urea levels were evaluated.
Results. HS diet-fed mice were associated with significantly increased renal inflammation, TGF-β1 expression levels, myofibroblast differentiation, glomerulosclerosis, interstitial fibrosis, TIMP-1 levels and vascular rarefaction (determined by morphometry of Masson's trichrome- or immunohistochemically-stained sections and/or Western blotting), total kidney collagen concentration (hydroxyproline analysis) and plasma urea compared to that measured from NS diet-fed counterparts (all $P<0.01$ vs NS group). RLX or HFI-419 significantly reduced most measures of HS-induced renal fibrosis and plasma urea levels back to that measured in mice fed the NS diet (all $p<0.05$ vs HS group). RLX or HFI-419 demonstrated similar, if not greater, anti-fibrotic effects compared to that offered by PERIN, but which also reduced blood pressure, body weight and worsened plasma urea levels at the dose used ($p<0.01$ vs HS group). CAND, however, did not demonstrate any marked anti-fibrotic effects in the model/organ studied.
Discussion. RLX or HFI-419 offers improved anti-fibrotic efficacy and renoprotection compared to CAND and safer anti-fibrotic efficacy compared to PERIN in the setting of HS-induced kidney damage.¶

478 Systemic and cardiac-selective targeting of histone deacetylase 4 (HDAC4) to limit diabetic cardiomyopathy

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Introduction: Diabetic cardiomyopathy is characterised by left ventricular (LV) diastolic dysfunction and structural changes, including cardiomyocyte hypertrophy and interstitial fibrosis. Epigenetic modifications, such as histone deacetylation, have been implicated in the molecular pathways that drive structural changes in this setting. HDAC4 is associated with the pathological cardiac remodelling similar to that observed in diabetic cardiomyopathy.
Aims. To determine whether inhibiting HDAC4, via a cardiac-selective approach using adeno-associated virus (AAV), or globally (by tasquinimod), ameliorates diabetic cardiomyopathy in a murine model of type-1 diabetes (T1D).
Methods: T1D was induced in 6 week old male FVB/N mice with streptozotocin (5 days, 55mg/kg/d or vehicle, i.p.). Echocardiography was performed at 6 (baseline), 14 (pre-treatment), and 22 (endpoint) weeks of age in anaesthetised mice (ketamine/xylazine/atropine, 60/6/0.6 mg/kg). The first approach utilised cardiac-selective rAAV6-dnHDAC4 ($2x10^{11}$ genomes or null vector). The second approach utilised tasquinimod (10mg/kg/d or vehicle administered via daily i.p.). Both approaches commenced after 8 weeks of diabetes with a follow-up period of 8 weeks.
Results: Blood glucose and HbA1c levels were increased with diabetes ($P<0.0001$). Diabetes reduced heart mass, however rAAV6-dnHDAC4 significantly increased LV mass compared to untreated diabetes ($P<0.05$). Diabetes-induced prolongation of isovolumetric relaxation time and increased LV connective tissue growth factor (CTGF) gene expression; both were attenuated by rAAV6-dnHDAC4 ($P=0.08$ and $P<0.05$, respectively) in T1D mice. Treatment with rAAV6-dnHDAC4 also blunted the diabetes-induced expression of hypertrophic genes including B-type natriuretic peptide (BNP) and β-myosin heavy chain (β-MHC, both $P<0.05$). Treatment with tasquinimod ameliorated diabetes-induced LV diastolic dysfunction with improved E/A and e'/a' in comparison to untreated diabetes (both $P<0.01$) and a reduction in deceleration time ($P<0.01$). Diabetes increased LV BNP gene expression ($P<0.05$) and superoxide levels ($P<0.001$) both of which were reduced by treatment with tasquinimod (both $P<0.05$).
Conclusions: Inhibition of HDAC4 attenuates characteristics of diabetic cardiomyopathy including cardiomyocyte hypertrophy, fibrosis, superoxide generation and LV diastolic dysfunction, in a model of T1D.¶

479 Role of TRPC3 in endothelium-dependent vasodilation of rat mesenteric arteries

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Introduction. Endothelium-dependent dilation (EDD) of arteries is an important auto-regulatory function of the microvasculature. Previous studies suggested a role for transient receptor potential canonical-3 channels (TRPC3) in EDD (Senadheera et al., 2012) using pyrazole-3, a TRPC3 blocker with poor selectivity (Schleifer et al., 2012).
Aims. The present study further examined the role of TRPC3 in both agonist and flow-stimulated EDD of arteries utilizing a new, more selective TRPC3 blocker, pyrazole-10 (PYR10; Schleifer et al., 2012).
Methods. Cumulative stimulus-response curves to ACh (1 nM/L - 10 µM/L) or intra-luminal flow (0-20µL/min) were performed in isolated, pressurized (60 mmHg), phenylephrine-constricted rat mesenteric arteries. Data is presented as % maximum dilation from baseline.
Results. In control arteries, increasing flow caused dilation, with peak dilation observed at 14uL/min (17.2 ± 3.2%, n=6). The flow-mediated dilation (FMD) was not altered by inhibition of nitric oxide (NO) synthase and guanylate cyclase using a combination of L-NAME (100 µM) and ODQ (10 µM). In the presence of PYR10 (1 µM), some FMD persisted at low flow rates (<10 µl/min), but at flow ≥12µL/min significant flow-induced constriction of vessels was observed (max constriction - 21.8 ± 10.5% $P\leq0.05$, n=4). ACh caused a concentration-dependent dilation of mesenteric arteries (pEC_{50} = 7.63 ± 0.09, max 95.1 ± 2.6%, n = 4). The ACh-induced dilation was inhibited by PYR10 (max 51.0 ± 1.5%, $P<0.05$, n = 4). The combination of L-NAME, ODQ and PYR10 further reduced ACh-induced dilation (max 10.0 ± 1.1%, $P<0.05$, n = 4). PYR10 did not alter phenylephrine-induced vasoconstriction of the arteries.
Discussion. These studies support a role for TRPC3 in mediating both agonist- and flow-induced EDD of rat mesenteric arteries. TRPC3 appears to be coupled to non-NO-dependent signaling pathways, presumably involving endothelium-derived hyperpolarization of vascular smooth muscle.

Schleifer H, *et al.* (2012). Br J Pharmacol 167**:** 1712-1722.
Senadheera S, *, et al.* (2012). Cardiovasc Res 95**:** 439-447.

480 An investigation of the vascular effects of Sailuotong, a standardised Chinese herbal formula, for vascular dementia

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Introduction. Sailuotong (SLT) is standardised three-herb formulation consisting of *Panax ginseng C A Mey*, *Ginkgo biloba L* and *Crocus sativus L* for the management of vascular dementia (VaD). Although SLT has been shown to increase cerebral blow flow in animal and clinical studies, the direct effects of SLT on vascular reactivity have not investigated.
Aims. To examine the vasodilatatory effects of SLT and its underlying mechanisms of action in rat isolated tail artery.
Methods. Male, 250-300g Wistar Kyoto (WKY) rat-tail artery was isolated for isometric tension measurement.
Results. Cumulative administration of SLT (0.1 – 5000 µg/mL) caused a concentration-dependent relaxation in phenylephrine-preconstricted tail artery. Pre-incubation of endothelium nitric oxide synthase inhibitor (N-nitro-L-arginine methyl ester, L-NAME; 20 µM) did not inhibit the SLT-induced vasodilatation. In contraction experiments, SLT (10, 100 and 1000 µg/mL) significantly attenuated phenylephrine (0.001 to 10 µM)- and KCl (10 – 80 mM)-induced contraction. In Ca^{2+}-free solution, SLT (5000 µg/mL) markedly suppressed Ca^{2+}-induced (0.001 – 3 mM) vasoconstriction in both phenylephrine (10 µM) and KCl (80 mM) stimulated tail arteries.
Discussion. Putting these together, our results suggested that SLT induces relaxation of rat isolated tail arterial rings through an endothelium-independent pathway, involving blockade of extracellular Ca^{2+} influx.¶

481 Clinically actionable CYP450 pharmacogenotypes relevant to analgesics used for alleviating rheumatoid arthritis pain in community-dwelling older Australians.

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Introduction. Genomic polymorphisms altering metabolic activity of isoforms of cytochrome P450 (CYP) enzymes may lead to heterogeneity in patient responses to analgesics ranging from therapeutic failure to drug toxicities (Ting and Schug, 2016). Pre-emptive array-based analysis of such pharmacogenotypes has potential for optimizing therapeutic outcomes while reducing unnecessary costs associated with adverse drug reactions (Van Driest SL et al, 2014).
Aims. To assess prevalence of clinically actionable polymorphisms with strong or moderate level evidence for opioids and non-steroidal anti-inflammatory drugs in 2121 Australians aged 55 years or older from the Hunter Cohort study.
Methods. Clinical actionability and risk level (medium- or high-risk) of drug-gene interactions with strong evidence (codeine-*CYP2D6*; rs28371705, rs28371725, rs28735595 and tramadol-*CYP2D6*; rs28371705) or moderate evidence (oxycodone-*CYP2D6*; rs28371705 and celecoxib-*CYP2C9*; rs1057910) were assessed from the Pharmacogenomic Knowledge Database, Clinical Pharmacogenomic Implementation Consortium or FDA recommendations using self-reported medication data and genotyping by Affymetrix Kaiser Axiom arrays or imputed from reference panels.
Results. One third of participants (33%, 698/2121; 95% CI: 31%–35%) had at least one medium- or high-risk clinically actionable genotypes of the *CYP2D6* or *CYP2C9* gene variants studied. In total 2% (47/2121; 95% CI: 1.6%–2.2%) were homozygous for strongly or moderately evidenced high risk actionable SNPs of *CYP2D6* or *CYP2C9* and were also considered as poor metabolizers. About 1% (20/2121, 95% CI: 0.5%–1.4%) of participants with actionable genotypes were exposed to codeine or celecoxib. Conversely, about 17% (44/256, 95% CI: 13%–22%) of participants taking these medications had at least one relevant actionable genotype.
Discussion. At least 3 in 10 participants have an actionable genotype and over 10% of those on analgesics have at least one actionable genotype that would prompt a change of standard therapy according to current international therapeutic guidelines. Taking genotype into account may provide better therapeutic outcomes for arthritis patients.
Ting S and Schug S (2016) J Pain Res 9:49-56 Van Driest SL et al (2014) Clin Pharmacol Ther 95:423-31¶

482 Cytochrome interactions between lumacaftor and ivacaftor undergoing Orkambi cystic fibrosis therapy

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Ivacaftor (Kalydeco) and ivacaftor-lumacaftor (Orkambi) combination are two new breakthrough cystic fibrosis (CF) drugs that directly modulate the activity and trafficking of the defective CF transmembrane conductance regulator-protein. However, there is still a dearth of understanding on the pharmacology of ivacaftor and lumacaftor. Since release of Orkambi several red-flags have been raised that highlight the clinical efficacy maybe be limited due to antagonistic drug-drug interactions e.g. cytochrome P450 interactions. For the cell-free CYP inhibition studies, P450-Glo luminescence assays have been used to measure the cytochrome P450 activity of ivacaftor, its major metabolites and lumacaftor at 10 µg/mL. Chloramphenicol, a known CYP3A4 inducer at 10 µg/mL was employed as a positive control. Ivacaftor-carboxylate (RLU = 274 394.4) and lumacaftor (RLU = 304 899.45) produced higher response luminescence units (RLU) than Chloramphenicol (RLU = 248 319.4). Interestingly, hydroxymethyl-ivacaftor (RLU = -1 368.895) and ivacaftor (RLU = 153 564.4) caused a decrease in RLU compared to the control. This would suggest potential antagonistic drug-drug interactions between lumacaftor and ivacaftor are at play where the former induces the metabolism of the latter. Overall, these factors maybe compounding together to limit the clinical efficacy of Orkambi therapy. This is clearly an important issue that requires attention given the modest benefits to the patient may not justify the high cost of therapy.

483 The role of efflux transporters in the transfer of drugs from mother to breastfed infant via breastmilk.

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Introduction. Although breastfeeding is advocated as the best nutritional start for an infant, there is always concern regarding the transfer of medications from mother to their breastfed baby via milk. Although most drugs are compatible with breastfeeding, cases of toxic drug exposure have been reported. This is thought to be due to active transport mechanisms whereby efflux transporter proteins expressed in the epithelial cells of the mammary gland actively secrete drugs into milk. An example of such efflux transporters is the breast cancer resistance protein (BCRP) which is strongly induced during lactation and this could result in contamination of milk with the substrates of this transporter which may place any suckling infant at risk of toxicity.
Aim. The objective of this study was to investigate the changes in the expression of four ATPase Binding Cassette (ABC) transporters namely BCRP, MDR1, MRP1 and MRP2 in the lactating human mammary epithelial cells at various time points during lactation and to explore whether cells derived from breastmilk can be used to develop an individualised, non-invasive model to predict drug transfer from mother to baby via breastmilk.
Methods. Milk samples were collected from nursing mothers at various times starting at one month post-partum (intended at 1, 3, 5, 9 and 12 months) until a maximum of 12 months post-partum or cessation of breastfeeding. Gene expression of the transporters was tested at both the mRNA (qRT-PCR) and protein levels (immunostaining). Cells obtained from breastmilk were isolated and successfully grown in culture using specialised media.
Results. Breastmilk derived cell gene expression of these transporters varied widely amongst participants. Our results indicate that there was a strong trend showing a monthly increase of +2.24 in MRP2 ($p=0.0002$), and a weaker (but significant) increase in MDR1 (+0.17 per month; $p=0.0102$). There was no evidence of a change in BCRP or MRP1 over time. The expression of these transporters also varied significantly in cultured cells compared to fresh cells.
Discussion. Our results show that the expression of some ABC transporters is stage dependent in humans and may exhibit some interpersonal variability.

484 Silencing *ABCC2* transporter gene enhances oxaliplatin chemo-sensitivity in colorectal cancer cells

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Introduction. Oxaliplatin as a first-line treatment for colorectal cancer has greatly contributed to improving patient outcomes. However, a member of the ATP-binding cassette (ABC) transporter superfamily, ABCC2, has been suggested to confer oxaliplatin resistance by pumping oxaliplatin out of cells.
Aims. To observe the effects of small interfering RNA (siRNA) targeting *ABCC2* gene in Caco-2 cells and interaction of ABCC2 with oxaliplatin.
Methods. Caco-2 cells were transfected with three different siRNAs of *ABCC2* and scramble-sequence negative control siRNA. *ABCC2* mRNA expression levels were measured by quantitative real time PCR. Flow cytometry was used to analyse the cellular accumulation of ABCC2 substrate, 5(6)-carboxy-2', 7'-dichlorofluorescein (CDCF). MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was undertaken to determine oxaliplatin sensitivity and half-inhibitory concentration (IC_{50}) of oxaliplatin was calculated.
Results. The *ABCC2* mRNA expression significantly decreased by 60% after siRNA transfection. The cellular CDCF accumulation significantly increased by 33%±7.0% ($P < 0.05$), 53%±3.7% ($P< 0.001$) and 45%±4.5% ($P<0.001$) in ABCC2-silencing Caco-2 cells compared with control. The IC_{50} values of oxaliplatin in transfected Caco-2 cells were decreased to 7.7±0.1 µM ($P < 0.05$), 8.4±0.2 µM ($P < 0.05$) and 7.0±0.9 µM ($P< 0.05$) compared with a control value of 13.83±1.5 µM.
Discussion. Our study provided the evidence that ABCC2 was identified as a targetable factor and transfection of *ABCC2* by siRNA, enhanced sensitivity of Caco-2 cells to oxaliplatin. Thus, silencing of *ABCC2* gene may reverse oxaliplatin resistance in colorectal cancer.¶

485 Raised urinary excretion of thymine following an oral load is a marker for severe fluoropyrimidine toxicity

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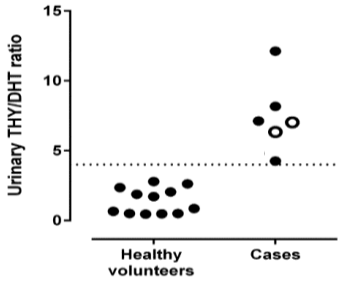
Introduction. Cancer chemotherapy continues to rely heavily upon 5-fluorouracil (5FU) and its prodrug capecitabine. Serious adverse effects have focussed dihydropyrimidine dehydrogenase (DPD), but this accounts for only about one-third of observed toxicity. The majority of 5FU toxicity remains unexplained.
Aim. To compare retrospective oral thymine (THY) loading of six cancer patients who had suffered severe 5FU toxicity, with a control range.
Methods. The THY oral loading regimen was published previously for twelve healthy volunteers (Duley et al, 2016). Briefly, after a light breakfast a 250 mg capsule of powdered THY was swallowed with tap water. Patients provided a pre-dose urine, then post-dose urines up to 5 hours. Urine THY and its catabolite dihydrothymine (DHT) were assayed.



Results. The figure shows the ratios of THY:DHT excreted in urine by the six patients, compared to twelve healthy volunteers. The mean (range) of urine THY:DHT for healthy subjects was 1.34 (0.36 - 2.84). Of the patients, two were later confirmed DPD-deficient carriers, with ratios of 6.35 and 6.93 respectively (unfilled symbols). The other four patients ranged from 4.8 - 12.1. Using a nominal THY:DHT ratio cutoff of 4 for healthy volunteers, all six patients had elevated urinary THY:DHT, post-THY load.
Discussion. Oral THY loading appears to provide a means of evaluating fluoropyrimidine sensitivity in cancer patients that is superior to other reported methods. Our trial in six cancer patients, who had suffered 5FU toxicity, found DPD deficiency in two of the patients. Three of the four other patients appeared to have an augmented thymine uptake phenotype. PK-based THY testing may facilitate prediction of the majority of cases of fluoropyrimidine sensitivity, until the pharmacogenetic factors are fully comprehended. A prospective trial is being undertaken in NZ.
Ref. Duley JA et al (2016) Eur J Pharm Sci 81:36-41¶

486 The ethics of direct-to-consumer pharmacogenomic screening in primary care

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Background: Direct-to-consumer pharmacogenomic screening is currently available in primary care through community pharmacies and general practice (e.g. https://www.mydna.life). Screening involves testing for an array of single nucleotide polymorphisms commonly implicated in variation in drug response. While the genetic contribution to pharmacodynamics and pharmacokinetics is increasingly well understood for many drugs, direct-to-consumer pharmacogenomic screening raises a number of ethical questions. A key ethical consideration is whether there is sufficient evidence for the benefit of these tests.
Aim: Assess the appropriateness of direct-to-consumer pharmacogenomic screening in primary care.
Method: The evidence for the benefit of direct-to-consumer pharmacogenomic screening is assessed by examining the evidence for screening in relation to common treatments such as warfarin and antidepressants. Pharmacogenomic studies for these medications are reviewed to identify challenges in applying the information provided in the literature to direct-to-consumer pharmacogenomic screening.
Results: The warfarin and antidepressant cases highlight a number of challenges for determining the clinical utility of direct-to-consumer pharmacogenomic screening. Specifically, the complexity of the contribution that genetic variation makes to other clinical variation; the methodological weaknesses of the studies conducted; limited frameworks for clinical implementation and weak measures for improved patient outcomes. [1,2]
Conclusion**:** The translation of pharmacogenomic research to the day-to-day management of patients has a number of opportunities, but also some challenges. More discussion is needed on how the current evidence base may be used to implement direct-to-consumer pharmacogenomic screening appropriately.

1. Alcalde MG, Rothstein MA. Pharmacogenomics: Ethical concerns for research and pharmacy practice. American Journal of Heal-System Pharmacy. 2002;59(22):2239–40.
2. Ma JD, Lee KC, Kuo GM. Clinical application of pharmacogenomics. Journal of Pharmacy Practise. 2012;25(4):417–27.

487 Therapeutic drug safety, important to 'Close the Gap' for Aboriginal and Torres Strait Islanders: an illustrative case of phenytoin hypersensitivity syndrome.

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Introduction. Australian Indigenous populations have high burdens of disease and consequent medication exposure. The evidence base for drug efficacy and safety in this population is limited (Thynne, Gabb 2016). Early identification of specific adverse drug reactions and strategies to mitigate risk in this population are important to 'Close the Gap'.
Aims. To describe a clinical case of a severe phenytoin hypersensitivity syndrome in a 30 year old Aboriginal woman.
Methods. Clinical case report of phenotypic features, cytochrome P450 and HLA testing.
Results. A 30 year old Aboriginal woman presented with six months of odynophagia, intermittent diarrhoea and 50 kilogram weight loss with a desquamating, ichthyoid rash, most severe on the palms and feet. She had been commenced on phenytoin six months previously following seizures. She had been reviewed by her general practitioner, as well as 3 speciality services (gastroenterology, cardiology, intensive care) without a diagnosis. Investigations revealed eosinophilia, acute kidney injury with electrolyte disturbance, vitamin and mineral deficiencies and abnormal liver function with cholestasis. A clinical diagnosis was made of drug rash with eosinophilia and systemic symptoms (DRESS) secondary to phenytoin and phenytoin was ceased. Cytochrome P450 testing was positive for CYP2C9*3 denoting a poor metaboliser, and HLA testing was positive for HLA-B*56-02 previously associated with phenytoin hypersensitivity in Aboriginal Australians (3 cases, 2 fatal) (Harding et al, 2012). This patient survived and recovered over the next twelve to 18 months.
Discussion. This case illustrates the importance of clinical recognition of adverse drug reactions in Aboriginal and Torres Strait Islanders; the relevance of personal genomic information that may contribute to risk and that phenytoin DRESS HLA is different to Caucasians and Asians. An organised pro-active approach to therapeutic drug safety in the indigenous Australians may assist in closing the gap in health outcomes for this population.

Thynne T, Gabb G (2016) MJA 2014:16-17
Harding DJ et al (2012) Aust Med J 197:411-4¶

488 Physiologically based pharmacokinetic modelling of atomoxetine in the different *CYP2D6* genotypes

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Atomoxetine is a norepinephrine reuptake inhibitor indicated in the treatment of attention deficit hyperactivity disorder. It is primarily metabolised by CYP2D6 to its equipotent metabolite, 4-hydroxyatomoxetine, which promptly undergoes further glucuronidation to an inactive 4-HAT-O-glucuronide. Clinical trials have shown that decreased CYP2D6 activity leads to substantially elevated atomoxetine exposure and more adverse reactions. The aims of this study were to analyse the pharmacokinetics of atomoxetine and to develop a pharmacologically based pharmacokinetic (PBPK) model of atomoxetine in different *CYP2D6* genotypes. A single 20 mg dose of atomoxetine was given to 19 healthy Korean individuals with *CYP2D6*wt/*wt* (**wt = *1* or **2*) or *CYP2D6*10/*10* genotype. Based on the results of this pharmacokinetic study, a PBPK model for *CYP2D6*wt/*wt* individuals was developed. This model was scaled to those with *CYP2D6*10/*10* genotype, as well as *CYP2D6* poor metabolisers. We validated this model by comparing the achieved pharmacokinetic parameters with diverse results from the literature. The presented PBPK model describes the pharmacokinetics after single and repeated oral atomoxetine doses with regard to *CYP2D6* genotype and phenotype. This model could be utilized for identification of appropriate dosages of atomoxetine in patients with reduced CYP2D6 activity to minimize the adverse events, and to enable personalised medicine.¶

489 No significant effect of *CYP3A, ABCB1, POR* and *NR1I2* polymorphisms on acute rejection and nephrotoxicity in the first 3 months post kidney transplantation in patients receiving tacrolimus

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Introduction. Tacrolimus is a first line immunosuppressant used after kidney transplantation but with extensive inter-individual variability in PK and PD. Low or high tacrolimus concentrations are associated with acute rejection and nephrotoxicity, respectively. SNPs in its major metabolising enzyme (*CYP3A4/5*), P-glycoprotein efflux transporter (*ABCB1*), their expression regulator Pregnane X Receptor (*NR1I2*), and cytochrome P450 reductase (*POR*), have been studied for their effects on tacrolimus PK (Hesselink et al, 2014; Kurzawski et al, 2017). However, there are few studies on their effects on PD, especially in the first 3 months post-transplantation when acute rejection occurs more frequently.
Aims. To investigate the impact of *CYP3A4/5*, *ABCB1*, *NR1I2* and *POR* SNPs on acute rejection and nephrotoxicity in kidney transplant patients receiving tacrolimus in the first 3 months post-transplant.
Methods. A total of 165 kidney transplant recipients and 129 donors were included in this study. Biopsy- or clinical observation-confirmed acute rejection, delayed graft function (DGF) and eGFR data were collected from case notes. Acute rejection and DGF were analysed as binomial outcomes (Y/N) while eGFR (unit: ml/min/1.73m^2) as continuous variables. Genotyping was performed for: *CYP3A5*3*; *CYP3A4*22*; *ABCB1* 61A>G, 1199G>A, 1236C>T, 2677G>T, 3435C>T; *POR*28*; and *NR1I2* 8055 C>T, -25385C>T, 63396C>T. Recipient and donor genotype and predicted *ABCB1* haplotype (PHASE) differences in recipients with acute rejection and DGF in 3 months post-transplant, and 1- and 3-month log-transformed eGFR, were tested by χ^2 or Fisher's exact tests, and linear mixed effects models, respectively.
Results. No recipient or donor genotypes/haplotypes had a significant effect on occurrence of acute rejection (P>0.2), DGF (P>0.1), or eGFR (P>0.02) after adjusting for multiple testing (False discovery rate (α=0.05), P=0.002).
Discussion. Tacrolimus metabolism- and transport-related genetic factors do not significantly affect acute rejection or nephrotoxicity in the first 3 months post kidney transplantation.

[1] Hesselink D.A. et al. (2014) Clin Pharmacokinet, 53(2): 123-39.
[2] Kurzawski M. et al. (2017) Pharmacogenet Genomics. 2017;27(10):372-7.¶

490 *ABCB1* pharmacogenetics in Papua New Guinea HIV/AIDS patients and association with efavirenz CNS/Psychiatric adverse effects

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Introduction. Papua New Guinea (PNG) has the highest prevalence of HIV/AIDS in the Pacific with efavirenz as the main treatment. *ABCB1* encodes the P-glycoprotein efflux transporter which is important for drug disposition, and *ABCB1* genotype has been linked to efavirenz CNS/Psychiatric adverse effects (Dickinson et al., 2016). However, nothing is known about key *ABCB1* SNPs in the PNG population. We hypothesised that *ABCB1* genetics would be associated with efavirenz CNS/Psychiatric adverse effects in PNG HIV/AIDS patients.
Aims. To determine the frequency of *ABCB1* 61A>G, 1199G>A, 1236C>T, 2677G>T and 3435C>T SNPs and haplotypes in PNG HIV/AIDS patients receiving efavirenz and examine genotype/haplotype differences in the incidence of CNS/Psychiatric adverse effects.
Methods. Demographic and clinical data, including CNS/Psychiatric adverse effects, and saliva were collected from 51 PNG HIV/AIDS patients. Salivary DNA was genotyped for *ABCB1* SNPs and allele frequencies compared to other populations (Caucasian, East Asian, African) (Auton et al., 2015). *ABCB1* haplotypes were inferred by PHASE. Incidence of CNS/Psychiatric adverse effects was compared between *ABCB1* genotypes and haplotypes by Fisher's exact tests.
Results. PNG HIV/AIDS patients have a high frequency of 1236T (82%), 2677T (62%) and 3435T (66%) alleles compared to other populations (14-63%, 0-13% and 15-52%, respectively). No variant alleles were observed for 61A>G and 1199G>A. There were no significant genotype/haplotype differences in CNS/Psychiatric adverse effects ($p>0.15$).
Discussion. PNG HIV/AIDS patients exhibit very high frequencies of key *ABCB1* SNPs which may have important implications for P-glycoprotein substrate drugs in this population. However, no significant association with efavirenz adverse effects was detected in this small study, and larger studies incorporating efavirenz PK are required.

Dickinson et al. (2016) Clin Pharmacokinet 55:861-873.
Auton et al. (2015) Nature 526:68-74.¶

491 Therapeutic drug monitoring of voriconazole

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Introduction. Voriconazole is used to treat invasive fungal infections. It is an ideal candidate for therapeutic drug monitoring (TDM) as it has non-linear PK, a narrow therapeutic index and large inter-patient variability. Guidelines for both voriconazole dosing and TDM (target trough concentrations 1-5 mg/L) are available.
Aims. To examine voriconazole dosing and monitoring at St Vincent's Hospital and determine compliance with guidelines.
Methods. A retrospective audit (1st Jan to 31st Dec 2016) of voriconazole dosing and TDM was undertaken. Patient demographics, voriconazole dosing history and plasma concentrations were collected from medical records. Voriconazole trough concentrations were predicted using a Bayesian dose prediction software (DoseMe, Brisbane).
Results. Serum samples (n=417) were collected from 94 patients during 127 courses of therapy. For po therapy, the first and second loading doses and initial maintenance dose were concordant with guidelines in 20%, 17% and 86% of courses, respectively. For iv therapy, the first and second loading doses and initial maintenance dose were concordant with guidelines in 22%, 26% and 42% of courses, respectively. The most commonly prescribed dose was 200 mg. Voriconazole concentrations were obtained for 104/127 therapies. There was marked variability in the timing of the first serum sample in the course of therapy (median: before 5th dose, range: before 1st – 22nd dose). Of the first serum samples collected, 24% were "true" trough concentrations (median: 1.7 mg/L, range: 0.1 – 4.3 mg/L). Approximately 80% of the predicted trough concentrations were within the target range; 20% were <1 mg/L. Only one of the courses with sub-therapeutic predicted trough concentrations had a dose increases.
Discussion. Compliance with voriconazole guidelines was poor. Further, when trough concentrations were outside the guideline recommended therapeutic range no change was seen in dosing. The disparity between voriconazole prescribing and monitoring observed implies a need for improved guidance for clinicians to optimise patient outcomes. Bayesian dose prediction software has the potential to support TDM for voriconazole.

492 Antithrombotic drug-drug interaction alerts in MedChart™

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Introduction. Concomitant use of two antithrombotic medications (antiplatelets or anticoagulants) is associated with increased risk of bleeding. Public hospitals in Christchurch, NZ use MedChart™, a computerised physician order entry system that has been locally configured with prescribing alerts that trigger against antithrombotic drug-drug interactions (DDIs).
Aims. To evaluate the rate of antithrombotic DDI alerts and prescriber responses to these alerts.
Methods. MedChart™ alert data from 1 August to 31 December 2016 were extracted and rates of antithrombotic DDI alerts were determined. A subset of the alerts were reviewed in detail. Analysis of prescription changes attributed to the alert and issues contributing to alert fatigue were also performed.
Results. During the study period 1011 antithrombotic DDI alerts were recorded (mean 7 per day) corresponding to an alert rate of 48/10,000 total prescriptions. Oral anticoagulant-oral antiplatelet alerts comprised of 62% (624/1,011) of these DDI alerts. Of 280 alerts assessed, 81% (228/280) were 'clinically appropriate'. Prescribers changed antithrombotic prescriptions within 30 minutes of triggering DDI alerts on 28% (79/280) of occasions. The combination of enoxaparin and dabigatran was associated with 34 alerts, of which 88%(30/34) were 'clinically appropriate' and 74% (25/34) were associated with a change in antithrombotic prescription within 30 minutes of the alert firing.
Discussion. Targeted clinical decision support can reduce high risk prescribing. However, even carefully constructed alerts targeting the highest risk prescribing has specificity considerably below 100%. It is important to assess temporally proximal changes to prescriptions following an alert, and not just focus on the decision at the point of the alert. This data will inform further development of clinical decision support.

493 Prescribing based on the effective dose 50 (ED50)

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Introduction. Undesirable on- and off-target effects of pharmacotherapy are mostly dose-related. The prescriber may avoid these with knowledge of a drug's effective dose 50 (ED50), which can be defined as the mean population dose necessary to achieve half of the maximum possible drug effect (Emax). ED50 may be based on receptor occupancy or effect, clinical surrogates or outcomes and can guide dose optimisation.
Aims. To provide an ED50 reference for routine cardiovascular drugs, to facilitate optimal prescribing.
Methods. Clinical studies which addressed dose response were sought through best Reviews and PubMed.
Results.

	ED50 (mg)	Approved doses (mg)	Approved doses as a ratio of ED50
LDL-lowering Rx			
Simvastatin	15	5 - 80	0.3 - 5.3
Pravastatin	40	10 - 80	0.25 - 2
Atorvastatin	2	10 - 80	5 – 40
Ezetimibe	0.3	5 - 10	17 - 33
PLATELET ANTI-AGGREGANT Rx			
Aspirin	40	50 - 100	1.2 - 2.5
Clopidogrel	30	75	2.5
ANTIHYPERTENSIVE Rx			
Hydrochlorothiazide	10	12.5 - 25	1.2 - 2.5
Metoprolol	30	50 - 100	1.6 - 3.3
Amlodipine	2	5 - 10	2.5 – 5
(T2DM: Metformin	2000	500-2000	0.25 – 1.0)

Discussion. ED50 centres a drug's dose response curve for a population. Benefits plateau above ED50, whilst a variety of adverse events continue to increase. Mid-range low density lipoprotein (LDL) concentrations and blood pressure (BP) correlate with cardiovascular outcomes but total mortality at low LDL and BP plateaus and may increase related to adverse events. Benefit and risk of a drug should be established by commencement at ED50 and careful measurement of the clinical effects with titration down if not tolerated and up to achieve required efficacy.¶

494 'Mrs Have-A-Chat': Pilot study showing that following up an "AdherenceCheck" every two weeks for 9 months improves the management of medicines in the older-aged living in a rental retirement village

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Introduction. We have shown that the older-aged living in a low socioeconomic, rental retirement village have a low adherence to medicines and a poor understanding of their illnesses (Doggrell & Kairuz, 2012), and that this is not improved by an "AdherenceCheck, which was based on the "MedsCheck" (Doggrell, 2017).
Aims. The aim was to determine whether following up an AdherenceCheck every two weeks for 9 months had any effect on the ongoing management of medicines by the older-aged living independently in a rental retirement village.
Methods. After we assessed the management of medicines by the older-aged living in the village, using semi-structured interviews, we delivered to each of them a personalized "Action Plan" to help them manage their medicines. Then, we followed-up 2 weekly with phone calls or visits to discuss the Action Plan. Nine months later we reassessed their management of medicines.
Results. The 27 participants at the rental retirement village had a mean age of ~80 years, 59% were non-adherent or at risk of being nonadherent, and only 33% had a good knowledge of their medicines/illnesses. After 9 months, 9 participants were lost to the study: 6 had left the village, 2 withdrew, and 1 had died. Of the remaining 18 subjects; at baseline, 50% were nonadherent and 31% had a good knowledge of their medicines/illnesses. After the 9 months of follow-up, only 17% participants remained nonadherent, and 57% had a good knowledge of their medicines/illnesses.
Discussion. The management of medicines by the older-aged living in a low socioeconomic rental retirement villages is poor, and there is evidence from this pilot study, that following up an AdherenceCheck/Action Plan every two weeks for 9 months improves this.

Doggrell SA, Kairuz T. (2012) J Pharmac Pract Res 42:208-12.
Doggrell SA. (2017) Int J Clin Pharmac 39:443-9.

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495 Making Adverse Drug Reactions Visible

Matthew Doogue[1,2], Richard McNeill[2], Mary Young[2], John Wilkinson[2], Sandra Pugh[2], Valya Sylevych[2], Chris Warren[2], QianYi Chua[2], Niall Hamilton[2], Ignatius Chua[2], Heather Isenman[2], Murray Barclay[1,2], Paul Chin[1,2]. Department of Medicine, University of Otago[1]; Clinical Pharmacology, Canterbury District Health Board[2], Christchurch, New Zealand.

Introduction. Adverse drug reactions (ADRs) cause harm to patients and add unnecessary costs to the health system. Every patient admitted to hospital has their ADR history recorded on the medication chart. Every patient discharged from hospital has diagnoses coded, including ADRs, using the "International Statistical Classification of Diseases and Related Health Problems" (ICD-10). However, this information is of variable quality and under used in patient care.
Aims. To increase the accessibility and utility of ADR data. To describe the incidence and prevalence of ADRs in a tertiary hospital.
Methods. Stakeholder user requirements were established. An ADR incidence dashboard of ADR coding data extracted from hospital coding data was developed. An ADR prevalence dashboard of patient ADR histories extracted from the ePrescribing software was developed. The ADR dashboards were evaluated by clinical governance groups. Canterbury District Health Board (CDHB) data were evaluated using the dashboards. One year of coding data and 3 months of prescriptions were examined.

	Incidence (new ADRs)	Prevalence (n=4,421)
Total ADRs	4,427 per year (7.6 per 100 admissions)	8,643
ADRs to Penicillin	201 per year	1,293
Anaphylaxis to penicillin	11 per year	98

Results. The incidence of coded ADRs at CDHB is 7.6% of admissions. The prevalence of ADRs in CDHB inpatients is 50% with a median of 1 ADR per patient (range 1-21). Penicillin antibiotics is the most prevalent class of drugs with ADRs. Non-drug reactions were 8.8% of ADR entries in electronic medication charts.
Discussion. Clinical dashboards were successfully developed to provide access to ADR data. Limitations include inconsistent taxonomies between data sources and non-coded text fields (e.g. the reactions recorded in the prescribing software). The dashboards allow rapid interrogation of hospital ADR data including time trend and data quality. The next step is implementation of these tools in clinical practice to improve the veracity of ADR diagnoses.

496 Severe cutaneous adverse reactions and the (in)accuracy of medicines information sources.

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Introduction. In the preparation of New Zealand consumer medicines information (CMIs) we find frequent inconsistencies in adverse drug reactions (ADRs) recorded in commonly used sources of medicines information. Severe cutaneous adverse reactions (SCAR) is the most feared type of ADR. The drugs causing SCAR have been systematically identified by the RegiSCAR group (www.regiscar.org) and this has been used as the definitive source to inform our CMIs (www.mymedicines.org.nz/cdhb). Incorrect warnings about SCAR in medicines information can lead to incorrect clinical decisions.
Aim. To evaluate the accuracy of medicines information sources' warnings of potential severe cutaneous adverse drug reactions.
Methods. For medicines in the MyMedicines database, we defined their SCAR risk using RegiSCAR publications. We compared this list with the SCAR ADRs recorded in four widely used sources of medicines information: Micromedex®, Lexidrugs®, the New Zealand manufacturer's product information (PI) and the New Zealand Formulary (NZF).
Results. There were 41 medicines in the MyMedicines database causally associated with SCAR. Of these 37, 34, 32 and 34 had SCAR warnings in Micromedex®, Lexidrugs®, the PI and the NZF, respectively – true positives. Of 321 medicines not causally associated with SCAR 24, 30, 25 and 18 had SCAR warnings in Micromedex®, Lexidrugs®, the PI and the NZF respectively – false positives.
Discussion. ADRs are inconsistently recorded in medicines information. The limited clinical data at the time of registration results in under-recording of ADRs. Conversely, medico-legal caution and failure to control for placebo data results in over-recording of ADRS. Consideration of individual ADRs across all drugs lead to more accurate risk assessment than consideration of all ADRs in individual drugs. For most ADRs there is an existing body of literature that can be used to provide more accurate risk assessments than are currently provided.¶

497 Peri-operative medication dosing in obese elective surgical patients: a systematic review of clinical studies

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Introduction. Despite an increasing number of obese patients requiring elective surgery, there is a lack of guidelines about medication dosing in such patients.
Aims. To systemically review the dosing and outcomes of peri-operative medications used in obese elective surgical patients, and develop practical dosing recommendations for commonly used medications.
Methods. Medical subject headings and general key words were used to systematically search multiple databases (PubMed, EMBASE, Cochrane Library and CINAHL). Studies comparing the dosing of medications in obese patients undergoing surgery were included if they had a non-obese control or comparative dosing scalar group.
Results. Thirty-three studies of six drug classes were include; anaesthetics (n=6), muscle relaxants (n=10), neuromuscular reversal agents (n=3), analgesics (n=2), antibiotics (n=5), and anticoagulants (n=7). A variety of dosing scalars and/or recommendations were identified. Ideal body weight was the preferred dosing scalar for non-depolarizing muscle relaxants, and neuromuscular reversal agents. For anaesthetic agents, lean body weight was used for induction of anaesthesia and total body weight was preferred for the maintenance of anaesthesia. Total body weight was found to be suitable for dosing muscle relaxants whereas corrected or ideal body weight were suitable weight scalars for dosing morphine. The standard 2 g dose of cefazolin appeared effective in the prevention of surgical site infections. For anticoagulants, body mass index stratified dosing of enoxaparin appeared effective for venous thromboembolism prevention.
Discussion. Limited data suggest that clinicians should consider each class of medication when selecting a dose for obese surgical patients. Routine use of fixed dosing regimen is likely to under- or overdose obese patients thus predisposing them to adverse drug events or treatment failure leading to patient harm.

498 Audit of Individual Patient Use Applications for High Cost Medicines at a Tertiary Hospital

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Introduction: High-cost medicines are often used for rare diseases and non-approved indications. Clinicians in South Australia must apply to their Drug and Therapeutics Committee (DTC) for individual patient use (IPU) of medicines which exceed >$10,000 per treatment course.
Aims: To examine the characteristics of IPU applications for high-cost medicines at a large teaching hospital.
Methods: We conducted a retrospective audit of IPU applications for high-cost medicines between January 2015 and December 2015 at the Royal Adelaide Hospital, South Australia. Information obtained included the medicine name, approval status, cost, level of evidence to support the application and nominated monitoring outcome. Monitoring outcomes were categories into subjective and objective and a judgment made on whether a third party could determine the efficacy of the funded treatment from the patient's medical records using the information provided.
Results: A total of 87 IPU applications were examined. All except one (n=86, 98.85%) of these applications were approved resulting in an annual cost of $1,339,203. The most common high-cost medicines were rituximab (n=33, 37.93%), abacavir/dolutegravir/lamivudine (n=10, 11.49%), infliximab (n=8, 9.20%) and posaconazole (n=5, 5.75%). Half of all applications (n=45, 51.72%) provided no supporting evidence and when evidence was included it was often NHMRC Level III or below (n=28, 32.18%). Of all applications, approximately half (n=41, 47.13%) proposed an objective monitoring outcome but very few (n=8, 9.20%) contained sufficient information for a third party to make a determination from the patient's medical records on whether the approved medicine had been efficacious. For renewal applications, the efficacy of the previously funded treatment course was described in over half of all cases (n=15, 68.18%).
Discussion: This study confirms the considerable cost associated with funding high-cost medicines. Applications were often based on low levels of evidence, making it difficult to assess the benefit and cost-effectiveness of funding these treatments. Furthermore, the monitoring outcomes provided are rarely objective or evaluable, posing challenges for auditing the efficacy of the course being funded. These findings have informed change to the IPU application process for clinicians seeking funding for high-cost medicines at our institution.¶

499 Cannabinoid toxicity post human intraperitoneal injection

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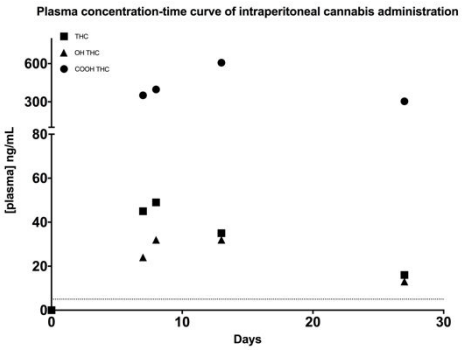

Introduction. Medicinal cannabis is able to be prescribed under the provision of a controlled drug in the Australian Poisons Standard. However, multiple laws must be navigated in order for patients to obtain access and imported products can be expensive. Dose-response information for both efficacy and toxicity pertaining to medicinal cannabis is lacking. The pharmacokinetics of cannabis administered by traditional routes has been described but to date, there is no literature pertaining to pharmacokinetics of an intraperitoneal cannabinoid emulsion.
Case description. A cachectic 56-year-old female with stage IV ovarian cancer and peritoneal metastases presented to hospital with fevers, abdominal distension and severe pain, vomiting, anorexia, dehydration and confusion. The patient reported receiving an intraperitoneal injection, purported to contain 12 g of mixed cannabinoid (administered by a deregistered medical practitioner) two days prior to presentation. Additionally, cannabis oil oral capsules were administered in the hours prior to hospital admission.
Results. THC concentrations were consistent with the clinical state but not with the known pharmacokinetics of cannabis nor of intraperitoneal absorption. THC concentrations at the time of presentation were predicted to be ~ 60 ng/mL. Evidence suggests that blood THC concentrations > 5 ng/mL are associated with substantial cognitive and psychomotor impairment. The predicted time for concentrations to drop < 5 ng/mL was 49 days post administration.
Discussion. The unusual pharmacokinetics of the case suggest that there is a large amount unknown about cannabis pharmacokinetics. The pharmacokinetics of a large amount of a lipid soluble compound given intraperitoneally gave insights into the absorption and distribution of cannabinoids, particularly in the setting of metastatic malignancy.¶

500 Clinical relevance of drug-drug interaction alerts

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Introduction. Computerised alerts trigger at the point of prescribing to warn prescribers that a drug-drug interaction (DDI) may occur. Although useful in principle, users become desensitised to DDI alerts due to the high number being triggered (Van der Sijs *et al.* 2006)
Aims. To evaluate the potential alert burden and clinical relevance of severe DDI alerts if they were to be enabled in a Sydney hospital.
Methods. Firstly, alert burden was measured by identifying how often DDI alerts would hypothetically be triggered in the system if alerts were operational. Secondly, drug pairs that triggered alerts in the hospital system were entered common DDI compendia to determine the severity of the interaction. Thirdly, a subset of patient cases was presented to an expert panel to determine the perceived clinical relevance of DDI alerts.
Results. In total, 40% (31/78) of patient admissions would experience at least one severe DDI alert. On average, these admissions would have triggered 4.7 DDI alerts. The most frequently triggered DDI alert was between "opioid agonists and opioid antagonists" (25% of all alerts). There was poor agreement between the compendia on what constituted a severe DDI (Fleiss' kappa= -0.01). The expert panel determined the relevance of DDI alerts is dependent on the context, for example, the patient's age and lab results would be important for determining when certain alerts fire.
Discussion. Context factors such as age and lab data should be used to ensure alerts only trigger when relevant to the patient and clinician.

Van der Sijs H (2006) J Am Med Inform 13:138-147

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501 A chiral UHPLC-MS/MS method to investigate the pharmacokinetics of enantiomeric ketorolac in human plasma

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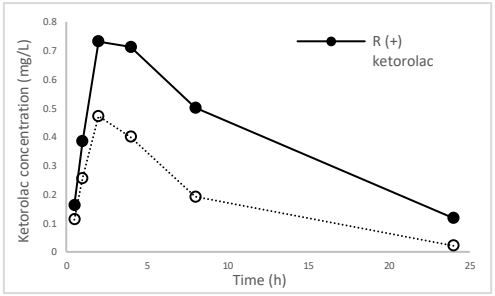
Introduction. Ketorolac tromethamine is used for joint infiltration by orthopaedic surgeons as part of multimodal analgesia. Ketorolac is a racemic mixture of S (-) ketorolac and R (+) ketorolac enantiomers, with the S (-) isomer providing pharmacological activity.
Aims. Establish a simple and validated LC-MSMS method for the determination of S (-) ketorolac and R (+) ketorolac in plasma, suitable for application to a clinical PK study.
Methods. Plasma samples were collected from patients receiving intra-articular ketorolac during total knee or total hip replacement surgery. 100 µL of plasma was extracted using an acetonitrile protein precipitation method, followed by a dichloromethane wash to remove unwanted lipophilic compounds. A chiral Phenomenex Lux Cellulose-3, 50 x 2mm (3µm) column separated S (-) and R (+) ketorolac and the internal standard, [$^2H_4$]-ketorolac. Quantitation was performed using a Shimadzu UHPLC-MSMS 8030+ with electrospray ionisation, using an SRM at 256>>105 (reference ion 256>>77).



Results. The assay met the requirements for a bioanalytical method validation for the measurement of S (-) ketorolac and R (+) ketorolac in plasma across the concentration range of 0.02 to 5 mg/L. Precision was within 10% and accuracy within 3% for both S (-) and R (+) ketorolac. The figure shows the ketorolac PK profiles for a patient undergoing total hip replacement, with maximum concentrations (C_{max}) of 0.47 mg/L for S (-) ketorolac and 0.73 mg/L for R (+) ketorolac, minimum concentrations (C_{min}) were 0.021 mg/L for S (-) ketorolac and 0.117 mg/L for R (+) ketorolac.
Discussion. This accurate and rapid method for the quantification of the enantiomers, (S)-ketorolac and (R)-ketorolac, in plasma has been successfully validated and applied to clinical samples from a PK study of patients undergoing total hip or knee replacement surgery.

501.1 A suite of LC-MSMS assays to investigate the pharmacokinetics of meropenem in critically ill patients receiving renal replacement therapy

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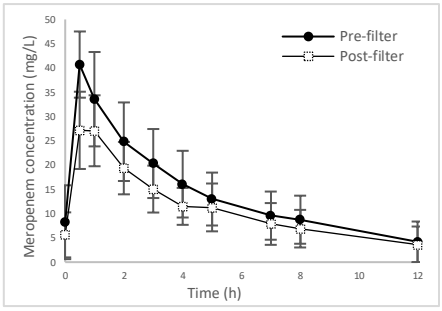

Introduction. Maximising effectiveness of and limiting resistance to meropenem requires an understanding of the patient group. Patients requiring renal replacement therapy may experience sub-optimal antibiotic exposures as meropenem is largely renally excreted and recommended dosing regimens have not been validated in these patients.
Aims. To establish validated LC-MSMS methods for bioanalysis of meropenem in plasma, renal replacement therapy effluent (RRTE) and urine, for a clinical PK study [Roberts et al, 2016].
Methods. 10 µL of plasma, RRTE and urine from critically-ill patients undergoing RRT and receiving meropenem were treated with acetonitrile. Meropenem and the internal standard, [$^2H_6$]-meropenem were separated using a SeQuant zic-HILIC 2.1 x 20 mm (5.0µm) column. Quantitation was performed using Shimadzu Nexera 8030+ LC-MSMS, equipped with an electrospray ionisation source, monitoring SRM 383.5>>68 (ref 383.5>>141) for meropenem, and 390>>147 (ref 390>>68) for [$^2H_6$]-meropenem
Results. The assays using microsample volumes for each matrix met requirements for a bioanalytical method validation over the concentration range of 0.2 to 100 mg/L in plasma and RRTE, and 20 to 10,000 mg/L for urine. The figure presents the pre- and post-filter plasma results from three ICU patients on RRT receiving IV meropenem b.i.d. Maximum concentrations (C_{max}) were 37.4 ± 3.2 mg/L for plasma, 18.3 ± 1.6 mg/L for RRTE and 926 ± 532 mg/L for urine.
Discussion. Application of this method may lead to improved clinical study participation for patients with challenging phlebotomies or where the collection of small volumes of sample can reduce the burden of study participation. This suite of methods has been successfully used in a multinational clinical PK trial.
Roberts et al (2016) BMC Infect Dis 16:103

502 Current antimicrobial stewardship (AMS) practices in the Australian community pharmacies

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Introduction**.** Increasing antimicrobial use is one of the modifiable causes of antimicrobial resistance (AMR) that is expected to claim 10 million human lives by 2050. Though the majority of antimicrobials are prescribed in community, little is known about the antimicrobial stewardship (AMS) practices in the Australian pharmacies. Therefore, we report the development, validation and piloting of the first Australian survey to measure the current AMS practices. Method. A questionnaire to measure community pharmacists' current practices of AMS and perceived importance, barriers and facilitators to participate in AMS initiatives was developed based on a literature review and expert opinion. A convenience sample of 140 community pharmacists was selected who were invited to complete the online survey. Cronbach's alpha and exploratory factor analysis (EFA) were used to measure the reliability and validity of the questionnaire, respectively. Results. 85 out of 140 (61%) pharmacists responded to the survey. The majority of the pharmacists were female practicing in metropolitan areas. EFA identified three components confirming a single factor solution for the three scales. Cronbach's alpha for each scale is; perceived importance 0.65, barriers 0.82 and facilitators 0.68. The majority of respondents require better access to patient records (92%), guidelines (62%) and training (52%) to enable their participation in AMS initiatives. The majority of respondents (54%) agreed that GPs are more receptive of a change in dose or duration of an antibiotic recommendation but less receptive if a change in antibiotic choice is recommended (63%). Discussion. A reliable and valid tool was developed to measure community pharmacists' perceptions of AMS that can be used to conduct larger national and international studies. Tasmanian pharmacists were more willing to participate in AMS initiative if additional training, access to locally developed antibiotic guidelines and better access to patients' clinical data is provided to them.
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503 Inappropriate drug prescribing in elderly hospitalised patients with falls and fractures

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Introduction. Falls and osteoporotic fractures are two major health problems in the elderly. Medications significantly contribute to both risks and prevention of these serious complications.
Aims. To investigate the prevalence of prescribing potentially inappropriate, especially psychoactive, medications and prescribing omissions (especially anti-osteoporotic drugs) in older patients with falls and fractures.
Methods. Data on medications used, demographics and comorbidities were collected in two cohorts of elderly patients (≥ 60 years): 587 medical (mean age 85.7±6.9 years, 63.6% female) and 854 orthogeriatric patients with non-vertebral fractures (mean age 78.10±9.51 years, 73.0% female). In medical patients risk of osteoporosis was estimated (Osteoporosis Self-assessment Tool, OST). The appropriateness of medication use was assessed by Beers Criteria 2015.
Results. Amongst medical patients, there were 137 (23.3%) with history of falls and 98 (16.7%) with previous fractures. At admission, 55 (40.1%) of fallers and 23 (23.5%) of patients with fractures have been using at least one of the following classes of medications known to be associated with falls and fractures: antidepressants (selective serotonin reuptake inhibitors, tricyclics), benzodiazepines or antipsychotics (haloperidol, risperidone, olanzapine, quetiapine). High risk of osteoporosis (OST<-3) was found in 352 (66.9%) patients, 122 (34.7%) of whom were receiving falls-risk medicines, but only 66 (18.8%) have been prescribed anti-osteoporotic drugs (bisphosphonates, denosumab). OST<-3 was significantly and independently associated with age >75 years (OR 15.0, 95%CI 6.05-37.21, p<0.001), history of fractures (OR 2.5, 95%CI 1.23-4.90, p=0.011) and falls (OR 1.9, 95%CI 1.09-3.44, p=0.024). Anaemia (haemoglobin<120g/L), mostly iron deficient, was found in 72 (52.6%) fallers and 54 (55.1%) patients with fractures, but iron supplements were used only by 8.3% and 13.0%, respectively; at discharge the prescriptions improved minimally. Among orthogeriatric patients, prior to admission, 239 (28.0%) were using falls-risk medicines, 620 (72.6%) were anaemic, but only 139 (16.3%) received anti-osteoporotic drugs and only 41 (6.6%) anaemic individuals were prescribed iron supplements.
Discussion. Deprescribing potentially inappropriate drugs and rationale use of anti-osteoporotic and anti-anaemic therapies is essential for prevention falls and fractures in the elderly.

504 A time and motion study of phlebotomists' work

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Introduction. Therapeutic drug monitoring (TDM) involves adjusting drug dosage regimens according to circulating concentrations of the drug. Critical to this process is obtaining blood samples (usually collected by phlebotomists) at the appropriate time in the dosing interval. Due to the busy nature of the clinical setting, TDM samples are often collected at the incorrect time. No research has explored phlebotomists' work and their role in the TDM.
Aims. To collect time and motion data on the work of phlebotomists and to explore the opinions and experiences of phlebotomists about their working environment.
Methods. Observational time and motion data of ward phlebotomists (n=5, 45 hours) was collected using the Work Observation Method by Activity Timing (WOMBAT) software. Descriptive statistics were used to determine the proportion of total observed time spent on tasks. Participating phlebotomists also partook in a focus group.
Results. Phlebotomists predominantly spent time collecting blood (54%), in professional communication (15%) and in transit (15%). Phlebotomists spent 14% of their time multitasking and were interrupted every 19 minutes. Social activities, including lunch and bathroom breaks, accounted for 13% of their time. Phlebotomists raised concerns about their high workload, predominately attributed to understaffing and patient care tasks beyond blood collection, which contributed to physical fatigue and stress.
Discussion. Phlebotomists spent the majority of their time in blood collection. Professional communication was also an important component of their daily tasks and was most commonly associated with multitasking and interruptions. This finding was recognised by the phlebotomists. Phlebotomists displayed similar multitasking to nurses (14% vs 12%) however had a much more frequent interruption rate (19 mins vs 49 mins)[1]. The heavy workload experienced by the phlebotomists contributes to the difficulty of collecting TDM samples at regimented times. Increasing the number of ward phlebotomists and/or recruiting a dedicated TDM phlebotomist may help overcome this challenge.
[1]Westbrook J (2009) Int J Med Inform 78:S25-33¶

505 Impact of deprescribing interventions in older hospitalised patients on prescribing and clinical outcomes: a systematic review of randomised trials

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Introduction. Polypharmacy and potentially inappropriate medications (PIMs) are prevalent in older inpatients, and are associated with adverse drug reactions, falls, confusion, hospitalisation, and death. Deprescribing is one intervention to reduce polypharmacy and PIM use.
Aims. To investigate the efficacy of deprescribing interventions to reduce PIMs and impact on clinical outcomes in older inpatients.
Methods. MEDLINE, Embase, Informit, International Pharmaceutical Abstracts, Scopus, PsycINFO, Cochrane Central Register of Controlled Trials, and CINAHL were searched for randomised controlled trials (RCTs) in English from 1996 to April 2017. Two researchers independently screened retrieved articles. RCTs reporting on deprescribing interventions to reduce PIMs in older hospitalised adults were eligible. Data was extracted and study quality of all included RCTs was assessed. The primary outcome of interest was reduction in PIMs. Where available other clinically relevant outcomes were assessed.
Results. Nine RCTs (n=2522 subjects) met the inclusion criteria. A high risk of bias was present among the studies. Deprescribing interventions were either pharmacist-led (n=4), physician-led (n=4), or multidisciplinary team-led (n=1). Six studies used an explicit tool to identify PIMs. In terms of the primary outcome, 7 of the 9 studies reported a statistically significant reduction in PIMs in the intervention group. There was significant heterogeneity in outcome measures and reporting. Other reported clinical outcomes included impact on drug related problems, health related quality of life (n=2), mortality (n=3), hospital readmissions (n=4), falls (n=3), and functional status (n=2). The results were mixed with most reporting no statistically significant difference between control and intervention groups.
Discussion. The available evidence suggests that deprescribing interventions are feasible and safe in older adults in hospital, and are efficacious at reducing PIMs. However, the current evidence is limited and of low quality. High quality RCTs with clinical outcomes relevant to older adults are required.

506 A Multicentre Open-Label Pharmacokinetic-Pharmacodynamic Study of Febuxostat in Patients with Chronic Gout

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Introduction. There are conflicting data concerning the effect of renal function on the pharmacokinetics and response to febuxostat (Fbx).
Aims. To explore relationships between the concentrations of serum urate (SU) and plasma Fbx in patients with chronic gout and examine the influence of renal function on the plasma concentrations of Fbx and the efficacy of Fbx.
Methods. Baseline demographics including SU and serum creatinine concentrations were collected. Plasma Fbx concentrations and SU were measured at four times during long term treatment with Fbx over the dosage interval (24 h). Data is presented as mean ± S.D.
Results. Chronic gout patients (20 males, 6 females) were recruited. The duration of Fbx treatment (40-120 mg/day) was 6 weeks to 66 months. Baseline SU and eGFR were 0.59 ± 0.09 mmol/L and 61 ± 24 mL/min, respectively. Fbx 40 (n=8), 80 (n=17) and 120 (n=5) mg/day achieved similar reductions of SU; 0.34 ± 0.09, 0.36 ± 0.11 and 0.31 ± 0.07 mmol/L, respectively. Target SU ≤ 0.36 and ≤ 0.30 mmol/L were achieved by 90% (24/26) and 77% (20/26) of patients, respectively, with Fbx doses of up to 120 mg/day. At Fbx 80 mg daily, the reduction in SU was 0.37 ± 0.09 and 0.34 ± 0.13 mmol/L in patients with eGFR < 60 (n=9) and ≥ 60 (n=8) mL/min, respectively. At Fbx dosage of 80 mg daily, trough concentrations of Fbx were significantly higher in patients with eGFR < 60 mL/min (0.17 ± 0.11) than those with eGFR ≥ 60 mL/min (0.03 ± 0.01) (P= 0.009). Renal function had no significant effect on peak Fbx concentrations. There was a 50-fold fluctuation in plasma Fbx over 24 h while SU did not fluctuate significantly over this time.
Discussion. Higher trough Fbx concentrations in patients with eGFR < 60 mL/ min may be due to the retention of Fbx-glucuronide and the subsequent regeneration of the parent drug. Renal function does not influence the hypouricaemic response to Fbx. We suggest that the small fluctuation in SU over 24 h is due to the long half-life of urate (20 to 30 h). A larger sample size is required to confirm present results. The small fluctuation in plasma urate over 24 h is due to long half-life of urate and possibly prolonged hypouricaemic effects of Fbx.

1. Mayer MD et al (2005) Am J Ther 12(1):22-34.
2. Hira D et al (2015) J Pharmacology 96(1-2):90-8.
3. Becker MA et al (The CONFIRMS trial) (2010) Arthritis Res Ther 12(2):R63.

508 The perceived impact of medicines, foods and substances taken by mother on their breastfed baby.

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Introduction. It has been shown that the types and rate of adverse drug reactions (ADRs) experienced by breastfed infants whose mothers are taking medications has not been well defined and are notoriously under reported. Those that are reported often have crucial information missing which makes it difficult to draw valid conclusion mainly due to a lack of a standardized reporting system.
Aim. The objective of this study was to ascertain whether the breastfed baby of a nursing mother experienced any ADRs due to the medications taken by the mother and the impact of such a reaction on the continuation of breastfeeding and/or mother's treatment.
Methods. This study was conducted as an anonymous online survey consisting of 42 questions divided into 6 sections. Data was collected over a period of 6 months. Data was analysed using SPSS software.
Results. A total of 360 responses were obtained. Approximately 40% of the breastfeeding mothers surveyed indicated that they took one or more medication(s) while breastfeeding. About one third of the respondents indicated that they were concerned about the transfer of medications to their baby. An ADR in the breastfed baby led to discontinuation of treatment in 20 women and cessation of breastfeeding in another 8 women. Only 2 reports of an adverse drug reaction was confirmed to have been reported by the healthcare professional to a regulatory body.
Discussion. Medication use in breastfeeding is quite prevalent and consequently the occurrence of ADRs in the breastfed infant is also possible. An adverse drug reaction in a breastfed baby can lead to treatment discontinuation for the mother or cessation of breastfeeding, with neither being ideal. However, the identification of ADRs in breastfed infants and reporting of these ADRs is likely to be understated.
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509 The effect of deprescribing after chronic polypharmacy on locomotor activity and cognition in a preclinical model

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Introduction. Two-thirds of Australians over the age of 75 are prescribed 5 or more drugs concurrently (polypharmacy). Clinical observational studies have shown polypharmacy and increasing exposure to anticholinergic and sedative effects, which can be measured using Drug Burden Index (DBI), are associated with adverse geriatric outcomes, including falls, frailty and cognitive impairment. In older people, interventional studies of deprescribing (withdrawal of medications) are logistically difficult to conduct. A preclinical model to test the effects of deprescribing would help to provide evidence as to the safety and efficacy of deprescribing for global health outcomes.
Aim. To determine the effects of deprescribing on locomotor activity and cognition after chronic exposure of ageing mice to polypharmacy regimens with increasing DBI.
Methods. From 12 to 21 months of age, male C57BL/6 mice were given control diet or feed containing therapeutic doses of commonly used medications. Polypharmacy treatment groups included zero DBI (simvastatin, metoprolol, omeprazole, paracetamol, irbesartan), low DBI (simvastatin, metoprolol, omeprazole, paracetamol, citalopram) and high DBI (simvastatin, metoprolol, oxybutynin, oxycodone, citalopram). The single drugs from the high DBI group were also administered as monotherapy. At 21 months, half of the animals in treatment groups had medications deprescribed (n=14-20 per group). The open field and Barnes Maze were performed at 21 and 24 months.
Results. Preliminary results are shown for a subgroup of the cohort (n=9-12 per group). Deprescribing the high DBI with polypharmacy regimen and citalopram resulted in locomotor activity improvement ($P<0.005$ and $P<0.05$ respectively) when compared to mice that continued treatment. Non-significant improvements in short and long term memory were seen in mice deprescribed the high DBI polypharmacy regimen when compared to those that continued treatment ($P<0.3$).
Discussion. This is the first reported pre-clinical model to examine the safety and efficacy of deprescribing. Our preliminary results show deprescribing medications in the absence of disease does not appear to be harmful and could improve outcomes. Completion of the study is required to confirm these findings in a larger sample.

510 A simple, sensitive and rapid LC-MS/MS method for the simultaneous measurement of anthracyclines, cyclophosphamide and taxanes in breast cancer patient samples

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Introduction. Tailoring drug dose for anticancer drugs is known to provide better outcomes by maximising drug benefit and minimising toxicity, especially in patients with altered phenotypes such as obesity, advanced age or organ dysfunction. Dose individualization with chemotherapies has been shown to improve patient outcomes and minimise adverse events, yet dose individualization is a challenging task, methodologically and practically. New simple, sensitive, rapid and reproducible methods to simultaneously measure drugs levels in small volumes of patient samples are needed to make it convenient and practical for patients and clinicians.
Aims. To develop a simple, sensitive and rapid LC-MS/MS method for the simultaneous measurement of anthracyclines, cyclophosphamide and taxanes in small volumes of blood samples.
Methods. Deuterated internal standards in acetonitrile are added to small volumes of blood samples (10-50µl) for extraction. Chromatographic separation is achieved using a Kinetex C18 50 x 2.1mm, 1.7µm column with gradient elution of mobile phase starting at 20% acetonitrile with 0.1% formic acid. The compounds are detected by a triple quadruple mass spectrometer (Shimadzu 8060), operating in positive electrospray. Total run time is 5.0 min.
Results. The method is linear over a range of 1–500ng/mL for doxorubicin, epirubicin, docetaxel and paclitaxel, 0.1-50µg/mL for cyclophosphamide covering the expected concentrations in patient samples. Inter-assay precision is between 1 - 15% and inter-assay bias is between -11 – 9% for all compounds. Intra-assay precision was between 0.4 - 6% and intra-assay bias was between -5 – 11% for all compounds.
Discussion. An LC–MS/MS method for determination of anthracyclines, cyclophosphamide and taxanes in very small volumes of blood was developed and validated. This methodological improvement will facilitate personalised dosing of chemotherapy for each patient to provide the best response and reduced side effects. Methods can be adapted for a variety of agents, including novel targeted anticancer therapies.¶

511 Relationship between plasma dolutegravir concentration and cause of anti- HIV therapy discontinuation

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Introduction. Dolutegravir (DTG) is a second-generation HIV-1 integrase inhibitor that is high potent against both wild-type and drug-resistant HIV-1 strains. DTG has a good toleration, few drug interactions, minimal drug resistance and once-daily dosing for treatment-naive patients. Therefore, DTG is approved for use in a broad population of HIV-infected patients. However, there are few data for reasons of DTG discontinuation during antiretroviral therapy.
Aims. In this study, we intended to investigate major causes of DTG discontinuation and relationship with plasma DTG concentrations.
Methods. We examined 656 HIV-1-infected patients (male:female=604:52) who were treated with DTG containing regimen from 2014 to 2016, retrospectively. All patients had been administered with 50mg DTG once daily in combination with other antiretrovirals. Plasma DTG concentrations were determined by our developed LC-MS method. Adverse events were assessed by laboratory data and interview at outpatient clinic.
Results. In 656 patients, 149 patients were therapy-naïve and 507 patients were therapy-experienced for HIV. Of 656 patients, 17 patients (male:female=15:2) discontinued DTG, and switched from DTG to other antiretrovirals. The reasons of DTG discontinuation were adverse events (15 patients), drug interactions (1 patients), and pregnancy (1 patient), respectively. The median duration of DTG administration was 40 weeks (2-77 weeks). The adverse events were rash (7 patients), CNS side effects (3 patients), vomiting (1 patients), diarrhea (1 patient), ALT/AST elevation (1 patient), arthralgia (1 patient), and renal dysfunction (1 patient), respectively. The plasma DTG concentrations were significantly higher (2.5-fold) in patients who had adverse events than in those with no events.
Discussion. These findings suggest that we have to pay attention for rash and CNS side effects during DTG containing antiretroviral regimen, especially. As the patients with adverse events had higher DTG plasma concentrations, Therapeutic drug monitoring for DTG will be useful for preventing various adverse events.

512 Preclinical models to understand the risks of single and multiple concurrent medicines in old age

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Introduction. Chronic medication use is common in older people. Older people, particularly those with polypharmacy (use ≥ 5 drugs) for multi-morbidity, are rarely included in clinical trials to determine efficacy and safety. Observational studies indicate polypharmacy and increasing Drug Burden Index (DBI: measures total anticholinergic and sedative medication exposure) are associated with impaired physical function in older people. Preclinical models of clinically relevant drug exposures in ageing would be useful to screen for adverse geriatric outcomes prior to marketing.
Aim: To develop a preclinical mouse model to determine whether chronic use of therapeutic drugs (monotherapy or polypharmacy) and/or increasing DBI exposure impair translatable functional outcomes in ageing.
Methods: From 12 months of age, male C57BL/6 mice were fed control diet or feed/water containing therapeutic doses of study drug(s). We tested regimens of five drugs that had Zero DBI (simvastatin, metoprolol, omeprazole, paracetamol, irbesartan), Low DBI (simvastatin, metoprolol, omeprazole, paracetamol, citalopram), High DBI (simvastatin, metoprolol, oxybutynin, oxycodone, citalopram) and single drugs from the High DBI regimen as monotherapy. Functional tests are performed every 3 months throughout life. Power calculations estimate that a sample size of 10-12 per group is required to detect changes in functional measures with treatment.
Results. For the subgroup of animals with data currently available after 6 months of treatment (age 15 and 18 months), compared to control, measures of spontaneous activity in the open field (distance and midzone entries), grip strength (wire hang), nesting scores and frailty score were reduced in the Low DBI, High DBI and citalopram groups (n=25-40, $p<0.05$). Compared to control, muscle endurance (rotarod) was significantly reduced in Low DBI and citalopram after 6 months of treatment (n=25-40, $p<0.05$).
Discussion: We have developed a preclinical model that can detect impaired functional outcomes following chronic treatment with polypharmacy regimens or monotherapy in ageing mice. These methods can be applied to determine and understand mechanisms and reversibility of the risks of medicines to global health outcomes in old age. ¶

513 Prevalence and prediction of adverse drug reactions in older inpatients with hyperpolypharmacy

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Introduction. Adverse drug reactions (ADRs) in hospital carry serious health consequences and increase the burden on the health care system. As the risk of ADRs increases with old age and the number of medications, older patients with hyperpolypharmacy (taking 10≤ medications), are at particularly high risk. Additional clinical and pharmacological risk assessments could help prioritise inpatients for medication review to prevent potentially avoidable ADRs.
Aims. To determine the prevalence of ADRs in older inpatients with hyperpolypharmacy and explore potential predictors for identifying patients who are most likely to have an ADR in hospital.Methods. Patients >65 years with hyperpolypharmacy on admission were recruited prospectively from a tertiary referral teaching hospital in Sydney, Australia. Data collected included gender, age, current medications, Reported Edmonton Frail Scale and Charlson Comorbidity Index. ADRs were detected by review of medical records, assessed for causality using the Naranjo criteria, and classified by drug class. Data was verified by a multidisciplinary panel. T-tests were used to compare clinical characteristics and medication use between patients who did and did not have an ADR.
Results. Sixty nine patients were recruited with a median age of 78 years with 56.5% being female .Preliminary results showed 22.1% of inpatients had an ADR during their admission. Average Naranjo rating for each of the experienced ADRs was 6.9 ± 0.4, with an average of 3.4± 0.2 unknown factors, where a score of 5-8 is probable. Of the 23 ADRs, drug classes responsible were antibiotics (26.1%), diuretics (26.1%), cardiac drugs (21.7%), opioids (8.7%), anticonvulsants (8.7%), and anticoagulants (8.7%).There was no significant difference in frailty, comorbidity index or measures of medication exposure, between patients with and without an observed ADR in hospital. However, those who experienced an ADR in hospital had a significantly longer length of stay (11.1 verses 5.7 days, P<0.05).
Discussion. ADRs in hospital were common in this high risk group. Average Naranjo ratings suggest probable causality but assessment was limited by a high prevalence of unknown factors. Preliminary results show no significant association of patient and medication characteristics with ADRs for older patients with hyperpolypharmacy in the acute setting. Future studies will need to investigate larger sample sizes to confirm these findings. ¶

514 Pharmacometrics to address weaknesses in Australian medical countermeasure product development

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Introduction. Medical countermeasure (MCM) products are defined as diagnostics, vaccines and therapeutics for the protection of personnel against chemical, biological and radiological (CBR) threats, and emerging infectious diseases.
Aims. To understand Australia's MCM product development capability and capacity and to identify new opportunities in the ecosystem for pharmacometrics.
Methods. An electronic survey of 145 questions was conducted together with 30 min minute structured interviews. Surveys were analysed using descriptive statistics. Technology readiness level (TRL)-guided 'impressions' of capability and capacity were established, de-identified, and presented using heat maps (individual) and summarized (national).
Results. There were 131 completed surveys. Two-thirds identified their primary and secondary positions on the MCM product development value chain as 'translational research/pre-clinical research'. The greatest concentration of activity was at TRL4 and earlier. Late-phase capabilities (i.e., clinical development and manufacturing) were weaker, and very few respondents identified market access as their primary position (5/131). Interviews were conducted with 49 of the survey respondents (37%). Interviews confirmed strong BSL2/3+ *in vitro* capabilities but minimal GxP examples in DMPK, immunoassay, bioanalysis, PD or safety. *In vivo* GLP-compliant non-clinical services had limited diversity and availability. The 2 areas of MCM product development that could benefit most from increased pharmacometrics were non-clinical *in vivo* PK ± PD and toxicology studies (e.g., to replace/inform studies in larger animals that require high level containment, ABSL3/4) and early clinical development (e.g., phase 1 and phase 2a/b).
Discussion. Australia has a dispersed, relatively small but experienced discovery and development community. Pharmacometric approaches could be utilized more broadly to address capability and capacity gaps in the Australian MCM product development ecosystem.¶

515 Intravesical mitomycin C enhances spontaneous phasic contractile activity in the murine bladder

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Introduction. Mitomycin C (MMC) is the most common cytotoxic drug used for intravesical bladder cancer treatment, but 34.5% of patients experience urological adverse effects including increased urinary frequency, urgency and pain. The effects of MMC on normal bladder function are poorly understood and there are currently no proven treatment options to manage these side effects.
Aims. This study aimed to determine the effects of intravesical MMC on normal murine bladder function 7-days following treatment.
Methods. Aged female C57/BL6JArc mice (Age 36 weeks, n=4 per group) were randomly allocated into 2 experimental groups (Saline/Control or MMC) and given a 1-hour intravesical treatment with saline or MMC (1mg/mL). After 7 days, a whole bladder preparation was used to assess spontaneous contractile activity, and intravesical pressure changes in response to bladder filling (30µl/min), electrical field stimulation (EFS: 1,5,10, 20Hz) and pharmacological agents.
Results. Spontaneous phasic contractile activity was observed in saline treated bladders with a frequency of 2.5±0.29 contractions/min and amplitude of 0.11±0.1 mmHg. Spontaneous activity was increased in MMC treated bladders with a 1.7-fold increase in frequency (4.25±0.48 contractions/min, $p<0.05$) and 3.4 fold increase in the amplitude (0.36±0.13 mmHg, $p>0.05$) of phasic contractions. Pressure responses to EFS, the muscarinic agonist carbachol, the purinergic agonist α,βmATP and the β-adrenoceptor agonist isoprenaline were unchanged by MMC treatment.
Discussion. An increase in the rate and amplitude of spontaneous non-voiding bladder contractions may explain the increased frequency and urgency in patients following treatment with MMC.

516 The influence of perioperative opioids on cancer metastasis

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Introduction. The possibility that opioids can modulate the tumour microenvironment and thereby influence tumour growth and metastasis is of intense interest. The μ-opioid receptor (MOR) and toll-like receptor-4 (TLR4) can be activated by opioids or their metabolites, are expressed on cancer cells as well as tumour-associated cells, and control signalling pathways that play a key role in modulating cancer metastasis. In this study, we quantified the ability of opioids and their metabolites, present in clinical samples, to activate the MOR and TLR4. We also evaluated the effect of opioid administration to patients, on their circulating proteolytic profile.
Aim. The research aims to elaborate upon the potential interplay between opioid analgesia and tumour metastasis through MOR, TLR4, and matrix degradation.
Methods. Plasma samples were collected from 60 patients undergoing elective lower limb joint replacement pre-operatively and at 3, 6 and 24 h after the surgery. Plasma was also collected from 10 healthy volunteers. Opioid administration was recorded and converted into morphine IV equivalents. AlphascreenTM cyclic AMP (cAMP) assay and MOR-overexpressing HEK293 cells were employed to quantify MOR activation. Cells engineered to express TLR4 and co-receptors essential for TLR4 activation (HEK-Blue™ hTLR4) were utilised to measure TLR4 activation. Circulating matrix metalloprotease (MMP) and Tissue Inhibitor of Matrix Protease (TIMP) activities were assessed by zymography and reverse zymography, respectively.
Results. Post-operative plasma samples displayed MOR activation potential and, the ability to inhibit LPS-induced TLR4 activation. These samples also displayed altered circulating matrix-degrading enzymes activity potential.
Discussion. Evaluating the effect of perioperatively administered opioids on circulating parameters likely to affect the biology of cancer cells and other prominent tumour-associated cells is a novel and promising approach to understanding whether perioperative analgesia of cancer surgery patients can influence the risk of long term metastasis or recurrence.¶

517 Simultaneous determination of adalimumab and infliximab in human serum by liquid chromatography/tandem mass spectrometry

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Introduction. Adalimumab and infliximab are monoclonal antibody biologic drugs that work against tumour necrosis factor alpha (TNF-α) and are used to treat autoimmune diseases. Pharmacokinetic studies and therapeutic drug monitoring of adalimumab and infliximab in patients require a well validated analytical method to analyse the concentrations of adalimumab and infliximab in serum.
Aims. The objective of this work was to develop and validate a simple and sensitive LC-MS/MS method for the simultaneous determination of adalimumab and infliximab in human serum.
Methods. The method was based on a combination of trypsin digestion of serum and LC-MS/MS analysis of unique peptides produced by trypsin digestion of the therapeutic monoclonal antibody drugs. Serum samples were processed by denaturation, reduction, alkylation and trypsin digestion to break down proteins into peptides. Digests were injected into the LC-MS/MS system. The tryptic peptides were separated under gradient elution using an analytical column. The mass spectrometer was operated in the positive ion mode. The tryptic peptides that are unique for adalimumab and infliximab were utilized for their quantification.
Results and Discussion. For both adalimumab and infliximab, standard curves were linear over the concentration range 1.0 to 100 mg/L ($r > 0.99$) in serum, biases were $<\pm 10\%$, and intra- and inter-day coefficients of variation (imprecision) were <10%. The limit of quantification was 1.0 mg/L in serum. The assay has been successfully tested to monitor adalimumab and infliximab concentrations in patients on adalimumab or infliximab.¶

518 Ibuprofen in Infants younger than 6 Months: What is the Efficacy and Safety Profile?

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Introduction. Ibuprofen is a non-steroidal anti-inflammatory drug frequently administered to children of various ages for relief of fever and pain and is approved as over-the-counter medication in many countries worldwide. Although there is extensive data on its efficacy and safety in children and adults, there are divergent dosing recommendations for analgesia and treatment of fever in infants, especially in the age group between 3 and 6 months of age.
Aims. To assess the safety and efficacy profile of ibuprofen in this age group in an attempt to optimise pain and fever management.
Methods. A comprehensive PubMed search was conducted in order to identify publications concerning the use of ibuprofen in infants younger than 6 months of age. Identified studies were reviewed so that only those presenting original clinical data regarding the pharmacokinetics, safety or efficacy of ibuprofen in this age group are included.
Results. The literature search identified 5 pharmacokinetic and 10 efficacy and safety studies which met the review inclusion criteria. Eligible PK studies presented data of 243 children, which included at least 18 infants under the age of 6 months. Eligible efficacy and safety studies contained data of 39,234 children including minimum 207 children younger than 6 months. The most common underlying pathological condition was fever. The most common clinical setting was outpatient care.
Discussion. Based on the current evidence, short-term use of ibuprofen is considered safe in infants older than 3 months of age having a body weight of more than 5-6 kg when special attention is given to the patient's hydration. Ibuprofen should be prescribed based on body weight using a dose of 5-10 mg/kg. This dose can be administered 3-4 times a day resulting in a total daily dose of maximally 30-40 mg/kg. The rectal route has been shown to be less reliable because of erratic absorption, especially in young infants. Since most efficacy and safety data have been derived from paediatric trials in infants with fever, future studies should focus on the efficacy of ibuprofen in young infants with pain.¶

519 The Safety and Pharmacokinetics of Metformin in Heart Failure

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Introduction. Metformin, a type II diabetes (T2DM) drug, is contraindicated in heart failure (HF) in Australia due to a perceived increased risk of lactic acidosis. The safety of metformin in HF, described in epidemiological studies, has facilitated approval of its use in HF patients in the US, UK, Canada and New Zealand. Detailed prospective data on the safety and PK of metformin is required to confidently remove this contraindication in Australia.
Aim. To explore the safety and PK of metformin in HF patients and compare with healthy subjects (Timmins et al, 2005) and T2DM patients without HF (T2DM Control) (Duong et al, 2013)
Methods. This cross-sectional study consisted of two cohorts of HF subjects; those with T2DM receiving metformin (n=10), and those without T2DM and metformin naïve (n=26). Biochemical parameters (including lactate, anion gap and bicarbonate) and plasma metformin concentrations were determined. Metformin PK parameters were determined using NONMEM.
Results. In HF patients with T2DM, plasma lactate, anion gap and bicarbonate concentrations did not correlate with plasma metformin concentrations. The apparent CL of metformin (37 ± 17 L/h) was similar to the T2DM patients (49 ± 26 L/h), but significantly lower than healthy subjects (75 ± 14 L/h; $p < 0.05$). The peripheral V was significantly lower in HF patients compared to healthy subjects ($p=0.04$). Lactate concentrations of HF patients without T2DM (1.5 ± 0.7 mmol/L) were significantly lower than in T2DM patients with or without HF (1.9 ± 0.9 mmol/L; $p < 0.05$).
Discussion. The PK of metformin in T2DM HF patients are similar to those in T2DM patients without HF. Additionally, hyperlactatemia was not associated with HF patients both with and without T2DM. These results provide the support for a larger interventional study with metformin in HF patients.
Duong JK et al (2013) Clin Pharmacokinet 52:373-384
Timmins P et al (2005) Clin Pharmacokinet 44: 721-729¶

520 The Safety of Metformin in Haemodiafiltration

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Introduction. The cardioprotective effects of the anti-hyperglycaemic agent metformin may be of great benefit to patients with type 2 diabetes mellitus (T2DM) and end-stage kidney disease (ESKD) who require haemodiafiltration (HDF). Metformin is extensively cleared from plasma during HDF (Smith et al, 2016). This indicates that metformin may be safely given to these patients if administration matches extraction during HDF, thereby preventing metformin accumulation and lactic acidosis. Further studies are required to confirm this.
Aims. To monitor the safety of metformin in patients with T2DM and ESKD undergoing HDF.
Methods. Patients received metformin (IR, 250 mg) after each HDF session (thrice weekly; 750 mg/week) for 6 months. Regular blood samples were collected prior to the start of HDF to monitor safety parameters (plasma lactate <5 mmol/L, plasma metformin <5 mg/L). Metformin concentrations were quantified by HPLC.
Results. Plasma lactate concentrations remained below 5 mmol/L in all patients (n=7) for the duration of treatment. Plasma metformin concentrations remained below 5 mg/L, except for 2 occasions in Patient 3 (max=5.3 mg/L). Unfortunately, Patients 1 and 6 passed away from cardiac events in the fourth month of the study. The study was subsequently ceased by local governance. No safety data from these patients was suggestive of lactic acidosis.
Discussion. Cardiovascular disease is the leading cause of death in HDF patients. Additionally, there is no evidence to date that associates metformin with an increased risk of cardiovascular events. Prior to study cessation, all data collected supported the safety of metformin in HDF. This information, particularly given the safety data collected from Patients 1 and 6, suggests it is unlikely that metformin contributed to these deaths. Regardless, further studies are required to investigate any potentially deleterious interactions between metformin and the rapid shifts in biochemistry and body fluid that take place during HDF.
Smith F et al (2016) Am J Kidney Dis 68:990-992¶

521 Discharge summaries: an untapped resource for optimising Adverse Drug Reaction identification

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Introduction. Adverse drug reactions (ADRs) are the most common type of iatrogenic injury and cause substantial morbidity, mortality and financial costs. Most pharmacovigilance systems are based on voluntary reports, however under-reporting is a well-recognised limitation. In December 2016, a complementary pathway was introduced for generating ADR reports using International Statistical Classification of Diseases (ICD-10) coding data from electronic discharge summaries.
Aims. To determine the effect of the introduction of a clinical coding surveillance system on the identification of ADRs, considering differences in reaction severity and patient follow-up.
Methods. All encounters at our health service coded with ICD-10 codes Y40-Y59 were captured by the clinical coding surveillance system as potential ADR reports. These reports are reviewed by a multi-disciplinary committee which assigns ratings of causality and severity and decides whether any follow-up is required. ADR data from before (pre-intervention period) and after the introduction of coding surveillance (post-intervention period) were compared.
Results. Two six-month periods were compared. In the pre-intervention period, 104 ADRs were reported by clinicians. In the post-intervention period, 281 ADR notifications were generated and of these, 109 reports were from clinicians, 113 were from coding and 59 were detected in both systems. The review of this volume of ADR notifications was possible because of the use of electronic medical records in our organisation. The proportion of reactions rated as at least "moderate" severity was the same for both periods (78.9% vs. 82.6%; difference 3.7%; 95% CI -6—13.4%; p=0.49). There was no difference in the proportion of reactions that resulted in an ADR Committee intervention (55.8% vs. 48.0%; difference 7.8%; 95% CI -4.1—19.6%; p=0.22).
Discussion. Clinical coding surveillance complements the existing voluntary reporting process by providing valuable information which would otherwise has been missed. The additional detection of ADRs of at least moderate severity by this process may impact patient morbidity and safety. This could have system-wide implications for the uptake of pharmacovigilance in hospital systems.

522 GLP-1-induced anorectic and emetic responses are mediated via exendin (9-39)-sensitive mechanisms in *Suncus murinus*

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Introduction. GLP-1 receptor agonists can be associated with nausea, emesis and reduced appetite in man. Our previous studies showed that the GLP-1 receptor agonists, GLP-1 (7-36) amide and exendin-4, inhibited feeding and water intake, and induced emesis in *Suncus murinus*.
Aims. In the present study, we examine if the action of GLP-1 (7-36) to induce emesis and inhibit feeding are mediated via central GLP-1 receptors using the potent GLP-1 receptor antagonist, exendin (9-39).
Methods. *Suncus murinus* were anaesthetised with sodium pentobarbitone (40 mg/kg, i.p.) and then stereotaxically implanted with a guide cannula into the lateral ventricle and allowed a 7-days recovery before experimentation. Animals were fasted 12-h prior to administration of drugs. On the day experimentation, they were administered exendin (9-39) (30 nmol, i.c.v.), or saline (5 µl i.c.v.) 15 min prior to GLP-1 (7-36) (3 nmol, i.c.v.), or saline (5 µl, i.c.v.). Food and water consumption and behaviour were measured for 1-h.
Results. GLP-1 (7-36) inhibited food and water intake ($P<0.001$) and induced emesis in 1 out of 6 animals. GLP-1 (7-36) also reduced significantly the distance moved when compared with the control group ($P<0.05$) and increased the duration of lying flat behaviour ($P<0.001$). Exendin (9-39) antagonized the effect of GLP-1 (7-36) on feeding and drinking, and lying flat behaviour ($P<0.01$). None of the animals pretreated with exendin (9-39) exhibited emesis.
Discussion. The data suggests that the action of GLP-1 (7-36) in the brain is probably mediated via GLP-1 receptors. The studies were fully supported by a grant from the Research Grants Council of the Hong Kong SAR, China (Project no. UGC/FDS11/M02/15).
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523 Polyphenol reversal of amyloid-induced neurite dysfunction

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Introduction. The amyloid beta protein (Aβ) that accumulates in Alzheimer's disease induces neuronal death and neurite dysfunction. Potential anti-amyloid drugs have typically been tested for their ability to prevent cell death, but not reversal of neurite dysfunction. One such compound is epigallocatechin gallate (EGCG) due to its neuroprotective effects that reduce and prevent amyloid beta (Aβ) induced toxicity and cell death. Current research with EGCG primarily focusses on prevention of cell death in late stages of the disease while EGCG's potential to reduce or even prevent Aβ toxicity at the earlier stage of the neuronal dysfunction, has been largely ignored.
Aims and Hypotheses. This research aims to test EGCG's potential to prevent or reverse Aβ-induced neurite dysfunction and to test whether this can be achieved at lower concentrations than what is needed to prevent cell death. It is hypothesised that EGCG will prevent Aβ from hindering axonal growth and neurite development and stimulate the repair of dysfunctional neurites.
Methods. PC-12 Ordway cells were grown in Roswell Park Memorial Institute medium, under standard conditions. These cells were passaged every 3-4 days. Cells were then either plated into 96 well plates or onto coverslips in six well plates. Cell death was monitored in 96 well plates using the MTT assay. Cells on coverslips were exposed to Aβ (100nmol/L and 1µmol/L) and EGCG (20nmol/L and 200nmol/L) during development and differentiation for 7 days (O'Neil et al, 2016). Neurite number and length were initially counted manually and will be analysed later by image J.
Results. Preliminary results show that Aβ stops neurite formation at concentrations much lower than those that produce cell death (100nmol/L vs 1µmol/L). EGCG did not have any adverse effect on neurite outgrowth and we expect it will reverse the neurite dysfunction caused by Aβ.
Discussion. The finding that Aβ is more potent on neurite formation suggests that early intervention with Anti-amyloid drugs may be more effective than later. Compounds such as, EGCG may potentially slow the progression of the disease if used early.

O'Neil K et al (2016) Basic Clin Pharmacol Toxicol 120(4): 390-397¶

524 Endosomal trafficking kinetics of orexin receptors as measured by BRET trafficking assay.

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Introduction. Studies of the two orexin G protein-coupled receptor (GPCR) subtypes (OXR1 and OXR2) report differences in temporal interactions with β-arrestin-ubiquitin complexes, with OXR1 exhibiting transient interactions and rapid recycling relative to OXR2 (Dalrymple et al, 2011). These differing kinetics are likely reflected in divergences in spatial and temporal endosomal trafficking, however, no such comparative study has yet been conducted.
Aims. To investigate the temporal and spatial aspects of OXR1 and OXR2 trafficking.
Methods. Trafficking kinetics were investigated using a novel trafficking assay (Lan et al, 2012; Tiulpakov et al, 2016) with bioluminescence resonance energy transfer (BRET) technology. This assay allows temporal and spatial trafficking of a protein of interest to be observed through proximity to tagged compartment marker proteins. This assay was also used to construct dose-response curves.
Results. Statistically significant differences in BRET signals between OXR1 and OXR2 were observed in response to stimulation with 1 µM orexin A (OxA) for proximity to Rab4 (early endosome to recycling), Rab5 (early endosome) and Rab11a (recycling endosome) (Two-way repeated measures ANOVA of plateau, n=5, $p<0.05$). OXR1 exhibited an increased signal relative to OXR2 with Rab4, and a decreased signal relative to OXR2 with Rab5 and Rab11a.
Discussion. Subtle differences in the endosomal trafficking kinetics of OXR1 and OXR2 have been observed. These results increase our understanding of the molecular characteristics of OXR1 and OXR2, further illustrating molecular differences between the orexin receptor subtypes.

Dalrymple MB et al (2011) J Biol Chem 286:16726-16733.
Lan TH et al (2012) Traffic 13:1450-1456.
Tiulpakov A et al (2016) Mol Endocrinol 30: 889-904.¶

525 Splicing regulation and function of cytosolic sulfotransferase: SULT4A1

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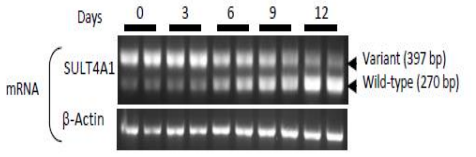
Introduction. In many tissues and cell lines, Sulfotransferase 4A1 (SULT4A1) is transcribed as an aberrant splice variant that does not translate into a functional protein. By contrast, neuron- and enterocyte-like cells splice SULT4A1 pre-mRNA into the protein coding wild type transcript. SULT4A1 protein is also known to regulate other co-expressed paralogue sulfotransferases (SULTs).



SULT4A1 switching pattern in SH-SY5Y cells with 12 days of RA treatment

Aims. To decipher the splicing events dictating the preferential expression of SULT4A1 protein and to understand the mechanisms of SULT4A1 regulation of other SULTs.
Methods. Human neuroblastoma cell lines SH-SY5Y and SK-N-MC were used as models in this study. Differentiation of cells was with 10 µM retinoic acid (RA) up to 14 days. A minigene splicing assay using a series of deletions and site-directed mutagenesis of SULT4A1 intronic sequences 5' and 3' of exon 6 were used to map splicing factor binding sites identified by bioinformatics. Expression patterns of endogenous SULTs and splicing factors were profiled by Western blot and semi-quantitative RT-PCR. SULTs activities were monitored using HPLC and MS techniques.
Results. Minigene deletion assays and overexpression assays in SH-SY5Y cells identified Muscleblind-like proteins (MBNL) and CUG-BP and ETR-3-like factor (*CELF*) proteins as potential regulators of SULT4A1 splicing. In line with SULT4A1 switching, increased nuclear translocation of MBNL proteins and cytosolic retention of CELF proteins were seen in SH-SY5Y cells with RA treatment. It was also found that SULT4A1 protein targets other co-expressed SULTs to the autophagosomal degradation pathway in SK-N-MC cells.
Discussion. The pattern of their intracellular compartmentalisation following RA-induced neuronal differentiation suggested MBNLs as primary regulators of SULT4A1 splicing, at least in this cell model. Our results also demonstrated the central role of SULT4A1 protein in modulating the function of other SULTs by influencing their stability in vivo. ¶

526 Diverse approaches to understand functional pharmacology- examples of the serotonin 5-HT1B and 5-HT2A receptors

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Introduction. Serotonin (5-hydroxytryptamine, or 5-HT) receptors mediate a wide range of physiological processes in the central and peripheral nervous systems. The serotonin receptor family is composed of both G-protein coupled receptors (GPCRs) and ligand-gated ion channel (LGIC) superfamilies. Serotonergic dysfunction has been implicated in many neuropsychiatric disorders. Due to the extensive distribution of serotonin receptors in the CNS and periphery, a complete understanding of the selectivity and the pharmacological actions of compounds of interest is critical.
Aims. In this poster, we present two examples involving the 5-HT1B and 5-HT2A receptor sub-types to demonstrate how the use of diverse pharmacology platforms can provide a more precise evaluation of compound/receptor interactions.
Methods. In the example of 5-HT1B receptor, we modulated the cAMP assay conditions to quantify reference compounds-induced changes of cAMP production. In the example of 5-HT2A receptor, we applied GTPgammaS, IP1 and calcium flux methods to detect % response or % inhibition patterns and potency changes of a series of reference compounds.
Results. In the example of 5-HT1B receptor, an increase of Na^+ in the assay buffer decreases constitutive activity in cellular models. It decreases agonist potency without affecting antagonist IC50s. In the example of 5-HT2A receptor, full or partial agonist-induced response patterns and potency vary by assay types whereas antagonists potency are relatively consistent among the three assay types and inverse agonism can only be revealed by GTPgammaS method.
Discussion. In order to have a complete understanding of the selectivity and the pharmacological actions of compounds of interest, an appropriate screening platform is extremely important in drug discovery. This poster shows the importance of carefully selecting models as pharmacological tools. A complete scanning of references in diverse formats to select ideal screening tools is also suggested to prevent assay type-dependent bias.
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527 Statins exhibit diverse effects on behaviour and cytokine levels in an in vivo model of LPS-induced neuroinflammation

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Introduction. The effect of statins in the CNS has received much attention in recent years, with recent *in vitro* studies implicating the reduction of neuroinflammation as a potential protective mechanism associated with statin use. Despite this, there is a lack of *in vivo* studies which explore the relationship with statins and neuroinflammation; hence many aspects of statins' effects, including comparisons between individual statins and the differences between short-term and long-term administration, remain unclear.
Aims. To compare the short-term and long-term effects of atorvastatin (ATO), pravastatin (PRA), rosuvastatin (ROS), or simvastatin (SIM) on lipopolysaccharide (LPS)-induced neuroinflammation *in vivo*.
Methods. C57BL/6J male mice were randomly assigned to one of seven groups (n=6). Each group received either saline or statin (equivalent max. human daily dose) over 3 or 21 days. At 24 h post-final treatment, 1.5 mg/kg LPS from *E. coli* (055:B5) was used *i.p.* to induce neuroinflammation and behavioural despair. Behavioural assays (open field test, forced swim test, tail suspension test) were performed within 3 h of LPS. Animals were sacrificed by cervical dislocation 3 h post-LPS. Serum & brain samples were tested for TNF-α and IL-1β concentrations by ELISA.
Results. After 3 days, PRA, ROS, and SIM (but not ATO) improved at least one aspect of LPS-induced behavioural deficit in the OFT, FST and TST ($P<0.05$). Only ROS showed improvement in all 3 tests ($P<0.05$). PRA, ROS and SIM decreased serum TNF-α ($P<0.01$). While 3 day ATO did not affect behaviour, it reduced brain IL-1β and TNF-α ($P<0.05$). After 21 days all statins improved behaviour vs. LPS, which correlated with reduced brain IL-1β ($P<0.01$). PRA decreased brain IL-1β to below baseline. Brain TNF-α only reduced in ATO, ROS and SIM ($P<0.05$).
Discussion. Our findings are the first *in vivo* evidence which support the notion that the neurocognitive effects of statins could be non-equivalent across the class. PRA, ROS & SIM reduced LPS behavioural deficit at 3 days, while ATO only showed significant effect at 21 days. Differences between behaviour and cytokine release profile statins may suggest anti-neuroinflammatory mechanisms differ between statins, and require further investigation. ¶

528 The role of caveolae in glioblastoma invasiveness

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Introduction. Glioblastoma (GBM) is the most common brain cancer. The average survival time for most patients is approximately one year after diagnosis. A major feature of GBM that contributes to its poor prognosis is its high invasiveness. Caveolae are plasma membrane subdomains that participate in numerous biological functions. Caveolin-1 and polymerase I and transcript release factor (PTRF) are both necessary for caveola formation. We hypothesized that high expression of caveola-forming proteins in GBM promotes invasiveness *via* modulation of the production of matrix-degrading enzymes.
Aims. (i) to investigate the relationship between mRNA expression of caveola-forming proteins and GBM aggressiveness in patients and (ii) to compare the proteolytic profile and invasion *in vitro* among GBM cell lines expressing, of devoid of, caveolin-1 or PTRF.
Methods. The mRNA expression of caveola-forming protein in GBM samples, and survival after stratifying patients according to caveolin-1 or PTRF expression, were analyzed from TCGA and REMBRANDT databases. The proteolytic profile of different cell lines was investigated using zymography and real-time qPCR. The knockdown of caveola-forming proteins was performed using small interfering RNA (siRNA) transfection. Invasion through basement membrane-like protein was investigated *in vitro* using Transwell™.
Results. Expression of both caveolin-1 and PTRF was increased in GBM compared to normal samples. High expression of caveola-forming proteins was associated with lesser survival time. GBM cell lines that formed caveolae expressed more urokinase-type plasminogen activator (uPA) and matrix metalloproteinases-2 (MMP-2) than GBM cells devoid of caveolae. Conversely, knockdown of caveolin-1 or PTRF in GBM cells decreased the expression of uPA and/or MMP-2.
Discussion. Caveolae may modulate GBM cell invasion by regulating uPA and MMP-2 expression. ¶

529 Air on a G-String: Guanine Oxidation as a Stress Sensor in Relation to Depression and Neurological Disorders

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Introduction. Aberrant BDNF (Brain-Derived Neurotrophic Factor) signalling has been implicated in depression and neurological disorders that are often associated with chronic inflammation and stress. Expression of the BDNF gene is up-regulated by H_2O_2, a ubiquitous signalling molecule. BDNF signalling stimulates the Keap1-Nrf2 transcription factor system, the master regulator of oxidative stress responses, which creates a pair of regulatory feedback loops. The guanine (G)-rich BDNF promoter has the potential to form G-quadruplexes (GQs). GQs are stacks of two or more sets of four co-planar Gs that usually exist in dynamic equilibrium with alternative stem-loop (SL) structures. Guanine (G) is the most readily oxidised of the canonical nucleobases, 8-oxoG being a major product. Oxidation of G residues can act as a molecular switch by changing the equilibrium between GQ and SL structures, which results in the recruitment of proteins that couple DNA damage repair to transcriptional responses. Re-setting molecular switches requires repair of oxidative 8-oxoG "lesions", which in this context are increasingly being recognised as epigenetic markers[1,2].
Methods and Aim. Various bioinformatics resources that are freely accessible *via* the internet were used to identify and analyse regulatory elements in the BDNF, Keap-1 and Nrf-2 genes, as well as other genes that are involved stress responses and maintenance of the intracellular G nucleotide pool, with the aim of devising a theoretical model.
Results. The BDNF proximal promoter contains a poly G tract homologous to putative redox-sensing poly G tracts that are located promoters of key stress response genes, most notably SOD2 (mitochondrial Superoxide Dismutase). G-rich sequences with the potential to form GQs in competition with alternative SL structures were identified in promoters of all relevant genes. Testable dynamic interaction models were generated from this information.
Discussion. BDNF expression is predicted to be controlled at the transcriptional level by an oscillatory redox sensing system involving cycles of G oxidation and repair-coupled transcription driven by GQ-SL transitions, for which the intracellular ratio of oxidised:reduced G derivatives is critical. The system is robust due to complex feedback and internal redundancy, but must fail when its capacity to remove 8-oxoG and/or supply (d)GTP is exceeded.
Fleming AM et al (2017) PNAS 114:2604-2609.B 2. Fleming AM & Burrows CJ (201) DNA Repair 56:75-83.¶

530 Behavioural, pharmacologic and histologic characterisation of a rat model of mechanical low back pain

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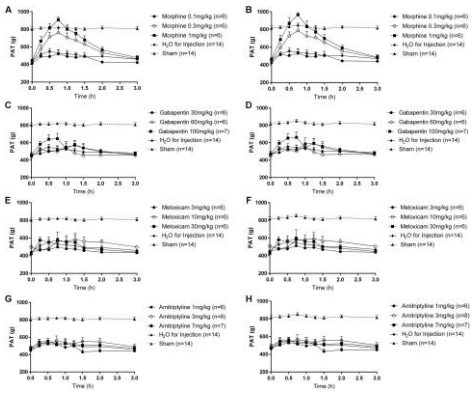

Introduction. Low back pain (LBP) is a common health problem affecting humans globally. Hence, I have used behavioural, histological and pharmacological methods to characterise an optimised rat model of mechanical LBP established at the CIPDD.[1]
Aims. To use behavioural, histological and pharmacological methods to characterise our new rat model of mechanical LBP.
Methods. Ten small punctures (0.5 mm o.d.; 2 mm deep) were induced in the L4/L5 and L5/L6 intervertebral discs (IVDs). Sham rats had the same surgery but there was no IVD puncture. Pressure algometry thresholds (PATs) at L4/5 and L1 were assessed. Additionally, paw withdrawal thresholds (PWTs) were measured in the bilateral hindpaws using calibrated von Frey filaments. PATs and PWTs were measured at weekly intervals until study completion. Dosing solutions of morphine (0.1, 0.3, and 1.0 mg/kg; sc), gabapentin (30, 60, and 100 mg/kg; ip), amitriptyline (1, 3, and 7 mg/kg; ip), meloxicam (3, 10, and 30 mg/kg; ip) and vehicle (2 mL/kg; ip) were administered to rats by the first person and testing was undertaken in a 'blinded' manner by the second person. Both LBP and sham rats were also characterised using histologic methods.
Results. Mechanical hyperalgesia developed progressively at L4/L5 and L1 in LBP-rats but not sham-rats. Importantly, PWTs remained unaltered for the study period. Histological analysis of the IVDs from LBP-rats showed an apparent loss of sharp boundaries between the nucleus pulposus and annulus fibrosus. In LBP-rats, single bolus doses of morphine produced dose-dependent relief of primary and secondary mechanical hyperalgesia in the lumbar axial deep tissues at L4/L5 and L1, respectively, whereas gabapentin, amitriptyline, meloxicam and vehicle were inactive.
Discussion. We have characterised a new rat model of chronic mechanical LBP using behavioural, pharmacologic and histologic methods.
[1]Muralidharan A, Park TSW et al (2017) Front Pharmacol 8:493¶

531 Morphine dosing affects development of antinociceptive tolerance and motor behaviour

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Introduction. Clinical development of antinociceptive tolerance after repeated administration morphine limits its chronic use. Despite growing knowledge about the molecular mechanisms of morphine tolerance, we know little about the influence of dosage regimen in its development.
Aims. We hypothesized that morphine dose, as well as dose increments, contribute to tolerance development. In addition, morphine-induced behavioural changes also might follow similar pattern of antinociception and tolerance.
Methods. Four groups of male Sprague Dawley rats received different daily doses of intermittent subcutaneous morphine for 14 days. After the development of antinociceptive tolerance, different increments of morphine doses were administered until tolerance redeveloped (Group A: 2.5 (b.i.d) → 5 → 10 mg/kg/day, Group B: 5 (b.i.d.) → 10 mg/kg/day, Group C: 5 (b.i.d.) → 15 mg/kg/day and Group D: 10 (b.i.d.) → 20 mg/kg/day). Antinociceptive responses were measured daily by tail-flick and hot-plate assays pre-treatment and at various post-treatment time-points. Motor behavioural effects were also measured using automated open-field paradigm and visual observations.
Results. Animals treated with lower starting-doses of morphine developed antinociceptive tolerance faster than those started on higher doses. Higher starting-doses and higher dose-increments after tolerance development resulted in more sustained antinociception and delayed the re-development of tolerance. These results were replicated by both anti-nociceptive assays and are therefore not assay-specific. The kinetics of morphine-induced motor suppression and desensitization were similar to those of antinociception and antinociceptive-tolerance respectively.
Discussion. These results suggest that morphine dosing regimen in rats significantly influences the manifestation of antinociceptive tolerance and the total antinociception (Paul et al., 2017). Our results also indicate that repetitive morphine dosing leads to desensitization of motor suppression in all major motor-behavioural parameters and manifests desensitization in conjunction with antinociceptive tolerance. Therefore, our results highlight that an optimized morphine dosing strategies can delay antinociceptive tolerance and reduce behavioural adverse effects.
Paul AK et al (2017) Neuropharmacology 121:158-166

532 Pharmacokinetics and metabolism of dabrafenib and trametinib in BRAF V600E/K metastatic melanoma

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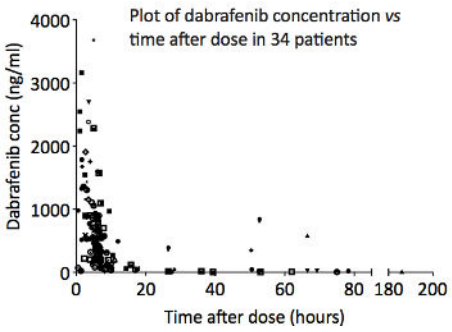
Introduction. Combination of BRAF inhibitor, dabrafenib and MEK inhibitor, trametinib (CombiDT) has improved survival outcomes compared with chemotherapy or BRAF inhibitor monotherapy in advanced BRAF V600E/K melanoma. However, the use of CombiDT has a high incidence of pyrexia, causing treatment delays (Menzies, 2015). The pharmacokinetics and metabolism of dabrafenib and trametinib may give clues relating to side effects such as pyrexia.



Aims. To measure plasma drug concentrations of dabrafenib and trametinib in melanoma patients treated with CombiDT.

Methods. Patients treated with CombiDT were recruited onto Neo Adjuvant Combi Trial (protocol ID: 200332). Their plasma samples were analysed for drug and metabolite concentrations using LC-MS. Standard plasma solutions were made for the range of 1ng/ml to 1000ng/ml for dabrafenib and 1ng/ml to 100ng/ml for trametinib. Vemurafenib was used as an internal standard.

Results. A total of 198 samples from 34 patients were analysed. Dabrafenib (4.0-3680ng/ml) and trametinib (1.0-66.2ng/ml) concentrations were measurable in 151 samples. Three dabrafenib metabolites (carboxy-, hydroxyl- and N-desmethyl-dabrafenib) were also measurable and showed a high degree of inter-patient variation.

Discussion. An analytical method was successfully established to measure dabrafenib, trametinib and metabolite concentrations. Further analysis using absolute metabolite concentrations, or more likely parent drug-metabolite ratio, may provide useful information regarding PK associations with toxicities such as pyrexia.

Menzies AM et al (2014) Annals of Oncology 26: 415–421.

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533 Optimisation of a meropenem plus tobramycin combination dosage regimen against hypermutable and non-hypermutable *Pseudomonas aeruginosa via* the hollow-fibre infection model and mechanism-based modelling

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Introduction. Hypermutable *Pseudomonas aeruginosa* are prevalent in patients with cystic fibrosis and rapidly become resistant to antibiotics when used as monotherapy. Antibiotic combinations are currently chosen empirically.

Aims. To optimise a combination dosage regimen against hypermutable and non-hypermutable strains utilising the dynamic hollow-fibre infection model (HFIM) and mechanism-based modelling (MBM).

Methods. The PAO1 wild-type strain and its isogenic hypermutable PAOΔ*mutS* strain ($MIC_{meropenem}$ 1.0mg/L, $MIC_{tobramycin}$ 0.5mg/L, for both) were assessed using 96h static concentration time-kill studies (SCTK) and 10-day HFIM studies (inoculum ~$10^{8.4}$ cfu/mL). MBM of SCTK data was performed in S-ADAPT to predict expected HFIM outcomes. Regimens studied in the HFIM were: meropenem 1g 8-hourly (0.5h infusion), meropenem 3g/day continuous infusion, tobramycin 10mg/kg 24-hourly (1h infusion) and both combinations; meropenem regimens delivered the same total daily dose. Time-courses of total and less-susceptible populations and MICs were determined.

Results. For PAOΔ*mutS* in the HFIM, all monotherapies resulted in rapid regrowth to >$10^{8.7}$ cfu/mL with near complete replacement by less-susceptible bacteria by day 3. Meropenem 8-hourly with tobramycin caused >7-log_{10} bacterial killing followed by regrowth to >6-log_{10} cfu/mL by day 5 and high-level resistance ($MIC_{meropenem}$ 32mg/L, $MIC_{tobramycin}$ 8mg/L). Continuous infusion meropenem with tobramycin achieved >8-log_{10} bacterial killing without regrowth. For PAO1, meropenem monotherapies suppressed bacterial growth to <4-log_{10} over 7-9 days, with both combination regimens achieving near eradication.

Discussion. As predicted by the MBM, an optimised meropenem plus tobramycin regimen was required to achieve synergistic killing and resistance suppression against the hypermutable *P. aeruginosa* strain when subjected to human pharmacokinetics in the HFIM, whereas both combination dosage regimens resulted in near eradiation of the non-hypermutable *P. aeruginosa* strain.¶

534 Evaluation of optimised piperacillin plus tobramycin combination dosage regimens against *Pseudomonas aeruginosa* (Pa) for patients with altered pharmacokinetics *via* the hollow fibre infection model and mechanism-based modelling

Rajbharan Yadav[1], Kate E. Rogers[1,2], Phillip J. Bergen[2], Jürgen B. Bulitta[3], Carl M. J. Kirkpatrick[2], Steven C. Wallis[4], David L. Paterson[5], Roger L. Nation[1], Jeffrey Lipman[4], Jason A. Roberts[4] and Cornelia B. Landersdorfer[1,2]. Drug Deliv, Dispos and Dynamics[1], Centre for Medicine Use and Safety[2], Monash Univ, Parkville, VIC, Australia; Dept of Pharmaceutics, University of Florida[3], Orlando, FL, USA; Burns, Trauma Crit Care Res Centre[4], Cent for Clin Res[5], Univ of Queensland, Brisbane, Australia.

Introduction. Augmented renal clearance (ARC) in critically-ill patients can result in suboptimal drug exposures and potential treatment failure.
Aims. This study aimed to design and evaluate optimised combination dosage regimens of piperacillin (PIP) and tobramycin (TOB) against a Pa clinical isolate in the hollow fibre infection model (HFIM) for patients with ARC.
Methods. We studied clinically relevant PIP and TOB concentrations, alone and in combinations in *in vitro* static concentration time-kills (SCTK), against a Pa clinical isolate at two inocula ($10^{5.7}$ and $10^{7.5}$ cfu/mL) over 72h. We optimised PIP + TOB regimens *via* mechanism-based modelling (MBM) of SCTK data. The effect of optimised PIP (4g q4h, 0.5h infusion) plus TOB (5 mg/kg q24h, 7 mg/kg q24h and 10 mg/kg q48h as 0.5h infusions) regimens on bacterial killing and regrowth was evaluated in the HFIM for patients with ARC (creatinine clearance 250 mL/min) over 8 days.
Results. PIP monotherapy (4g every 4h) in the HFIM provided 2.4 $\log_{10}$ killing at 13h followed by rapid regrowth at 24h with resistance emergence. TOB monotherapies displayed rapid initial killing (≥5 $\log_{10}$ at 13h) followed by extensive regrowth. The PIP + TOB dosage regimens were synergistic and provided ≥5 $\log_{10}$ killing with resistance suppression over 8 days in the HFIM.
Discussion. Optimised PIP + TOB regimens provided significant bacterial killing and suppressed resistance emergence as predicted by MBM, and therefore translated well from SCTK to the dynamic HFIM. This highlights the utility of MBM to select optimised regimens that maximise bacterial killing and minimise resistance emergence against Pa, an especially important finding given that Pa can rapidly develop MDR. Thus, these regimens are highly promising for effective and early treatment, even in the near-worst case scenario of ARC.¶

535 High-throughput assay for simultaneous quantification of the plasma concentrations of Omeprazole, Dextromethorphan, Midazolam, Losartan and their metabolites using liquid chromatography/tandem mass spectrometry (LC-MS/MS)

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Changes in pharmacokinetics of critical medications administered during surgeries involving cardiopulmonary bypass (CPB) have been reported. The impact of CPB on the activities of cytochrome P450 (CYP) enzymes is the key factor requiring further investigation. The rate of metabolism of dextromethorphan (DXM) to dextrorphan (DXR), midazolam (MDZ) to α-hydroxy midazolam (α-HM), omeprazole (OME) to 5-hydroxy omeprazole (5-HO) and Losartan (LOS) to EXP-3174 (EXP) are specific measures of *in vivo* CYP 2D6, 3A4, 2C19 and 2C9 activities, respectively. The aim of the study was to develop and validate a sensitive LC-MS/MS method to measure the concentrations of analytes of interest in plasma samples collected from patients to study the impact of CPB on the activities of major CYPs. Methods: To aliquots of human plasma (100 µL), internal standards (OME-d3 (10 ng/ml), DXM-d3 (50 ng/ml), LOS-d4 (40 ng/ml) and α-HM-d4 (10 ng/ml), 100 µL) and 10% methanol (MeOH) in 75mM ammonium acetate (AA) (pH=7, 100 µL) were mixed and loaded on solid phase extraction cartridges (Waters Oasis® HLB) which were then washed using 5% MeOH in 75mM AA pH=7 before elution with 20% propanol in MeOH. The samples were dried under the stream of nitrogen and reconstituted in 200 µL of 10% MeOH in AA pH=7. The mobile phase comprised 0.1% formic acid in water and 0.1% formic acid in acetonitrile with a flow rate of 0.4 mL/min using a 6.5 min run time. A C-18 XTerra® analytical column (Waters) was used and detection was performed using a QTrap 5500 mass spectrometer (AB SCIEX) with both positive & negative electrospray ionization. Results: The method demonstrated acceptable within-run and between-run precision and accuracy for all analytes of interest quality control samples (n=6, at 3 different days). Analytes were stable for 48 h in the autosampler, after 3 freeze-thaw cycles and after 6h at room temperature. The recovery was 88.6%-114.4% for all the analytes. Discussion: The fully validated high-throughput LC-MS/MS assay method using small volume of patients' blood (200 µL) in a relatively short run time met all validation requirements based on 2012 EMEA guideline on Bioanalytical method validation.¶

536 The influence of sampling time on estimated tobramycin exposure in cystic fibrosis patients

Yanhua Gao[1], Stefanie Hennig[1,2], Michael Barras[1,2] .[1]School of Pharmacy, University of Queensland, Brisbane, QLD, Australia, [2]Princess Alexandra Hospital, Brisbane, QLD, Australia.

Introduction. Current practice favours using the log-liner regression (LLR) method to estimate exposure (AUC) and subsequent dose adjustment of tobramycin in patients with cystic fibrosis (CF). This requires a minimum of two blood samples. Bayesian forecasting (BF) methods have been shown to be more accurate and precise in estimating the AUC; however the best sampling times for either method are unknown.
Aims. To investigate the influence of sampling time on the precision and accuracy of tobramycin exposure estimation and to determine the best sampling times.
Methods. Adult patients with CF, treated with once daily IV tobramycin, were intensively sampled to measure serum concentrations over one 24-hour dosing interval (50, 70, 100, 160, 280, 520 and 640 mins post-dose) to determine true exposure. AUCs were estimated using both LLR and BF methods with all combinations of sampling time points; 21 combinations per patient. All estimated AUCs were compared against the true exposure. The relative prediction errors (RE) were calculated, standardised to the true exposure.
Results. Twelve patients, with a median age and weight of 25 years and 66.5 kg respectively, contributed 84 tobramycin concentrations - a total of 504 estimated AUC combinations (LLR and BF methods). The average RE ranged from -35.4% to 34.5% for the LLR method and from -17.6% to 5.7% for the BF method. The most precise sampling time combinations were 100mins and 520mins for the LLR method and 70mins and 160mins for the BF method.
Conclusions. Sampling times markedly influence the precision and accuracy of AUC estimates and vary between methods. Overall, the change of sampling time impacts more on the precision and accuracy of AUC estimation with the LLR method. ¶

537 Tumour expression of copper transporters in colorectal cancer patients

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Introduction. Human copper transporters have been implicated in the transport of platinum-based anticancer drugs, including hCTR1, ATP7A and ATP7B. We have previously shown the positive contribution of hCTR1 to cellular uptake of oxaliplatin in hCTR1-overexpressing colorectal cancer cells, and the enhancement of oxaliplatin cytotoxicity by Cu chelators via hCTR1-mediated transport mechanism. However, information on endogenous expression of Cu transporters in tumour tissues of colorectal cancer is limited, but of clinical importance.
Aims. To quantify tumour expression of Cu transporters in tumour and matched normal colonic tissues of colorectal cancer patients.
Methods. Colorectal cancer patients were recruited based on pre-set eligibility criteria. Tissue specimens were collected to prepare paraffin-embedded sections for standard DAB immunohistochemistry. A semi-quantitative analysis was performed on de-convoluted DAB-stained images to measure histogram profile, score and percentage of positive hCTR1, ATP7A and ATP7B immunostaining.
Results. Eight patients aged 74 (65-86) had primary cancer at sigmoid, caecal and right colon. The immunoreactivity of hCTR1, ATP7A and ATP7B was limited to the adenocarcinoma. The percentage of hCTR1 positive staining in tumour tissues was 35 ± 4.9%, 41 ± 2.9%, 45 ± 1.8%, 74 ± 4.5, 53 ± 2.9% and 56 ± 3.7% in six patients. Higher tumour hCTR1 expression was detected than normal tissue in 2/6 cases. ATP7B immunostaining in tumour tissues was 69 ± 2.5%, 65 ± 1.4%, 55 ± 4.1%, 73 ± 4.3, 63 ± 4.5%, 69 ± 2.1% in six colorectal cancer patients. Higher tumour ATP7B expression was than normal tissue in 4/6 cases. ATP7A immunostaining in tumour tissues was 53 ± 1.9%, 40 ± 7.9%, 57 ± 3.5%, 55 ± 6.1, 62 ± 3%, 64 ± 5.4%. Higher ATP7B expression was detected in tumour than normal tissue in 2/6 cases.
Discussion. Cu transporters hCTR1, ATP7A and ATP7B are expressed in tumour tissues of colorectal cancer. There is no significant difference in hCTR1 or ATP7A expression between tumour and normal tissues in the majority of cases. ATP7B expression is lower in tumour tissues in most cases. Interpatient variability of expression of these transporters implies varying Cu demand and disposition in colorectal cancer, therefore, personalised strategy is needed for targeting Cu transporters. Supported by Royal Hobart Hospital Research Foundation and Cancer Council Tasmania.¶

538 Investigating the optimal initial dose of gentamicin in paediatric oncology patients considering efficacy and reduction in renal function

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Introduction. Selection of an optimal initial dose of gentamicin in paediatric oncology patients is challenging with patients often receiving long courses of this agent on multiple occasions, increasing the risk of nephrotoxicity.
Aims. This study aimed to estimate an optimal initial dose of gentamicin targeting specific efficacy endpoints while minimising the risk of renal function reduction.
Methods. Individual's gentamicin exposure was predicted using a population pharmacokinetic model[1] and bacterial killing was predicted using a semi-mechanistic pharmacodynamic model[2]. A utility function balancing probability of efficacy after the first dose and extent of reduction in renal function on day seven, if this dose was repeated daily for 7 days, was implemented in NONMEM® software. Different efficacy targets were considered: A maximal gentamicin concentration (C_{max}) to bacteria minimum inhibitory concentration (MIC) ratio of ≥ 10 and an area under the concentration-time curve from 0 to 24 hours post-dose (AUC_{24}) to MIC ratio of ≥ 70 – 100; and bacterial count reduction of 2-$\log_{10}$ Colony Forming Unit (CFU)/mL at 24-hours post-dose.
Results. An estimated initial dose of gentamicin of 7.1 mg/kg for bacteria with an MIC of 0.5 mg/L, 9.5 mg/kg for an MIC of 1 mg/L, 10.8 mg/kg for an MIC of 2 mg/L and 14.6 mg/kg for an MIC of 4 mg provided ≥ 75% probability of achieving C_{max}/MIC ≥ 10 and AUC_{24}/MIC ≥ 70 – 100. With an estimated dose of 12.8 mg/kg, 81.7% of patients achieved a 2-$\log_{10}$ bacterial count reduction at 24-hours post-dose. Under these different dosing scenarios reduction in renal function ranged on average from 6.9% to 14.5% on day seven.
Discussion. An initial gentamicin dose of 7.5 mg/kg/24 hours given in clinical practice may not achieve adequate efficacy for microorganisms with a MIC > 0.5 mg/L. With the highest dose estimated in this study (14.6 mg/kg/24 hours), 63.2% of patients had some reduction in their renal function on day seven. Therapeutic drug monitoring is recommended to individualised treatment after a high initial dose to ensure efficacy and minimize toxicity.

1. Llanos-Paez C.C (2017) AAC 61(8)
2. Mohamed A.F (2012) AAC 56(1):179-88.¶

539 Interaction of mangosteen extract and alpha mangostin with metformin in diabetic rats: PK/PD studies

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Introduction. Diabetes mellitus (DM) is a metabolic disorder disease and the first choice drug for type 2 diabetes is metformin. Nowadays, combining drugs with herbs becomes more popular, including for diabetic treatment. Mangosteen pericarp containing α-mangostin is one herb that shown antidiabetic effect.
Aims. To evaluate the pharmacokinetic and pharmacodynamics (PK/PD) interactions of mangosteen extract (ME) and α-mangostin (AM) with metformin (MFN) when used simultaneously in alloxan induced diabetic male rats.
Methods. Studies were performed on 5 groups of diabetic male rats (n = 5 for each group). Group I, II and III were single dose groups and given MFN (100 mg/kg BW), α-mangostin (37.2 mg/kg BW) and ME (248 mg/kg BW), respectively. Group IV and V were combination groups and given MFN & AM and MFN & ME, respectively, and group VI served as a control. MFN and AM levels in plasma were determined by HPLC and plasma glucose levels were determined by the GOD-PAP method. PK profiles and parameters of MFN and AM were calculated, % blood glucose decrease were calculated and dose-response curves were made.
Results. PK profiles of MFN followed 2 compartment model from either single or combination administrations, so did the AM from pure AM or EM administrations. Surprisingly, AM plasma levels could not be measured when AM given in combination with MFN (Group IV & V), while PD effects were observed. Based on AAC_{0-12}, MFN did not show significant change ($p>0.05$) in PD effects when combined with pure AM (Group IV vs I), interestingly significant changes ($p<0.05$) were observed when combined with EM (Group V vs I). AAC_{0-12} of Group IV and V increased when compared to Group II and III, these showed that AM increased the effects of MFN although the AM plasma concentrations could not be detected.
Discussion. Combination of MFN and EM showed a better hypoglycemic effects when compared to combination of MFN and AM, this could be due to other constituents in the extract that produced a potent hypoglycemic effects than AM itself. The current findings might lead to a benefit in using the mangosteen extract than the pure alpha mangostin.¶

540 Early prediction of chemotherapy efficacy in liver cancer cells by specific ROS levels

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Background: Liver cancer is the second most common cause of death from cancer worldwide. The most common type of primary and secondary liver cancer is hepatocellular carcinoma (HCC), and colorectal cancer liver metastases (CLM), respectively. Current assessments of chemotherapy responses for liver cancer require weeks to months, causing delay in instituting alternative chemotherapy regimens. Thus, there is an urgent need to predict the chemotherapy response at an early stage to improve liver cancer prognosis. It is known that levels of cellular reactive oxygen species (ROS) correlate with the aggressiveness of tumour cells and prognosis of patients. Cancer cells with increased endogenous ROS stress are more sensitive to anticancer agents and high levels of ROS generated by chemotherapeutic agents can induce cell death. Hence, ROS levels before and after chemotherapy in cancer cells can be an early indicator of treatment efficacy, which has the potential to shed new light on the chemotherapy.
Methods: In this study, the specific ROS (H_2O_2) levels were monitored and quantified before and after various concentrations of oxaliplatin in human colorectal cancer cells (HCT116) and mouse HCC cells (Hepa1-6). After different time points (30mins, 1, 2, and 3 hrs) post treatment by oxaliplatin, specific ROS detection probe (CM-H2DCFDA) was added to cells for detecting ROS levels by confocal microscopy (Olympus FV3000) at the excitation of 490 nm and emission wavelength of 520-560 nm. The fluorescence intensity of single cell in each group was measured and quantified by Image J. In the meantime, the viability and proliferation of these cells treated by the same concentrations of oxaliplatin were measured by MTT cell proliferation assay. Finally, the changes of specific ROS levels was correlated to cell viability after chemotherapy.
Results and conclusion: H_2O_2 levels of HCT116 cells was significantly increased from 1 hrs after oxaliplatin treatment compared to control untreated group, while Hepa1-6 did not show obvious increase of H_2O_2 levels after oxaliplatin treatment. In addition, the higher levels of H_2O_2 within cells in 3 hrs correlated well with greater inhibition of oxaliplatin after 24 hrs. These results indicated oxaliplatin has better treatment response in HCT116 cells, and ROS levels could be an indicator for early prediction of chemotherapy efficacy in CLM.¶

541 Drug delivery to the intestinal lymphatics enhances the immunosuppressant effects of mycophenolic acid in mice.

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Introduction: The lymphatic vessels that drain the intestine, the gut associated lymphoid tissue (GALT) and the mesenteric lymph nodes (MLN) are central to gut immune surveillance. The intestinal lymphatics also serve to transport dietary lipids (triglycerides, TGs) from the gut to the systemic circulation (Trevaskis et al 2015).
Aim: To evaluate the pharmacodynamic benefit of targeting an immunomodulatory agent (mycophenolic acid, MPA) to gut lymphatic immune cells by mimicking the endogenous transport pathway of TGs into the lymph. This was achieved via the design of a TG mimetic prodrug of MPA (MPA-2-TG) (2) to target lymphocytes in intestinal lymph.
Methods: The intestinal lymph transport of MPA and MPA-2-TG, was assessed after intraduodenal infusion, by cannulating the mesenteric lymph duct of anaesthetised mice (100 mg/kg ketamine and 10 mg/kg xylazine, ip). Immunosuppression was studied by adoptive transfer of dye labelled CD8+ T cells, purified from lymph nodes (LN) from OT 1 mice, into syngeneic mice fed 50 mg ovalbumin (OVA). Mice were then administered MPA or MPA-2-TG (50 mg/kg) twice daily for 3 days. At the end of the treatment, T cell proliferation in mesenteric and peripheral LNs was evaluated using flow cytometry.
Results: The lymphatic uptake of MPA-2-TG (17.3 % dose) was higher than MPA (0.14 %). MPA-2-TG treatment significantly reduced the proliferation of CD8+ T cells in the MLN, with most dye labelled cells (~80%) being found in generation 4 or lower after OVA stimulation. In contrast, MPA had no significant effect on cell replication.
Discussion: Targeting lymphocytes in intestinal lymph, via the use of a lipid-mimetic prodrug significantly enhanced the immunosuppressive effects of MPA. This approach may have the potential to enhance the pharmacodynamic benefit of other drugs, such as cytotoxic or immunomodulators that act within the mesenteric lymphatics and MLN.

1. Han S et al (2014) J Control Release 177:1-10.
2. Trevaskis NL et al (2015) Nat Rev Drug Discov 14:781-803.¶

542 Polymer precipitation inhibitors can maintain drug supersaturation and increase in vivo absorption from lipid-based formulations

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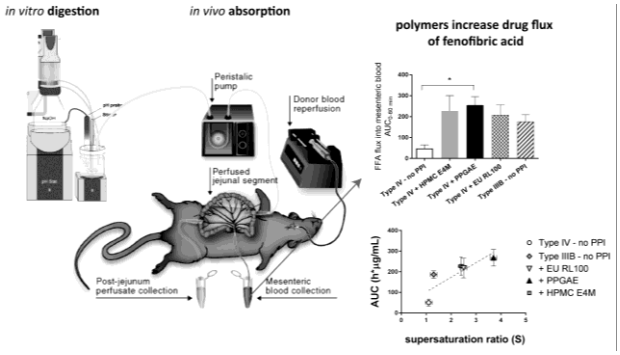

Introduction. Lipid-based formulations (LBFs) have emerged as a promising formulation strategy to overcome the issue of solubility-limited absorption, thereby improving the oral bioavailability of poorly water-soluble drugs (PWSDs). After oral dosing, supersaturation often arises with the potential for drug precipitation. To stabilize the metastable supersaturated state, polymer precipitation inhibitors (PPIs) may be added to LBFs to inhibit drug precipitation, potentially resulting in increased drug absorption.
Aims. The current project is exploring the solubility-supersaturation-absorption relationship when using PPIs in LBFs, by measuring drug flux in an *in vivo* experimental model.
Methods. A coupled *in vitro* digestion - isolated rat jejunum model, has been employed to evaluate in real time the impact of PPIs on drug flux. Fenofibrate and saquinavir were chosen as model PWSDs.
Results. Addition of selected PPIs prolonged supersaturation and led to increases in fenofibric acid absorption of up to ~ 4-fold. Reasonable correlation was evident between the degree of supersaturation and drug flux suggesting that increases in the intraluminal free drug fraction were driving increased absorption.
Discussion. This work demonstrates the utility of the coupled *in vitro* digestion-*in vivo* absorption model in developing a better understanding of drug absorption from polymer-containing LBFs. The data suggest that PPIs can support prolonged drug supersaturation and that this results in improved absorptive drug flux *in vivo*.¶

543 The effects of aging on polarization in collagen sandwich-cultured hepatocytes.

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Introduction. Hepatocytes have a unique polarized phenotype where apical domains of adjacent cells make up a tubular structure, known as bile canaliculus. This polarized morphology is important for hepatocyte function and viability. Loss of polarity can result in excessive accumulation of bile, toxins and metabolites that can lead to hepatocellular damage such as seen in drug-induced hepatotoxicity and liver diseases such as cholestasis, fibrosis and cirrhosis. Ageing is associated with increased susceptibility to impaired hepatic function, which can increase the risk of adverse drug reactions that are associated with hepatotoxicity and liver disease. However, the effect of ageing on hepatocyte polarization is unknown. Using collagen sandwich cultures of hepatocytes, we compared the reestablishment of hepatocyte polarization in isolated hepatocytes from young and old mice.
Methods. Hepatocytes were freshly isolated from young (3 months) and old (24 months) C57BL6 male mice and cultured in a collagen sandwich configuration. Polarization was assessed every 12 hours over 72 hours using lipid droplet staining and immunofluorescence of apical protein ATP-binding cassette sub-family B member 1 and tight junctional protein Zonula occludens-1. ATP levels were also quantified.
Results. Immunofluorescence revealed that hepatocytes from old mice polarized at a faster rate than young hepatocytes. Furthermore, there were significantly more and larger lipid droplets in the hepatocytes of old mice from the beginning of hepatocyte polarization. Lipid droplets remained large in old hepatocytes after 60 hours even after the formation of the bile canalicular network. In young mice, the reduction of lipid droplet numbers was evident after 24 hours. Polarization is an energy-dependent cellular process. ATP levels rapidly peaked within 24 hours in old hepatocytes whereas in young hepatocytes levels increased more slowly and peaked after 48 hours.
Discussion. Hepatocytes from old mice polarize and accumulate ATP more rapidly than young hepatocytes. These changes might contribute to age-related changes seen in hepatic function and susceptibility to drug-induced hepatotoxicity and liver diseases such as fatty liver. ¶

544 Tachykinin NK_2 receptor expression in the human colon; an insight into the influence of gender, age and disease

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Introduction. Neurokinin A (NKA) released from enteric neurons contracts intestinal smooth muscle via tachykinin NK_2 receptors (NK_2R). We have previously reported gender differences in the abundance of [^{125}I] NKA binding sites, and in the potency of the NK_2R antagonist ibodutant, in control human colonic smooth muscle (Burcher et al., 2008, Drimousis et al., 2016). We have also shown that NK_2R mRNA expression is downregulated, and contractility to NKA is reduced in the colonic smooth muscle of patients with diverticular disease (DD) (Burcher et al., 2008, Liu et al., 2011).
Aims. This study aimed to determine the existence of any gender differences in the cellular distribution and density of the NK_2R in the human colon in control and DD.
Methods. In human colon specimens, fluorescent immunohistochemistry and Western blot were conducted to localise and quantify the NK_2R protein expression.
Results. NK_2R immunoreactivity was densely expressed in the colonic smooth muscle layer, where it is colocalised with cell markers for smooth muscle (α-smooth muscle actin), nerve cell bodies (Hu), neurons (β-tubulin) and glial cells (S100). There were no obvious differences between genders, and between control and DD. NK_2R protein expression in control colonic smooth muscle was slightly higher in females compared to males (P = 0.066). While no age-related differential expression was observed in males, there was an increased expression with age in females (r = 0.61, *P = 0.010), which was particularly noticeable in specimens older than 50 years of age compared to younger ones (*P = 0.0485). There was a significant increase in NK_2R protein expression in DD muscle (1.3 fold higher than its age- and gender-matched control counterpart, ***P = 0.0005).
Discussion. Increased NK_2R protein expression arises in females at the average onset of menopause, suggesting that sex hormones may influence NK_2R expression. In DD, negative feedback may explain the discrepancies between NK_2R mRNA and protein levels.
Burcher E et al. (2008) J Pharmacol Exp Ther 324:170-8
Drimousis S et al. ASCEPT-MPGPCR conference abstract, 27-30 Nov 2016, Melbourne
Liu L et al. (2011) Neurogastroenterol Motil 23:475-83¶

545 Antibiotic Guidelines for Urinary Tract Infections (UTI) and Hospital Length of Stay

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Introduction. UTIs and pyelonephritis accounted for 73300 separations Australia wide and 7365 hospitalizations across WA hospitals in 2014 to 2015. This in an audit of inpatient antibiotic treatment and its potential effect on hospital average length of stay (ALOS).
Aims. To determine whether compliance with antibiotic stewardship guidelines impacts on ALOS for patients treated for kidney and urinary tract infections with catastrophic or severe case complexity by the Department of Geriatric Medicine at a major metropolitan tertiary hospital.

Table 1. Antibiotic Prescribing and Compliance with Guidelines

	Number of cases (total n=20)	Percent of total cases (%)
Antibiotic compliant with guidelines	11	55%
Antibiotic not compliant with guidelines	9	45%
Total	20	100%
Reasons for non compliance		
Incorrect drug	2	10%
Incorrect drug combination	3	15%
Incorrect dose	1	5%
Incorrect frequency	2	10%
Total	8	40%

Methods. Audit medical records of patients discharged between 1st July 2016 and 30th December 2016 in Geriatric Medicine at Royal Perth Hospital for ICD-10-CM primary diagnosis of Australian Refined Diagnosis Related Group (AR-DRG) L63A: Kidney and Urinary Tract Infections with Catastrophic or Severe Case Complexity. Review the compliance of antibiotic prescription with "Therapeutic Guidelines: Antibiotic Guidelines".
Results. There were 20 admissions. 6 males (30%) and 14 females (70%), ages ranging from 65 to 98 years of age, 70% were over the age of eighty. Urinary catheter in situ on admission was 4 out of 20, 2 were male and 2 were female. 19 patients had a mid-stream urine collected during their admission. 13(68.4%) had their specimen collected before commencing their first dose of antibiotics. Of these 13, 9 had a pathogen identified and antibiotic sensitivity confirmed (69.2%). For the 6 specimens collected after commencing antibiotics, 3 had a pathogen identified and antibiotic sensitivity confirmed (50%). There was no significant difference between compliant vs noncompliant groups (Table 1) for ALOS variance (p=0.38). There was a significant difference in LOS variance (p=0.03) for parenteral vs oral antibiotic used between the 2 groups.
Discussion. In a group of older patients with multiple comorbidities and social issues non-medical factors play a significant role in ALOS. However, the use of oral vs parenteral antibiotics compliant with therapeutic guidelines for severe kidney infections or UTIs have an effect on ALOS for the older patients. ¶

546 Age-related variations in porcine bladder responses to clinical antimuscarinics.

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Introduction. Of all patients prescribed antimuscarinic therapy for overactive bladder, those aged under 60 are more likely to discontinue treatment earlier than their older counterparts. The cause of this reduced adherence to treatment regimens is unclear, however may be attributed to either lifestyle changes or age-related physiological changes, with the latter being of particular interest.
Aims. This project aims to investigate the influence of ageing on contractile responses of the urinary bladder detrusor and U&LP (urothelium and lamina propria) tissue layers in response to clinically prescribed antimuscarinics.
Methods. Porcine U&LP or detrusor strips from either juvenile bacon-pigs or aged sows were mounted in gassed Krebs-bicarbonate solution at 37°C and carbachol concentration-response curves were recorded in the presence and absence of selective muscarinic antagonists. Data obtained was analysed using paired Student's *t*-tests, with $P<0.05$ being significant.
Results. Aged U&LP demonstrated a greater contraction to carbachol, with peak contractions of 7.49±0.84g being reached at 78mM (n=8) compared to juvenile tissue at the same concentration 4.50±0.63g (78mM, n=7). Aged detrusor also exhibited a heightened contraction to carbachol (21.55±2.48g, 780mM, n=8) than the juvenile samples (14.85±2.90g, 78mM, n=8). In both detrusor and U&LP tissues, the presence of 4-DAMP (10nM, n=8), oxybutynin (1µM, n=7) and tolterodine (1µM, n=8) significantly inhibited the contraction to carbachol ($P<0.05$ for all), and this inhibition was more effective in aged samples, when compared to juveniles ($p<0.05$ for all).
Discussion. A greater response to carbachol was observed in aged U&LP and detrusor tissues, compared to younger samples. 4-DAMP, oxybutynin and tolterodine inhibited these contractions to carbachol, with an increased effectiveness in inhibiting responses of aged tissue compared to juvenile samples. This suggests that the observed increased persistence for overactive bladder treatment regimes in older adults may be attributed to a heightened effectiveness of antimuscarinic therapy.

547 Histamine receptors as regulators of urothelial and detrusor contractile activity

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Introduction. The mechanisms underlying bladder contractile disorders, such as overactive bladder, are not fully understood. It is apparent that acetylcholine release is involved, however, other mediators and regulator chemicals may also have a potential influence. As such, there is a particular interest in isolating which receptors, other than muscarinic, are involved in modulating spontaneous contractile activity. Aims. This study aimed to identify the specific influence of the H1, H2, H3 and H4 histamine receptors on urothelial and lamina propria (U&LP) contractile activity. Methods. Strips of porcine U&LP and detrusor were mounted in gassed Krebs-bicarbonate solution at 37°C and responses to histamine obtained in the absence and presence of selective antagonists. Data analysis of the responses was performed using paired Student's *t*-tests. This project was supported by the Australian Bladder Foundation.

		ΔTension (g)		ΔFrequency (cpm)		
Antagonist	Conc.	Absence	Presence	Absence	Presence	n
Pyrilamine	30nM	0.47±0.11	0.11±0.08*	1.32±0.38	0.01±0.21*	8
Cimetidine	1µM	1.32±0.56	1.84±0.65*	1.48±0.63	1.41±0.94	8
Thioperamide	1µM	1.20±0.29	1.75±0.29*	1.49±0.63	1.76±0.58	6

Results. The table shows U&LP responses to 100µM histamine in the absence and presence of selective antagonists. The U&LP contracted in the presence of histamine by 1.14±0.3g and the frequency of spontaneous contractile activity was increased by 1.53±0.38 cycles min-1 (cpm, 100µM, n=26). Pyrilamine (30nM, H1 antagonist) inhibited these contractile responses ($p<0.01$, n=8) and increases in spontaneous contractions ($p<0.01$, n=8). In the presence of cimetidine (1µM, H2 antagonist) maximal contractions to histamine were enhanced ($p=0.05$, n=10). Although thioperamide (1µM, H3 and H4 antagonist) initially showed a significant increase in contractions ($p=0.03$, n=6), selective antagonism revealed no influence of H3/H4. In detrusor preparations, H1 receptors were responsible for the majority of the contraction to histamine ($p<0.05$, n=8), with no influence from antagonism of H2,H3 or H4. Discussion. Histamine produces both a contractile and relaxation response in the U&LP. The contraction appears mediated by the H1 receptor, while relaxation is mediated by the H2 receptor. Preliminary data presents these receptors as potential targets in future therapeutic treatments for overactive bladder or other bladder contractile diseases. ¶

548 β-adrenoceptors in the urinary bladder vasculature

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Introduction. Ischaemia of the bladder has been suggested to play a role in the etiology of bladder dysfunction (Bayrak et al., 2015). Mirabegron, the newest treatment available to treat bladder dysfunction, mediates relaxation via an action on β_3-adrenoceptors in the bladder detrusor smooth muscle (Igawa & Michel, 2013). Whether this action on β_3-adrenoceptors is also seen in the bladder vasculature is unknown.
Aims. The aim of this study was to investigate whether β3-adrenoceptors are involved in relaxation of the bladder vasculature.
Methods. Rings of superior vesical artery (SVA) from 6-month old pigs were mounted in organ baths. Tissues pre-contracted with KCl (60mM) were relaxed with a β-adrenoceptor agonist, either isoprenaline (non-selective), salbutamol (β_2), CGP-12177A (partial β_3) or mirabegron (β_3), in the presence of phentolamine (10µM) and L-NNA (100µM). Isoprenaline relaxations were also performed in the presence of the β-adrenoceptor antagonists propranolol (non-selective), CGP-20712A (β_1 selective), ICI-118, 551 (β_2 selective) and SR-59230A (β_3 selective).
Results. Isoprenaline relaxed the SVA with high potency (pEC_{50} 7.6±0.14, n=6) and a maximum relaxation of 57.1±3.2% of the KCl pre-contraction. Salbutamol, CGP12177A and mirabegron also induced relaxations with high potency (pEC_{50} 7.45±0.2, 7.94±0.18 and 7.93±0.16 respectively), although maximum relaxations were significantly ($P<0.05$) smaller than for isoprenaline (salbutamol, 23.7±1.9%; CGP12177A, 20.3±1.2%; mirabegron, 20.7±1.1%; n=7). Propranolol (10 & 100nM), CGP-20712A (3µM), ICI-118, 551 (3nM) and SR59230A (10nM) antagonised isoprenaline relaxations with high affinity (pKB 9.52, 9.26, 8.28, 12.14 and 8.87 respectively, n=3-6).
Discussion. This study demonstrates that all three β-adrenoceptor subtypes mediate relaxation of the superior vesical artery and confirms that β3-adrenoceptors are present within the bladder vasculature. Mirabegron can relax these blood vessels and this action may contribute to the efficacy of this new treatment for bladder dysfunction.

Bayrak S et al. (2015) Naunyn-Schmiedeberg's Arch Pharmacol. 388: 47-54.
Igawa Y & Michel M (2013) Naunyn-Schmiedeberg's Arch Pharmacol. 386: 177-183¶

549 Ethanol as an intravesical vehicle: effects on bladder function

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Introduction. Capsaicin is usually dissolved in 30% (v/v) ethanol when used intravesically to treat some refractory bladder conditions. Previous work examining capsaicin in the bladder using 30% ethanol as a vehicle control reported that the ethanol was just as irritating as the treatment.
Aims. Our objective was to investigate the effects of luminal ethanol on bladder function.
Methods. 30% ethanol (in 0.9% saline) was applied to the luminal surface of porcine bladders for 30 minutes. Matched control tissues (treated with 0.9% saline) were also assessed. Treatment medium was assayed for ATP and Ach release, contractile responses of isolated tissue strips were recorded and tissues were compared histologically.
Results. Histological examination revealed significant thinning of the urothelium occurred after treatment (control =69.4±6.36µm v 30%=13.3±1.51µm, n=6, $p<0.0001$). Treatment medium contained significantly enhanced urothelial ATP (control=0.01±0.004µM v 30%=1.68±0.22µM, n=8, $p<0.0001$) and depressed Ach release (control=2.61±0.40µM v 30%=0.24±0.22µM, n=8, $p<0.0001$). Maximal contractile responses to carbachol were significantly greater in urothelial (control=31.33±2.74mN v 30%=41.14±3.47nN, n=7, $p<0.046$) and detrusor tissues (control=66.91±4.06mN v 30%=92.81±2.13mN, n=7, $p<0.0002$) treated with 30% ethanol. Neurogenic responses were significantly larger at maximum stimulation (control=56.93±9.99mN v 30%=119.10m±16.31mN, n=7, p= <0.006).
Discussion. Based on our study, 30% ethanol used as a vehicle produces significant urothelial damage, altered release of mediators and enhanced contractile activity. The damage and enhanced activity should be taken into consideration as part of treatment side effects.

550 Prazosin but not tamsulosin sensitises PC-3 and LNCaP prostate cancer cells to docetaxel

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Introduction: Docetaxel is currently the first-line chemotherapeutic agent available for the treatment of patients with advanced prostate cancer. While docetaxel has been shown to modestly improve survival times for patients; they also experience significant docetaxel-induced toxicities. If treatment failure occurs there is currently no effective alternative and therefore there is an urgent need for adjunct therapies. Some quinazoline-based α1-adrenoceptor (ADR) antagonists have previously been shown to have cytotoxic actions in prostate cancer cells, but there is no research into their effects on docetaxel-induced toxicity.
Aims: The aim of this study was to determine if the quinazoline ADR, prazosin altered sensitivity of prostate cancer cells to docetaxel *in vitro*.
Methods: PC-3 and LNCaP cells were pre-treated (1hr) with prazosin (30µM) or tamsulosin (30µM), followed by docetaxel (12.5µM to 100µM, 24hrs). Docetaxel-induced toxicity was measured in terms of changes in cell proliferation (resazurin reduction), autophagy (monodansylcadaverine staining) and apoptosis (caspase-3 activity) and the production of reactive oxygen species (2'7'-dichlorofluorescein diacetate fluorimetry).
Results: Prazosin sensitised both cell lines (PC-3 and LNCaP) to docetaxel-induced toxicity. This effect appears to be mediated by autophagy and may also involve apoptosis. These sensitising effects of prazosin appear to be largely independent of reactive oxygen species production. In contrast, tamsulosin did not effect docetaxel-induced toxicity.
Discussion: We have shown, for the first time, that prazosin increases docetaxel-induced toxicity in PC-3 and LNCaP cells. Prazosin may therefore offer a viable treatment option in combination with docetaxel in metastatic prostate cancer.
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551 Purinergic P2X7 receptor antagonist A804598 reduces the damage induced by acrolein in *ex-vivo* porcine bladders

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Introduction. Cyclophosphamide (CYP) is an antineoplastic agent commonly used in chemotherapy. Acrolein, the highly toxic metabolite of CYP, excretes into urine causing severe bladder cystitis. The purinergic P2X7 receptor (P2X7R) has recently come into attention for its role in inflammation. P2X7R is highly expressed by many immune cells and regulates the expression and secretion of various cytokines such as IL-1β and TNF-α.
Aims. In this study, we aimed to develop an *ex-vivo* model of urothelial inflammation by perfusing porcine bladders with acrolein, and to determine if urothelium inflammation affects bladder activities. We also aimed to investigate if P2X7 antagonism can protect against acrolein-induced inflammation and damage in the bladder.
Methods. The whole bladders (n=9) from 2 month-old porcine were placed in 100-ml organ baths containing Krebs-Henseleit solution maintained at 37°C and perfused for 4 hours with carbogenated RPMI culture media (in the presence or absence of 0.05% acrolein) via a fine tube that was inserted into the bladder from urethral orifice. After 4 hours perfusion, each bladder was dissected into intact, detrusor and mucosa strips and their contractility in response to acetylcholine (ACh) were measured. Histology staining was performed to determine the degree of tissue damage.
Results. ACh contracted intact, detrusor and mucosa strips in a concentration-dependent manner in fresh and perfusion control bladders. In acrolein-treated strips, the contractile responses to ACh were significantly diminished in intact strips and completely abolished in mucosal strips. Pre-treating bladders with the P2X7R antagonist A804598 (at 10 µM for 1 hour) significantly reversed acrolein-induced reduction in response to ACh. The histology staining showed that acrolein caused substantial damage to the urothelial and suburothelial layers.
Discussion. In this study, we have established an *ex-vivo* inflammatory model in the porcine bladder, which is a good model for the study of the human bladder. The damage to the muscle bundles and myofibroblasts in the mucosa layer by acrolein is likely to account for the reduced contractile response of the intact and mucosa strips to ACh. The protective effect of A804598 has provided strong evidence that P2X7R plays an important role in bladder inflammation and indicated that an inhibition of P2X7R activities could be a pathway for the treatment of bladder inflammation, and could potentially be co-administered with CYP for chemotherapy.¶

552 Histamine receptor (Hrh) subtypes mediate bladder afferent sensitivity in mice

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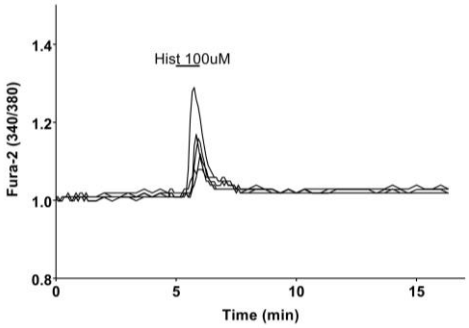

Introduction: Pelvic pain conditions such as overactive bladder syndrome and interstitial cystitis are associated with enhanced bladder sensation, leading to the symptoms of frequency, urgency and pain. Histamine, released from activated mast cells, is a key mediator of neurogenic inflammation and pain in the bladder and other visceral organs. However, the exact role and distribution of histamine receptor subtypes (Hrh1-4) in bladder sensory structures is unknown.
Aims: To determine the expression and function of histamine receptors in bladder sensory structures.
Methods: RT-PCR was performed on primary urothelial cells and mucosal and detrusor layers of mouse bladders. Retrogradely labelled bladder DRG neurons from mice were isolated and dissociated for single-cell RT-PCR and calcium imaging. *Ex-vivo* bladder afferent recordings determined bladder mechanosensitivity.
Results: RT-PCR revealed mRNA expression of Hrh1-3 in dissociated urothelial cells, and 10-fold higher expression in bladder mucosal and detrusor tissue. Hrh4 mRNA expression was 1000-fold lower in both cells and tissues. Single cell PCR data identified Hrh1 mRNA expression in 29% of bladder afferent neurons whilst histamine (100µM) induced significant calcium transients in 18% of bladder DRG neurons. Histamine (300µM) perfused into the bladder lumen induced mechanical hypersensitivity to bladder distension versus saline ($p<0.01$, n=6) which was attenuated by Hrh1 antagonist pyrilamine (100uM) and completely abolished by combined Hrh1 and Hrh4 antagonists.
Discussion: Histamine receptors are present and functional in bladder sensory structures, and their activation is able to induce calcium transients in isolated bladder neurons and enhance bladder mechanosensitivity to distension. This work provides valuable insight into the action of histamine, and the role of histamine receptors in the bladder, unravelling potential mechanisms of pelvic pain pathology.¶

553 Characterization of Na_v Channels in Colon-Innervating Dorsal Root Ganglion Neurons in Mice

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Chronic visceral pain is a poorly managed symptom of functional and inflammatory gastrointestinal disorders and there is a lack of analgesics that are efficacious without gastrointestinal side effects. Voltage-gated sodium (Na_v) channels regulate action potential generation and cell membrane excitability in sensory neurons, and they are implicated in several pain or loss-of-pain phenotypes in humans, which has inspired investigation into the therapeutic potential of Na_v channel modulation. In this study, we show that Na_v channels and their auxiliary β-subunits are abundantly expressed in dorsal root ganglia (DRG) neurons at thoracolumbar (TL) and lumbosacral (LS) levels from C57BL/6J mice, and heterogeneously expressed in colon-innervating DRG neurons. Using retrograde labeling and whole-cell patch clamp electrophysiology, we found that colonic TL and LS neurons exhibited comparable peak sodium current densities (TL: –894 pA/pF, n = 23; LS: –883 pA/pF, n = 14), however, colonic TL neurons were significantly less excitable compared to colonic LS neurons (rheobase: TL: 183 pA, n = 32; LS: 85 pA, n = 22. p = 0.0143). The Na_v channel blocker tetrodotoxin (TTX, 100 nM) significantly increased the minimum current required to fire an action potential in colonic TL and LS neurons, however, sodium current densities in colonic TL neurons were less affected by TTX compared to colonic LS neurons (TL: 50% reduction, n = 14; LS: 70% reduction, n = 8).

In conclusion, voltage-gated sodium channels and auxiliary β subunits are highly abundant in whole DRG and colonic DRG from T10–S1 spinal levels. However, TTX-S channels may have differing contributions to colonic DRG neurons innervating the thoracolumbar versus lumbosacral regions, which may underlie their differing functions.

554 Pharmacological effects of a jungle ginger on rat prostatic smooth muscle

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Introduction. Jungle ginger has been traditionally used by Sarawak natives to treat urological disorders. Since drugs that relax prostatic smooth muscle are used to manage urinary symptoms associated with urological disorders.
Aims. To assess the pharmacological effects of a jungle ginger on prostate contractility and to isolate its bioactive components.
Methods. This is original work reporting the biological effects of jungle ginger on isolated rat prostate contractility. Jungle ginger rhizome, roots, leaves and stem were harvested from Sarawak. Extracts of dried and ground plant materials were extracted using water at room temperature. Activity of these extracts was evaluated pharmacologically by assessing their effects on contractions of isolated rat prostate gland maintained in a modified Krebs solution at 37ºC and bubbled with carbogen gas. Nerve mediated contractions were evoked electrically (0.1-20 Hz, 0.5 ms pulse duration, 60 V) while direct muscle stimulation was achieved by application of the exogenously administered agonists. Pharmacological tools were used to identify mechanisms of action.
Discussion. Jungle ginger rhizome ($p=0.0004$, n=6), root ($p<0.0001$, n=6) and stem ($p=0.0057$, n=6) extract inhibited electrical field stimulation (EFS) induced contractions of rat prostatic smooth muscle, while leaf extract did not exhibit bioactivity ($p=0.0988$, n=6). Contractions mediated by exogenous administration of noradrenaline (1 nM-1 mM, n=6), acetylcholine (1 nM-1 mM, n=6) or ATP (0.3 uM-1 mM, n=6) were not inhibited by rhizome extract. Tyramine (10 nM-0.1 nM) induced contractions were also not effected by the rhizome extract (n=4). EFS-induced contractions were still attenuated by the rhizome extract in the presence of prazosin (300 nM, n=6), suramin (30 nM, n=6), yohimbine (1 µM, n=6), idazoxan (1 µM, n=6), propranolol (1 µM, n=6), atropine (1 µM, n=6), methysergide (1 µM, n=6), mepyramine (1 µM, n=6), hexamethonium (10 µM, n=6), desipramine (100 nM, n=6), 8-phenyltheophylline (10 µM, n=6), and AH6809 (10 µM, n=6). Jungle ginger rhizome, stem and root extracts inhibit contractility of rat prostatic smooth muscle by an indirect prejunctional mechanism that inhibits exocytotic release of neurotransmitter.¶

555 Inhibition of human $Ca_V3.2$ channels by synthetic cannabinoid MDMB-CHMICA *in vitro*

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Introduction. Methyl-2-[[1-(cyclohexylmethyl)indole-3-carbonyl]amino]-3,3-dimethylbutanoate (MDMB-CHMICA) is a synthetic cannabinoid associated with severe adverse effects including dozens of deaths (EMCDDA, 2016). We have recently shown that MDMB-CHMICA has a higher efficacy and 20-fold greater *in vitro* potency as a CB1 agonist than the psychoactive ingredient of cannabis, ∆9-tetrahydrocannabinol (Banister et al, 2016). The CB1 potency and efficacy may underlie some of the severe hallucinogenic and psychotomimetic effects of the drug, however, the mechanisms responsible for other symptoms such as heart arrhythmias and peripheral toxicity remain to be established.
Aims. Cannabinoids directly interact with several ion channel classes, and the aim of this study was to explore whether MDMB-CHMICA interact with human T-type calcium channels ($hCa_V3.2$), which are known to regulate rhythmicity in the heart and the brain (Catterall et al, 2008).
Methods. Whole-cell voltage clamp recordings were made from HEK293 cells expressing $hCa_V3.2$.
Results. When cells were stepped from a holding potential of -100 mV to a test potential of -30 mV, MDMB-CHMICA rapidly blocked $hCa_V3.2$ with an IC_{50} of 1.5 ± 0.2 µM (n=6 cells per point). When applied at a concentration of 1 µM, MDMB-CHMICA did not significantly affect the half-activation potential of $hCa_V3.2$ (-42 ± 1 mV in control, -43.3 ± 1 mV in drug) and had no obvious effects on channel kinetics (n=6).
Discussion. This is the first report of illicit synthetic cannabinoid inhibition of human T-type calcium channels. The mechanisms underlying synthetic cannabinoid toxicity are not firmly established, but many of the most severe symptoms, including arrhythmia, seizures and low blood pressure have been associated with altered $hCa_V3.2$activity (Catterall et al, 2008).
Banister SD et al, (2016) ACS Chem Neurosci 7: 1241-1254.
Catterall WA et al (2008) J. Neurosci 28:11768 –11777
European Monitoring Centre for Drugs and Drug Addiction (2016) EMCDDA–Europol Joint Report on a new psychoactive substance: MDMB-CHMICA.

556 Potential role of herb-herb interactions in hepatotoxicity

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Introduction. Despite the unknown safety of many complementary medicines, use of products continues to increase. Approximately 20% of all drug-induced hepatotoxicity cases have been linked to herbal medicines; a significant proportion of which are multi-herb traditional Chinese medicines (TCMs). Commonly perceived as natural, hence safe, TCMs are often taken with other non-prescribed substances; hence, it is imperative a causal relationship between adverse events and herbs is established. Currently, research focusses on herb-drug interactions, neglecting herb-herb interactions. 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), shown to increase Astragalus glycoside levels.
Aims. To establish CYP functionality and metabolic competency in our cell models with paracetamol and CYP inducers, rifampicin and phenobarbital, and to investigate the individual and combined toxicity of active components listed.
Methods. Psoralen and related chemicals, coumarin and 8-methoxypsoralen (8-MOP), were utilized in interaction experiments due to limited availability of psoralen. Psoralen, coumarin, 8-MOP, AST-IV and ATR-I were individually assessed for toxicity in model liver (HepG2) and intestinal epithelial (Caco2) cells. Following these experiments, combinations of the compounds were investigated. Cell viability was determined using colorimetric assays.
Results. Cell viability was significantly decreased with coumarin ($p=0.0002$; n=5) and 8-MOP ($p=0.002$; n=5) in HepG2 cells, and psoralen in HepG2 ($p<0.0001$; n=8) and Caco2 cells ($p<0.0001$; n=5). No significant effect was observed with AST-IV or ATR-I ($p<0.0001$; n=8), but they were toxic when combined (0.1mM AST-IV, 0.3mM ATR-I) in both cell lines ($p<0.05$; n=3). Previously non-toxic 0.2mM coumarin and 8-MOP decreased cell viability combined with AST-IV ($p<0.05$; n=3) and ATR-I ($p<0.05$; n=3) in both models. In three-component interactions, all combinations were no more toxic than two-component interactions ($p>0.05$; n=3).
Discussion. For the first time, this study showed that some major herbal components can be studied in relevant tissue culture models. Our results demonstrate that herbal components have the potential to produce severe toxic effects when combined in high, but plausible, concentrations.

557 QSAR models define Molecular Initiating Events for multiple AOPs

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Introduction. Molecular Initiating Events (MIE) comprise the first component of an Adverse Outcome Pathway (AOP), defining the interaction(s) between the chemical properties of a xenobiotic and a biological target that is mechanistically linked to an adverse outcome. This enables the application of Quantitative Structure Activity Relationship (QSAR) methodologies to predict potential MIEs for untested chemicals, which is essential to the practical regulatory use of AOP frameworks. Contemporary QSARs use multitask machine learning algorithms (MTML) which feature multiple prediction outputs to utilise salient information shared between similar tasks. This enhances performance, however, the capability of MTML techniques to model weakly related endpoints has not been studied.
Aims. This project investigates the performance of MTML QSAR in the prediction of *in vitro* assay results screening for endocrine disruption, steatosis, skin sensitization, and cardiac arrhythmia MIEs.
Methods. A 25293-structure dataset was collated from existing Tox21 (endocrine disruption, n=8014) and SkinSensDB (skin sensitization, n=402) datasets, in addition to searching public databases for nuclear receptor agonists (liver steatosis, n=7682) and hERG inhibitors (cardiac arrhythmia, n=9195). Single task and MTML algorithms modelled the 25 classes of this dataset to determine the effect of multitask learning for weakly related endpoints.
Results. Initial results with a Multitask Deep Neural Network model found 0.83±0.01, 0.79±0.04, 0.67±0.12, and 0.90 Mean (±SEM) AUC for predicting endocrine disruption, steatosis, skin sensitization, and hERG inhibition assay results in the external validation dataset, compared to a baseline Logistic Regression model which found 0.81±0.02, 0.64±0.08, 0.62±0.04, and 0.87 Mean (±SEM) AUC for those respective assays.
Discussion. MTML QSAR models feature enhanced prediction performance for weakly related outcomes, however, the results show the magnitude of this effect is highly variable. While this project is still ongoing, the degree of similarity between MIEs may determine the magnitude of performance enhancement. The current results show QSARs support the practical application of AOP paradigms with enhanced predictive performance for similar and distinct chemical domains.
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558 Functional Evaluations of Synephrine and Octopamine - Stimulants in Pre-Workout Supplements

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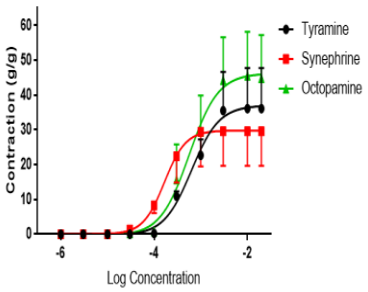
Introduction. Pre-workout supplements usually contain stimulatory botanical extracts for improved athletic performance. The rise in popularity of these supplements correlates with increased adverse health reports (Eudy et al, 2010). The biogenic amines synephrine and octopamine - found in plant extracts can increase blood pressure. However, the mechanisms involved in the vascular effect of these biogenic amines have not been fully established.



Aims. The purpose of this study was to evaluate whether vasoconstrictions were caused by synephrine and octopamine acting as indirect sympathomimetic agents (releasing the neurotransmitter, noradrenaline (NA)) - similar to tyramine, or whether these amines act directly on α- adrenoceptors.

Methods. The responses to synephrine and octopamine were investigated *in vitro* in rings of inferior mesenteric arteries of pigs.

Results. Synephrine (pEC50= 3.78 ± 0.21; *n=6*) was a more potent vasoconstrictor (*p<0.05*) than octopamine (pEC50= 3.25 ± 0.21; *n=6*). After depleting NA from the tissues, the maximum response for synephrine decreased by 67% (*p<0.05*), and its potency decreased (pEC50= 3.56 ± 0.18; *n=4*). However, neither the maximum contraction for octopamine nor its potency were affected by NA depletion (pEC50= 3.48 ± 0.26; *n=4*).

Discussion. The vasoconstriction induced by synephrine involves an indirect sympathomimetic pathway, whereas octopamine is likely a direct agonist at vascular α-adrenoceptors. These stimulants coupled with caffeine and strenuous exercise could explain the increase in adverse cardiovascular-related reports. Although synephrine is somewhat regulated in Australia, octopamine is an unregulated substance that is increasingly added to commercially available pre-workout supplements. Understanding the effects of these amines could lead to regulations of dietary supplement to protect vulnerable consumers.

Eudy AE et al (2013) Am J Health-Syst Ph, 70:577-588

559 The immunomodulation of dynorphin 3-14 on lipopolysaccharide-activated toll-like receptor 4 signalling pathway

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Introduction. In inflamed tissue, immune-derived dynorphin 1-17 (DYN 1-17) opioid peptide undergoes rapid metabolic degradation, producing a wide range of fragments with immunomodulatory functions. Dynorphin 3-14 (DYN 3-14) was found to be the most stable and prevalent fragment, however, there is little known of its cellular effects.

Aims. This study aims to investigate the presence of DYN 3-14 following biotransformation of DYN 1-17 in human inflamed tissues and to determine its effects on the LPS-activated toll-like receptor (TLR4) signalling pathway.

Methods. DYN 1-17 was incubated with human inflamed nasal tissue explants and its biotransformation was examined using LC-MS. The translocation of nuclear factor-kappaB/p65 (NF-κB/p65), the release of interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) were assessed in differentiated LPS-induced THP-1 cells treated with DYN 3-14. The involvement of DYN 3-14 on TLR4 activation was also examined using HEK-Blue™–hTLR4 cells stimulated with LPS.

Results. Incubation of DYN 1-17 with human inflamed tissues revealed that DYN 3-14 was one of the major hydrolysis fragments produced throughout the incubation period. Furthermore, DYN 3-14 inhibited LPS-induced NF-κB/p65 nuclear translocation ($P<0.05$) and differentially modulated the pro-inflammatory cytokines by inhibiting IL-1β and paradoxically augmenting TNF-α release ($P<0.05$). Intriguingly, DYN 3-14 showed significant concentration-dependent attenuation of TLR4 activation in HEK-Blue™-hTLR4 cells, albeit 300-fold lower than the potent TLR4 antagonist, LPS-RS.

Discussion. The abundant production of DYN 3-14 in human inflamed tissue homogenates highlights a rationale for its activity during an inflammatory response. Further observations reveal a mechanistic insight into the inhibition of NF-κB/p65 translocation and modulation of IL-1β and TNF-α release *via* the TLR4 pathway, following incubation of human activated macrophages with DYN 3-14. These findings thereby describe a potential role for DYN 3-14 as an antagonist at TLR4 and for its involvement in the regulation of inflammatory signals through a non-opioid mechanism in inflammation. ¶

560 Intranasal delivery of the TLR7 agonist, imiquimod, protects against influenza A virus-induced morbidity in mice

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Introduction. Influenza is a significant global burden with 5 million cases per year, 10% of which are fatal and thus, there is an urgent need for new therapeutics (WHO factsheet, 2017). Toll like receptor 7 (TLR7) is a pattern recognition receptor, which drives a powerful anti-viral signalling pathway that helps clear virus infections.
Aim. To determine the effect of the TLR7 agonist imiquimod on lung inflammation, oxidative stress and antibody production caused by influenza A virus (IAV) infection in mice.
Methods. Saline or imiquimod (50μg/mouse) was delivered intranasally to anaesthetised (inhaled isoflurane; 3%) male C57BL/6J mice one day prior to infection with a low (10^3PFU/mouse), moderate (10^4PFU/mouse) or high dose (10^5PFU/mouse) of the mouse adapted Hong Kong X31 (x-31) virus strain and everyday thereafter until mice were culled day 3 (d3) or 7 (d7) post-infection for analysis. Bronchoalveolar lavage (BAL) was performed to assess airways inflammation, and oxidative burst by L-012 enhanced chemiluminescence. In addition, BAL fluid and serum was used to determine antibody titres. The lungs were harvested and used to assess inflammation (H&E staining) and pro-inflammatory cytokine gene expression by qPCR. Bodyweights were recorded daily during the experimental process.
Results. Imiquimod significantly suppressed body weight loss caused by IAV infection with a maximum reduction of ~60% starting from day 4 (10^3 PFU/mouse, n=7-13, $p<0.001$). At d3 post infection, imiquimod treatment caused a significant reduction (~50-60%) in airway and peri-bronchial inflammation and BALF neutrophil populations (10^5 PFU/mouse, n=8-15, $p<0.01$) but had no effect on macrophage and lymphocyte populations, and the oxidative burst. TNF-α and IL-6 mRNA expression was supressed by ~60% ($p<0.01$ and $p<0.05$, respectively). Day 7 showed a modest but significant increase in IgE, IgM, IgG1, and IgG2a ($p<0.05$) in BALF following imiquimod treatment.
Discussion. Our findings highlight an exciting potential of imiquimod as a therapeutic option for the treatment of influenza disease.

561 IRAK3 modulates NFκB through its guanylate cyclase activity

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Introduction. Interleukin-1 receptor associated kinase 3 (IRAK3) acts as a negative regulator of inflammation. The role of IRAK3 is critical to maintaining homeostasis in the innate immune response and in preventing the development of autoimmune diseases. It is involved in various inflammation-associated disorders such as lung injury, metabolic syndrome and tumour growth. Prior studies identified IRAK3 as a potential novel guanylate cyclase (GC) catalyzing cyclic guanosine monophosphate (cGMP) synthesis. IRAK3 is predicted to be a mammalian representative of a new class of GCs containing a GC centre encapsulated within the kinase domain. (Freihat et al., 2014).
Aims. To investigate if IRAK3 is capable of generating cGMP and if modifying the GC centre modulates the downstream signaling pathways.
Methods. GC activity was assessed using the GE Amersham cGMP enzyme immunoassay kit. HEK BLUE hTLR4 cells containing a SEAP reporter system were transfected with either IRAK3 or IRAK3 mutant constructs, effects on NFκB activity in the presence of lipopolysaccharide (LPS) and cGMP were investigated.
Results. Recombinant IRAK3 protein produced significant amounts of cGMP in vitro, whilst the IRAK3 GC mutant did not. Overexpression of IRAK3 in HEK BLUE hTLR4 cells significantly reduced LPS induced, NFκB activation. Whereas IRAK3 GC mutants with reduced cGMP-generating capacity failed to inhibit LPS induced NFκB activity. The presence of cell-permeable cGMP restored IRAK3 function and significantly reduced NFκB activity in IRAK3 mutants with reduced cGMP-generating capacity.
Discussion. Low levels of cGMP are important for IRAK3 action and these findings are providing insight into the hidden functions of IRAK3 and may assist in explaining its selectivity and functionality in the inflammatory signalling cascade. Understanding how this novel GC function impacts the anti-inflammatory effect of IRAK3 is likely to be important when targeting this protein in different disease states.

Freihat, L., Muleya, V., Manallack, D.T., Wheeler, J.I., and Irving, H.R. (2014). Biochemical Society Transactions *42*, 1773-1779.¶

562 Pharmacological characterisation of small molecule C5aR1 inhibitors in primary human macrophages

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Introduction. The complement system is an essential component of innate immunity. The complement factor C5a is a core effector protein that exerts potent proinflammatory and immunomodulatory functions through its major receptor C5aR1. Over-activation of the C5a-C5aR1 axis has been implicated in a plethora of acute and chronic diseases, propelling the development of therapeutic inhibitors of C5aR1. Despite a number of these inhibitors being developed, to date, no systematic pharmacological characterisation of these compounds has been reported in human immune cells.
Aims. To compare the antagonistic potency and duration of inhibition of selected C5aR1 inhibitors against C5a-mediated cytokine release and phospho-ERK1/2 signalling respectively in primary human macrophages *in vitro*.
Methods. The peptidic (PMX53, PMX205, JPE1375) and non-peptide (W54011, NDT9513727) C5aR1 inhibitors were profiled in human monocyte-derived macrophages (HMDMs). IL-6 and IL-10 release in the co-presence of LPS was quantified using ELISA. Time-lapse pERK1/2 activity was examined using a AlphaLISA-based kit.
Results. The peptidic compounds were significantly more potent than the non-peptide small molecules in inhibiting the immunomodulatory effect of C5a. The rank order of potency was JPE1375 > PMX53 > PMX205 > NDT9513727 > W54011 for both IL-6 and IL-10 assays. In the wash-off study for pERK1/2 activity, PMX53 and JPE1375 possessed significantly longer duration of antagonistic activity ($t_{1/2}$ > 24 h) compared to the remaining inhibitors ($t_{1/2}$ ~ 5 h).
Discussion. The peptidic C5aR1 inhibitors are more effective at inhibiting C5aR1-mediated immunomodulatory effects in primary human immune cells, possibly due to their prolonged duration of receptor antagonism. The peptidic inhibitors may thus represent more ideal clinical drug candidates due to their potent and prolonged antagonistic activities. ¶

563 Chemical profile and anti-cancer potency of *Dendrobium* species from China and Australia

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Introduction. Some species of the *Dendrobium* and their chemical compounds especially bibenzyl derivatives have been reported to have anti-cancer activity. However, there are limited studies of chemical compounds and bioactivities of Dendrobium species from Australia. The aim of this study was to compare chemical profile and to explore anti-cancer potency of Dendrobium species from China and Australia.
Methods. Stems of *Dendrobiums* including twelve species commonly used in Chinese medicines from China and three species from Australia were extracted with pure ethanol and analyzed by TLC. Cytotoxicity and anti-proliferative activity assays were conducted with MTT and Incucyte method in LNCap cells.
Results. TLC profile indicated that each species has different chemical profile, and some compounds are likely common to all species tested. Only *D. chrysotoxum* contained erianin in high concentration. The ethanol extract, *D. chrysotoxum* showed the strongest cytotoxic activity against LNCap cells while *D. kingianum,* one of Australian species showed mild inhibitory activity with IC_{50} 67.8µg/mL and 36.5µg/mL at 24 and 72 hours respectively.
Discussion. Bibenzyl in *Dendrobium* are useful for quality standardization of *Dendrobium,* and contribute to cytotoxic effect of *Dendrobium*. Further study is needed to confirm active compounds and their potency.

Wang H et al (2016) Cell Death Dis 7:e2247
Ho C-K et al (2003) Cancer Investigation 21(5):729-36¶

564 Olfactory targeted mucoadhesive microparticles for enhanced brain uptake of phenytoin

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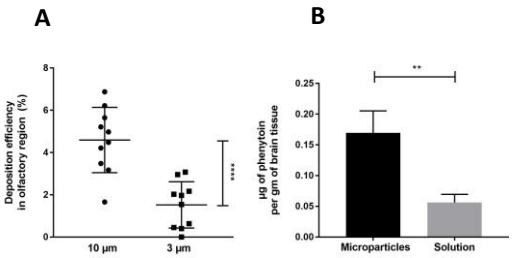

Introduction: Targeting and retention of drug in the olfactory region (OR) remain major challenges in nose-to-brain drug delivery and are a consequence of the geometrical complexity and rapid mucociliary clearance in the nasal cavity. Recently, we have developed a microparticle formulation that can address both these challenges by its specific size (10 µm) and mucoadhesive nature respectively (1).
Aims: Current study aims to package the poorly soluble anticonvulsant drug phenytoin into this microparticle formulation and determine its brain uptake after intranasal administration.
Methods: Spray drying parameters were optimised to produce 10 µm-sized phenytoin containing mucoadhesive microparticles with the naturally occurring polymer tamarind seed polysaccharide. Size, mucoadhesion and powder characteristics were investigated by laser diffraction texture-analysis, and X-ray diffraction respectively. A 3D-printed human nasal replica cast was used to study the deposition of microparticles in the OR. Male Wistar rats (3/group) were anesthetized (5% isoflurane) and phenytoin microparticles or solution was administered (~4 mg/kg) intranasally. Rats were sacrificed and tissues analyzed for phenytoin content by HPLC.
Results: A high entrapment of phenytoin (91% ± 4, n=5), in microparticles was achieved and the microparticles were amorphous. Microparticles demonstrated high mucoadhesion compared to phenytoin powder (n=3; $P<0.01$). Microparticles 10 µm in size showed significantly greater deposition (Fig. Panel A) in the OR (n=10; $P<0.001$) compared to 3 µm particles. The amount of phenytoin per gm of brain tissue administered as microparticles was significantly higher (Fig. Panel B) than the solution ($P< 0.05$).
Discussion: Phenytoin can be packaged into tailor-made 10 µm mucoadhesive microparticles to improve the olfactory deposition and retention and subsequent uptake into the brain. Furthermore, these amorphous microparticles can aid in solubility enhancement of phenytoin. Studies are underway to determine the efficacy in rat-seizure model.

(1). Yarragudi S B et al (2017) Carbohydrate Polymers 163: 216–226¶

565 Synthesis and characterization of a smart inulin hydrogel system for colon targeted drug delivery

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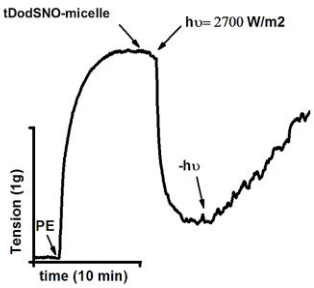
Introduction. Australia and New Zealand have the highest incidence of colorectal cancer in the world[1] and the incidence amongst young adults is increasing globally. Unfortunately, most chemotherapies for colorectal cancer use non-selective systemic delivery of the toxic drugs, resulting in significant side effects. Therefore, the development of a targeted drug delivery system for colon cancer treatment using oral administration is highly desirable.
Aims. To develop a smart inulin hydrogel that combines both pH and colonic bacteria dependent triggers for colon targeted drug delivery.
Methods. Inulin was crosslinked with dianhydrides via an esterification reaction. The physicochemical properties of the hydrogels were evaluated using Fourier-Transform Infrared Spectroscopy (FT-IR), mechanical analysis and equilibrium swelling tests. The loading of 5-fluorouracil (5-FU) into the hydrogel and subsequent *in-vitro* drug release in media mimicking both the conditions of the gastrointestinal tract and the colon was evaluated using HPLC as was the degradability of the hydrogel using inulinase.
Results / Discussion. FTIR was used to confirm the formation of the new hydrogel with new bands for the ester bonds and carboxylic acid groups observed. The hydrogel shows excellent water swelling following second order kinetics with water diffusion having a non-Fickian pattern. Increase gel strength with increase in crosslinker ratio. 5-FU was absorbed into swollen hydrogels at loadings of 8 and 18.1% dependant of crosslink density, having entrapment efficiencies 14.0 and 31.42% respectively. The *in-vitro* release studies showed a controlled release pattern with about 70 % release in the pH conditions of the colon. Furthermore, release of fructose from the hydrogel demonstrates degradability of the hydrogel by colon specific microbes.
Conclusion. These findings show that this hydrogel is a promising drug carrier for colon-specific drug delivery.
Reference
1. Boyce et al. Young-onset colorectal cancer population-based study. MJA, 2016;205(10):465-70. ¶

566 Induction of localized vasodilation and hyperpermeability using a novel nitric oxide donor nanoparticle.

Houman Alimoradi[1], Khaled Greish[2], Ivan Sammut[1], Anita Barzegar-fallah[1], Gregory I Giles[1]. [1]Department of Pharmacology and Toxicology, University of Otago, Dunedin, Otago, New Zealand. [2]Aljawhara centre for molecular medicine, Arabian Gulf University, Manama, Kingdom of Bahrain.

Nitric oxide (NO) donors can enhance drug targeting towards specific body compartments, especially for the uptake of nano-sized particles and proteins, and this has great potential in the treatment of many diseases such as solid tumours and central nervous system (CNS) disease. The main limitation, however, is controlling release of nitric oxide in target tissues. Hence, we have developed photoactive nanoparticles (NPs) by encapsulation of a bulky, hydrophobic and stable s-nitrosothiol, tert-dodecane s-nitrosothiol (tDodSNO), into polystyrene maleic acid which releases NO in a controlled manner. The NPs in the absence of irradiation had a $t_{1/2}$ of approximately 100 h, while photoactivation (cold light with the intensity of 2700 W/m^2) decreased this to just 4 min.



Theoretically NPs avoid metabolic breakdown of the s-nitrosothiol as well as trans-nitrosation reactions with other proteins, thereby inhibiting unspecific vasodilation and vascular hyperpermeability. To examine the effect of NPs on vasodilation, rat aortic rings were constricted with phenylephrine and then the vasoconstriction properties of different concentrations of NPs, in presence or absence of photoactivation, were assessed. The micellar system significantly relaxed the aortic rings only in the presence of photoactivation (graph inset). In addition, a rat mesenteric bed assay was used to determine the ability of the NPs to induce localized vasodilation when stimulated by light, as quantified by Evans blue dye leakage and florescent microscopy of mesenteric windows. Photo-irradiation of the NPs led to a significant increase in the dye extravasation compared with control NPs ($p < 0.01$), showing that photoactivation can control the release of NO in a target tissue and thereby cause localized vasodilation and hyperpermeability. In conclusion, tDodSNO-NPs are a novel form of NO donor, whose NO release characteristics can be modulated by photoactivation. They cause controllable and localized vasorelaxation and hyperpermeability, and therefore can potentially be used for tumour therapy and CNS disorders.¶

567 Scarring in horses – is there a way to objectively quantify an equine scar?

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Introduction. When a large wound on a horse heals it tends to develop inferior quality scar tissue. This scar tissue is fragile, easily damaged, stiff and cosmetically unappealing. While there have been numerous studies that have reviewed and tested measuring devices in humans for scar assessment, there have been no studies in horses. In order to effectively evaluate and monitor scar reduction treatments quantitative measurement modalities are needed.

Aims. To test the validity and reliability of the equipment used to quantify scars in humans, in horses.

Methods. A research study using different equipment to test horses' scars for flexibility, size and thickness was conducted. A cutometer, the gold standard for measuring pliability of human skin and scar tissue had the intra- and inter-rater reliability measured to ensure its validity in horses. The Silhouette Star, a laser beam wound camera was used to calculate the surface area of the scars and was compared for accuracy with the old method of using a tracing. Finally, ultrasound was compared to punch biopsy for measuring the epidermal, dermal and total thickness of the scar.

Results. The cutometer was reliable for measuring the pliability of some scars and normal skin however it was unreliable for severe scars due to a ceiling effect when rigid tissue was encountered. There was a low intra-rater reliability due to the difficulty relocating the device to the same measurement spot and the high sensitivity of the device. The Silhouette Star was not comparable to the tracing method for accuracy. The punch biopsy provided more information at a histological level than the ultrasound but was an invasive procedure and quite dangerous when trying to sample hind limbs.

Discussion. Objective scar measurement tools allow the accurate and reproducible evaluation of scars, which is important for both clinical and scientific use. However, no studies had been conducted to date to evaluate horse scars or validate equipment used for this purpose in humans and translate this to horses. Equipment validation is important in order to effectively evaluate and monitor scar reduction treatments in the future.

568 Prediction of skin permeation based on solute properties using machine learning and statistical tools

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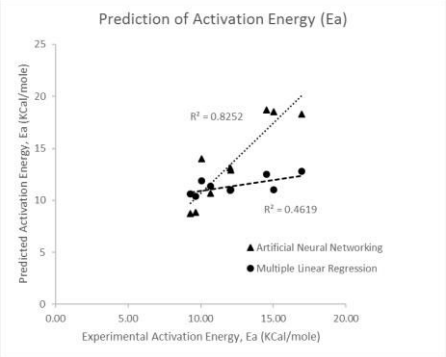
Introduction. The mechanism by which many solutes penetrate through the main human skin physical barrier, the stratum corneum, after topical application is currently poorly understood. Imaging, kinetic, quantitative structure –permeation relationships and computational dynamic studies suggest that pathways for transport include: directly through the corneocytes; between the corneocytes through the intercellular lipids; and via appendages, with deeper layers offering significant resistance for lipophilic solutes.
Aims. In this work, we used solute skin permeability coefficients determined at different temperatures to derive the thermodynamic parameters associated with their penetration. We then derived quantitative solute structure – human skin permeation relationships for these properties using both machine learning and statistical analysis.
Methods. Activation energy (Ea) and entropy (ΔS) for permeation were derived from literature and our own permeation data at different temperatures and related to solute physicochemical properties, including solute lipophilicity, molar volume and solubility). Artificial neural networking (ANN) was used for training and validation, with confirmatory statistical analyses using multiple linear regression (MLR) and multivariate analyses.
Results. Fig. 1 shows that the predicted Ea based on ANN better described the experimental data (r^2=0.82) than a MLR analysis (r^2=0.46). Solute molar volume and polarity were found to be the main determinants for Ea.
Discussion. Multiple linear regression is widely used to study quantitative solute structure – human skin permeation relationships. This work suggests that machine learning better predicts Ea. Our next step is to apply its findings in the better understanding of skin permeation mechanisms for different solutes.



569 Quality of levofloxacin tablets: in vitro dissolution testing and content evaluation

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Introduction. Levofloxacin is a broad spectrum antibiotic and used empirically to treat community acquired or nosocomial pneumonia. Low quality levofloxacin tablets can lead to treatment failure or bacterial resistance; hence the pharmaceutical evaluation of products available in the market should be conducted regularly.
Aims. To compare the *in vitro* dissolution and content uniformity of different brands of levofloxacin tablets manufactured and sold in India, Iran and Pakistan.
Methods. Innovator and generic brands of immediate release levofloxacin 500mg tablets were purchased from the authorized medicine wholesaler located at India, Iran and Pakistan as well as Australia. A total of 13 brands were tested for content uniformity and dissolution. For content uniformity analysis, levofloxacin tablets were dissolved in sufficient amount of 10% acetic acid which later was diluted prior to HPLC analysis. For dissolution, the tablets were added to Simulated Gastric Fluid prepared according to United State Pharmacopeia (USP) and sample was taken at 5,10,15,20,30,45, 60 and 90 minutes. *In vitro* dissolution data were collected with a Erweka USP 2 apparatus (37°C, 900ml, 50 rpm) The absorbance of all sample were measured at 270nm. Triplicate were used and each sample was tested twice. Dissolution profile comparison was performed using similarity factor (f_2) with $f_2 \geq 50$ indicate similarity.
Results. All the generic and brand-name levofloxacin passed content uniformity test stipulated by United State Pharmacopeia (USP) (specification: 90% to 110%). In term of ddissolution testing, 11 brands showed more than 80% dissolution within 30 minutes except two brands sourced from Pakistan.
Discussion. The content uniformity and weight uniformity of the levofloxacin brands from Iran, India and Pakistan were in accordance to USP standard. However, the dissolution profile of these brands were different, as indicated by the f_2 similarity factor. Levofloxacin is a Biopharmaceutics Classification System Class 1 compound characterised by high solubility and high permeability. Literature has not reported any risk of bioequivalence for levofloxacin tablets produced using different manufacturing methods. Clinically, levofloxacin has a wide therapeutic index and good oral bioavailability. Even though the tested levofloxacin tablets sourced from local suppliers exhibited different *in vitro* dissolution profiles versus the reference product, the potential effect on the *in vivo* performance will be minimal.

570 Evaluation of PLGA nanoparticles (NPs) uptake using Caco-2 cell monolayers

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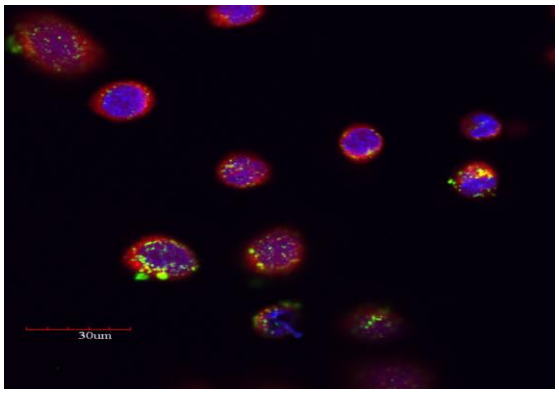

Introduction. PLGA NPs are solid colloidal particles made of biodegradable polymer. Therapeutic agents can be adsorbed to the surface or entrapped in the particles. PLGA NPs are suitable for delivering small molecular weight drugs by either localised or targeted delivery to the tissue of interest. Caco-2 cell is the most commonly used as in vitro cellular model for the studies of drug transport, uptake, metabolism and toxicity.
Aims. To investigate the uptake of PLGA NPs by Caco-2 cells.
Methods. A fluorescent material (FITC) was conjugated to PLGA using modified carbodimide method. Then FITC-labelled PLGA were prepared by the modified water-in-oil-in-water emulsion solvent evaporation technique. The lyophilized FITC-PLGA NPs were added to Caco-2 cells and the uptake of the NPs by the cells was investigated by fluorescence spectrophotometer and confocal laser scanning microscopy (CLSM).
Results. The uptake of PLGA NPs by Caco-2 cells seems to be mediated by endocytosis. Caco-2 cells took up PLGA NPs via a saturable, and temperature dependent process. The rate and extent of cellular uptake of PLGA NPs are mainly affected by particle size and hydrophilicity of particle surface. A decrease in particle size or particle surface hydrophilicity led to an increase in cellular uptake. The enhanced uptake is a consequence of better particles-cells interaction resulting in higher endocytotic uptake and/or higher retention time of NPs in cells. The CLSM image indicates that NPs were adsorbed on the cell membrane and a few of them were clearly localised in cytoplasm. The results are promising as they indicate that PLGA NPs of small particle size with hydrophobic surface may be employed as a drug delivery system for oral delivery of therapeutic agents to improve the drug bioavailability via oral administration.

571 Human NAT1 regulates invasion of MDA-MB-231 breast cancer cells by modulating the expression of MMPs

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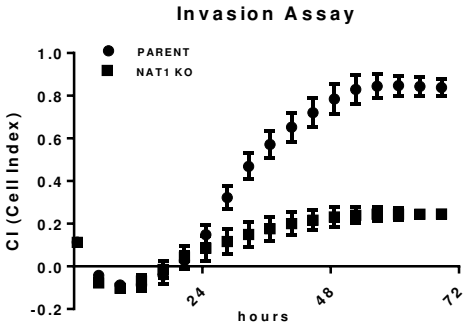

Introduction. Recent studies suggest that the phase II drug metabolising enzyme, arylamine N-acetyltransferase 1 (NAT1), may be important in cancer cell biology. To investigate its role in cancer cell invasion, NAT1 was knocked out in the highly invasive breast cancer cell line MDA-MB-231 using CRISPR technology. Matrix metalloproteinases (MMPs) are an important family of zinc-dependent endo-proteinases that degrade the extracellular matrix and promote cell invasion. They are secreted as latent pro-MMPs that are then activated by plasmin or other MMPs.
Aims. To determine the effect of NAT1 KO on 1) the ability of MDA-MB-231 cells to invade in vitro, 2) MMP gene expression.
Methods. Invasion and migration assays were performed using the ACEA xCELLigence system and Matrigel-coated membranes. Invadopodia degradation assays were performed on fluorescein-conjugated gelatin-coated coverslips and visualised by confocal microscopy. MMP gene expression was quantified by real-time RT-PCR and protein expression in cells and growth medium was determined by Western blot.
Results. Although cell migration was not different, NAT1 KO cells had a reduced ability to invade compared to parent cells. The gene expression of some MMPs changed significantly upon NAT1 KO, with MMPs 2, 7 and 9 all increasing, while MMP1 decreased. In addition, treatment of parent cells with the histone deacetylase inhibitor trichostatin A, indicated that these changes were associated with altered histone acetylation. Western blot of culture medium from NAT1 KO cells showed that MMP9 was significantly increased compared to parent cells, while MMP1 was significantly decreased compared to parent cells. MMPs 2 and 7 were not detectable for either NAT1 KO or parent cells.
Discussion. NAT1 KO cells have increased MMP9 expression, but unexpectedly, a reduced ability to invade in vitro. From Western blots, the size of the secreted MMP9 protein suggested that it is the latent pro-form of the protein in NAT1 KO cells. Further studies are required to explain this observation, as well as to determine the mechanism by which loss of NAT1 leads to changes in MMP gene and protein expression.¶

572 Effect of N-acetyltransferase 1 on the sensitivity of chemotherapeutics in breast cancer

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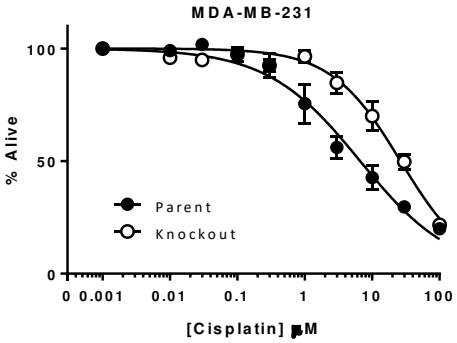

Introduction. Arylamine *N*-acetyltransferase 1 (NAT1) is a phase II drug-metabolising enzyme that acetylates drugs and carcinogens and has been associated with increased risk for some types of cancer. More recently, it has been linked to cancer cell growth and survival, as well as other characteristics of cancer progression, such as resistance to chemotherapeutics.

Aim. To determine if altering NAT1 levels in cancer cells can modulate their sensitivity to chemotherapy agents.

Methods. CRISPR technology was used to knockout NAT1 in the breast cancer cell lines MDA-MB-231 (ER-) and T47D (ER+). Cytotoxicity assays were performed using CyQuant NF Cell Proliferation Assay Kit following drug treatments of 72 hr.

Results. No change in toxicity was observed in the NAT1 KO with metformin, venetoclax, gemcitabine, etoposide and 5-FU. MDA-MB-231 NAT1 KO cells showed an increased resistance to cisplatin toxicity compared to parent cells (IC_{50} 26.6 ± 2.7 µM and 6.6 ± 0.9 µM, respectively), as was the case for T47D NAT1 KO cells (IC_{50} 26.7 ± 3.6 µM and 2.3 ± 0.4 µM, respectively). Similar results were found in both cell lines with daunorubicin. Combining multiple chemotherapeutics did not improve the sensitivity. No difference in cisplatin toxicity was seen between the parent and NAT1 KO in HT-29, 22RV1 and HeLa cell lines.

Discussion. Cisplatin has been shown to inhibit NAT1 irreversibly by binding to its catalytic cysteine. This inhibition occurred in a dose-dependent manner in-vitro but did not inhibit the enzyme significantly in cells. It is suspected that the NAT1-cisplatin interaction may have a role in the sensitivity of cells to the chemotherapeutic. However, the mechanism is not well understood and is still under investigation.

573 An Investigation of Sodium Fusidate and Recombinant Cytochrome P450 Enzymes Inhibition *In-Vitro*

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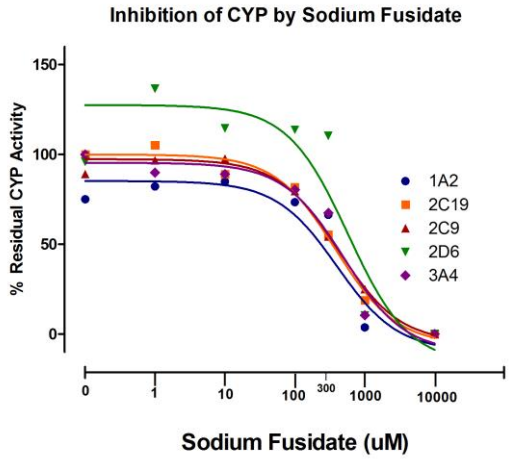

Introduction. Sodium fusidate is an antimicrobial agent that is used in the treatment of staphylococcal infections. Several case reports have noted a drug interaction between sodium fusidate and CYP3A4 metabolised statins, leading to statin toxicity. It is unclear whether sodium fusidate has the potential to cause interactions with other cytochrome P450 enzymes.

Aims. To investigate the effects of sodium fusidate on recombinant cytochrome P450 enzymes (1A2, 2C9, 2C19, 2D6 and 3A4) *in-vitro*.

Methods. A range of sodium fusidate concentrations (0.1µM, 1µM, 10µM, 100µM, 300µM, 1000µM and 10000µM) were tested to examine its activity on rCYP1A2, rCYP2C9, rCYP2C19, rCYP2D6 and rCYP3A4 using a luminescent assay with a luciferin substrate.

Results. Sodium fusidate inhibited all enzymes at tested concentrations which are relevant to those likely to be achieved in clinical practice. Further, sodium fusidate was found to be a time-dependent inhibitor of all the tested isoenzymes, with the exception of rCYP2C9.

Discussion. These findings suggest that there is a potential for sodium fusidate to cause drug interactions when used with other agents that are substrates for rCYP1A2, rCYP2C9, rCYP2C19, rCYP2D6 or rCYP3A4. Understanding the basis of this potential drug interaction will assist in safer use of sodium fusidate in clinical practice.

574 Physiologically-based IVIVC compared with conventional IVIVC for predicting in vivo pharmacokinetics of crushed paracetamol mixed with thickened fluids for swallowing disorders.

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Introduction. The aim of the study is to compare a conventional deconvolution method using *in vitro – in vivo* correlation (IVIVC) and a mechanistic physiologically-based *in vitro – in vivo* correlation (PB-IVIVC). The test product was crushed paracetamol tablets mixed with thickened water.
Methods. *In vitro* dissolution was performed in simulated gastric fluid using USP apparatus. *In vivo* PK parameters were calculated from salivary concentrations following a single 1 g dose in 20 adults. Conventional deconvolution IVIVC was performed using Level A correlation with Winnonlin®. Physiologically-based pharmacokinetic (PBPK) deconvolution was performed using the PK-Sim® model. The % prediction error (%PE) was calculated for AUC and C_{max} for the observed and predicted concentrations using the formula below. FDA guidelines recommend %PE < 15%.
Results. PB-IVIVC produced %PE < 15% for both AUC and C_{max}. The error associated with conventional IVIVC was higher than 15% for these parameters.
Discussion. The PB-IVIVC method includes corrections for *in vivo* dissolution, permeation, gut-wall metabolism and first-pass liver metabolism, while the conventional method does not separate the multiple mechanisms involved in the process of drug absorption. Incorporating these adjustments allowed PB-IVIVC to predict the *in vivo* profile more accurately than conventional IVIVC.

575 Release of somatostatin monomers from self-assembled hydrogels

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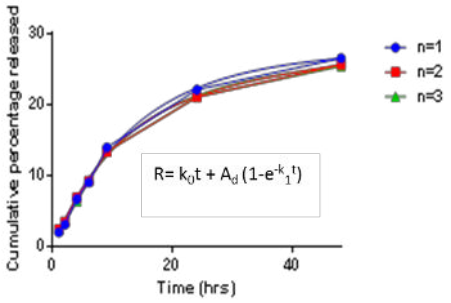
Introduction. Somatostatin-14 self-assembly has been demonstrated by in aqueous media (van Grondelle et al, 2007). Nanofibril self-assembly can be altered by the presence of electrolytes. While the reversibility of nanofibril formation has been demonstrated in the presence of heparin, an aggregation inducer (Anoop et al, 2014) there is currently no published data on the rheology and release kinetics of somatostatin hydrogels.
Aims. This study aims to investigate the release of monomers of somatostatin at higher concentrations that form a physical hydrogel in aqueous media and in the presence of electrolytes.



Methods. Rheological characterization of the somatostatin hydrogels was performed to determine a relationship between the viscoelasticity of the hydrogels and the release of somatostatin monomers. Transmission electron microscopy (TEM) was performed to characterise the morphology of the nanofibrils. Release of monomers was assayed by UV spectroscopy and fitted to a hybrid dual-order release model.
Results. This study showed that release of somatostatin monomers followed a two exponential release model. Somatostatin gels in water demonstrated relatively rapid release, which correlates to the lower storage modulus (G').

Anoop, A. et al, (2014) *J Biol Chem*, *289* (24), 16884-903
van Grondelle, W. et al,(2007) *J Struct Biol, 160* (2), 211-23
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576 L-arginine protects skeletal muscle against statin-induced myopathy.

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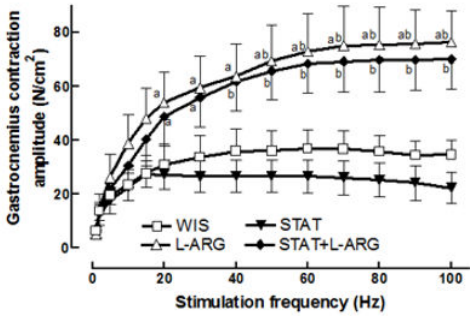

Introduction. Statin-induce myopathy (SIM) is reported to occur in 1 out of 10 000 statin users and be associated with high doses. Increases in inflammation, nitric oxide levels, mitochondrial dysfunction and reactive oxygen species are hypothesized as contributing factors for its development. Current literature indicates that L-arginine (L-arg) may be beneficial in protecting against such factors due to its antioxidant and vasodilatory effects.
Aims. To investigate whether the co-administration of L-arginine prevents the development of statin-induced damage to skeletal muscle.
Methods. 10-12 week old female Wistar rats were randomly assigned to one of four treatment groups; control (CON), SIM (80mg/kg/day of simvastatin), control+L-arg (L-arg; 100mg/kg/day) and SIM+L-arg (SIM+L-arg). After two weeks of treatment organ mass, muscle mass and serum samples were collected. Three skeletal muscles (gastrocnemius, soleus and tibialis anterior) were tested using electrical field stimulation to assess contractility and functionality while serum samples were analysed for biochemical markers of skeletal muscle damage.
Results. Improvements in skeletal muscle contractility and function from L-arg treatment was noted in gastrocnemius, soleus and tibialis anterior muscles compared to SIM treatment. The administration of L-arg to SIM animals attenuated serum albumin and significantly reduced creatinine levels (SIM+L-arg 30.29 ±1.48µmol/L; SIM 38.33 ±1.69µmol/L). Reductions in body mass after L-arg treatment (SIM+L-arg -1.19 ±6.72%; SIM 6.14 ±4.35%) and significantly increased gastrocnemius muscle mass (SIM-L-arg 6.13 ±0.12mg; SIM 5.66 ±0.17mg) were noted.
Discussion. Improved skeletal muscle function and mass of L-arg treated rats indicates its ability to reduce the impact of muscle wasting often associated with statins. Additionally, reductions in serum albumin and creatinine levels indicated less muscle damage occurring with the co-administration of L-arginine. Overall these factors demonstrate that L-arg can be utilised as a treatment to reduce the risk of developing SIM.

577 Intrinsic Dissolution Study of Aspirin

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Introduction. Dissolution is an important component of drug delivery, and thus, related lectures and laboratories are covered in Pharmacy Undergraduate education. In the laboratories, students not only begin to appreciate methods of study, together with data presentation and interpretation, but also learn to work as members of a team.
Aims. To determine the intrinsic dissolution of aspirin.
Method. Non-disintegrating disks of aspirin were prepared by compression. The disks were placed in rotating disk holders and then rotated at 50 rpm, 75 rpm or 100 rpm in two different dissolution media, acid (pH 1) and pH 6.8 phosphate buffer. Eight samples were taken at 6 min intervals. Samples were diluted with sodium hydroxide solution in order to hydrolyse aspirin to salicylic acid. A standard curve of salicylic acid was determined using UV spectroscopy, and concentrations of salicylic acid in the dissolution samples were determined. Aspirin dissolved was calculated using MW ratio.
Results. Dissolution profiles were linear and dissolution rates were calculated from gradients of dissolved aspirin v time. Dissolution was 4 to 5 times greater at pH 6.8 than in acid, reflecting the influence of solubility on dissolution rate. Dissolution rate increased with rotation speed.
Discussion. In this laboratory, the principles of an intrinsic dissolution study are explored. Surface area is maintained constant and dissolution occurs under sink conditions, thus giving a constant rate of dissolution.
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578 Effect of Storage on Release from Enteric Coated Diclofenac Tablets

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Introduction. Enteric coating is used on diclofenac tablets to protect the stomach from the drug and then release drug rapidly once in the intestine. Ideally, the function of enteric coating remains optimal until the tablets are consumed by the patient.
Aims. To determine the release characteristics of enteric coated diclofenac tablets before and after storage under different conditions.
Methods. Three brands of EC diclofenac tablets were studied. Storage was at 25^0C and 40^0C under 75% relative humidity and non-humid conditions for approximately 2 months. Release of diclofenac was monitored in acid (acid stage) and then in pH 6.8 buffer according to USP conditions. Samples were taken at different times to obtain release profiles, rather than just single point analyses. After storage, any weight gain/loss or change in appearance was recorded. Observations of tablets occurred throughout the release experiment. SEM was carried out on controls and some of the stored tablets.
Results. Storage changed the release characteristics of all brands of EC diclofenac tablets.
Discussion. As it is desirable for EC to protect the stomach and then give fast release upon gastric emptying, storage should not change release characteristics. ¶

579 Drug content and *in vitro* dissolution of ciprofloxacin tablets: Comparison and Evaluation

Deepali Sharma, Long C. Ming, Rahul P. Patel, Syed Tabish R. Zaidi. Pharmacy, School of Medicine, University of Tasmania, Hobart, TAS, Australia.

Introduction. Counterfeit and falsified drugs are easily available worldwide and the use of these medicines could lead to treatment failure and antibiotic resistance. Quality evaluation of drug products in the market could minimize the unwanted healthcare risk and adverse effect.
Aims. To evaluate the *in vitro* dissolution and quality of different brands of ciprofloxacin tablets that are available in the local market of India, Iran and Pakistan.
Methods. Innovator and generic brands of immediate release ciprofloxacin 500mg tablets were purchased from the authorized medicine suppliers located at India, Iran and Pakistan as well as from a local pharmacy in Hobart, Australia (reference product). A total of 15 brands were tested for content uniformity and dissolution. For content uniformity analysis, ciprofloxacin tablets were dissolved in sufficient amount of 10% acetic acid which later was diluted prior to HPLC analysis. For dissolution, the tablets were added to Simulated Gastric Fluid prepared according to United State Pharmacopeia (USP) and sample was taken at 5,10,15,20,30,45 and 60 minutes. Triplicate were used and each sample was tested twice. Dissolution profile comparison was performed using similarity factor (f_2) with $f_2 \geq 50$ indicate similarity.
Results. Content uniformity analysis indicated that all the tablets were within the limits of USP (within the range of 90-110%). Dissolution testing demonstrated that all tested brands, except one brand from Iran, followed the USP requirement of not less than 80% dissolved in 30 minutes. Out of the 14 brands, four have similar dissolution profile in comparison to the reference brand.
Discussion. Drug products purchased from authorized drug suppliers tend to have lower risk of counterfeiting. Complied drug content in 14 out of 15 brands tested, however, are not in congruent with the results of drug dissolution. Different dissolution profile could affect the *in vivo* ciprofloxacin absorption because physiological-based pharmacokinetics modeling confirmed that ciprofloxacin exhibited apparent “absorption window” in gastrointestinal tract. The inadvertent failure in drug release or dissolution compared to the reference product would yield lower bioavailability that eventually affect the desired minimum inhibitory concentration.
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580 Removal of interstitial hyaluronan with recombinant human hyaluronidase (rHuPH20) influences the systemic and lymphatic uptake of a monoclonal antibody in rats

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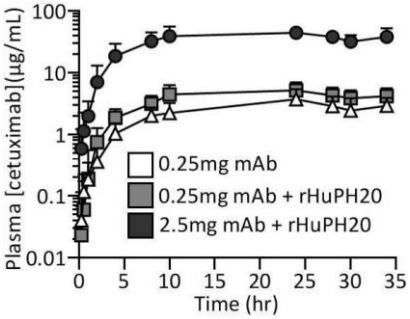

Introduction: Interstitial (e.g., intradermal (ID) or subcutaneous (SC)) administration of monoclonal antibodies (mAb) is less invasive than intravenous administration and leads to mAb uptake into both lymphatic and blood capillaries draining the injection site. Interstitial administration, however, is hindered by barriers to fluid transport that limit injection volumes. This study investigates the effect of transient removal of interstitial hyaluronan (HA), a major fluid barrier in the human interstitial space, via co-administration of recombinant human hyaluronidase PH20 (rHuPH20) on the lymphatic and systemic PK of the mAb, cetuximab, following ID or SC injection.
Methods: Male Sprague Dawley rats had cannulas inserted into the carotid artery (lymph 'intact' rats) and also into the thoracic lymph duct in lymph duct cannulated ('LDC') animals. Cetuximab (5 mg/mL) was dosed either ID or SC at volumes of 50 and 100 µL, respectively, and in the absence or presence of rHuPH20. Cetuximab was also administered at a 10-fold higher injection volume and dose in the presence of rHuPH20 (high dose). Lymph was collected for 30-34 hr and plasma sampled for 34 h.
Results: Cetuximab plasma exposure increased 1.8-fold in the presence of rHuPH20 after ID and SC administration. Cetuximab recovery in lymph was similarly increased. When the ID and SC injection volume/dose increased 10-fold, plasma AUCs increased 9.8 and 11.1-fold, respectively, consistent with approximately linear increases in absorption, although the proportional contribution of lymphatic transport appeared to reduce slightly.
Conclusion: rHuPH20 enhanced systemic and lymphatic absorption of cetuximab and enabled increases of injectable volumes up to 10-fold. At high injection volumes and doses, the relative role of lymphatic transport appeared to reduce slightly, however total cetuximab plasma exposure increased approximately linearly with dose.

581 Preparation of Viable and Metabolically Active Epidermal Membrane

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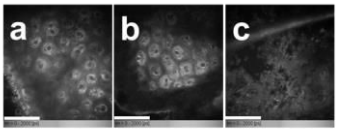


Autofluorescence of Stratum Granulosum imaged at an excitation wavelength of 760 nm (a) Dispase Separated Epidermis (DSE); (b) Dermatomed Skin (DTM); (c) Heat Separated Epidermis (HSE)*. Scale bar is 40 µm. *Specific layers not identifiable in Heat Separated Epidermis

Introduction: The skin acts as a limited route of entry for therapeutic substances and other xenobiotics and provides a metabolic defensive barrier. The current reconstructed human epidermis (RHEs) models for skin irritancy differ from the human skin in stratum corneum (SC) thickness and the permeation profile. A suitable skin model to be used for *in vitro* permeation testing (IVPT), evaluation of skin irritancy of topical formulations, and metabolic imaging studies should retain the viability and enzymatic activity of *in vivo* skin.
Aims: To separate a viable and metabolically active epidermal membrane from the excised human skin.
Methods: Epidermal membranes were separated from the excised human skin by enzymatic treatment. The viability evaluation of the separated epidermis was carried out by i) Multiphoton Microscopy-Fluorescence Lifetime imaging (MPM-FLIM) to measure intensities and lifetimes of endogenous fluorophores such as NAD(P)H, ii) MTT assay and iii) Hematoxylin and Eosin staining. The mapping of esterase enzyme distribution was carried out by staining with α-Napthyl acetate. The skin irritancy testing of known positive irritant 5% Sodium Dodecyl Sulphate (SDS) and TritonX 100 were performed according to Organisation for Economic Co-operation and Development (OECD) guidelines for new skin model development for skin irritancy testing.
Results and Discussion: The viability of the enzymatically separated epidermal membranes was confirmed by all three methods used. α- naphthyl acetate staining shows the retention of esterase activity and their distribution in the epidermis. The dose and incubation period for the positive irritants were optimised. The future studies will characterise the activity of the other main skin enzyme systems.

1. Kligman, A. M. Arch. Dermatol. 88, 702-705, (1963).
2. Sanchez et al. J Biomed Opt. 15(4):046008 (2010).
3. Manevski, N. et al. Drug Metabolism and Disposition 43, 126-139, (2015).
4. OECD. Test No. 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method. (OECD Publishing).¶

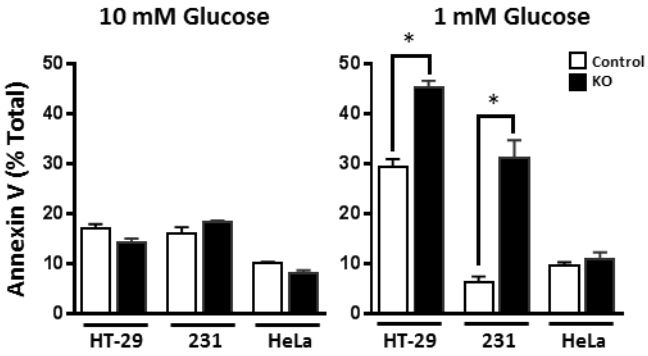
582 Arylamine N-acetyltransferase 1 regulates cancer cell survival via modulation of pyruvate dehydrogenase

LiLi Wang, Rodney F Minchin, Neville J Butcher. School of Biomed Sci, Uni of Queensland, Brisbane, QLD, Australia

Introduction. A growing body of evidence suggests that the phase II drug metabolising enzyme, arylamine N-acetyltransferase 1 (NAT1), plays a role in cancer cell biology. NAT1 has been closely associated with cancer cell growth and survival, as well as metastasis. It also has been associated with the methionine salvage pathway and palmitoleic acid homeostasis.

Aim. To determine the effect of NAT1 KO on cancer cell metabolism under normal and stressed conditions.

Methods. Cell proliferation assays used the CyQuant NF kit. Mitochondrial function was assessed using an XFe96 Analyser and Mito stress kit. CRISPR technology was used to generate NAT1 KO cell lines. Annexin V and caspase 3/7 assays were performed using a Muse analyser.



Results. Loss of NAT1 reduced cancer cell proliferation and increased apoptosis under nutrient deprivation. NAT1 KO led to decreased mitochondrial respiration via inactivation of pyruvate dehydrogenase, with concomitant increased levels of extracellular pyruvate and increased generation of reactive oxygen species. This may be via inhibition of the PI3K/AKT pathway as NAT1 KO caused a decrease in AKT phosphorylation. The above changes were observed for HT-29 and MDA-MB-231 cells, but not HeLa cells. One major difference between these cell lines is that the former have gain-of-function mutant p53 whereas the latter has wild-type p53.

Discussion. These results indicate that NAT1 is involved in the regulation of cancer cell metabolism and survival under stress, which may have implications for cancer treatment in the future. The exact molecular mechanism linking NAT1 to the observed effects is currently under investigation.

583 Biomedical applications of water-soluble pillar[*n*]arenes

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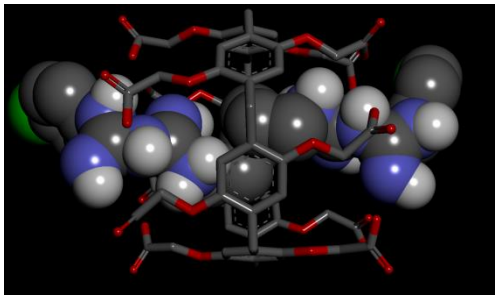
Introduction. Pillar[*n*]arenes are a new family of macrocycles that have shown potential in a range of different applications (Ogoshi 2016). Unfortunately, native pillar[*n*]arenes are not water soluble and therefore have few biomedical applications; but the development of water-soluble carboxylated-pillar[*n*]arenes has now opened up their potential applications (Wheate 2016).



Aims. To study host-guest complex formation between water soluble pillar[*n*]arenes and a range of drug and excipient molecules.

Methods. Host-guest complex formation was analysed by ^{1}H NMR, fluorescence spectroscopy, and molecular modelling. Toxicity to human cells was analysed using in vitro growth assays with the OVCR-3 and HEK293 cell lines.

Results. Both carboxylated-pillar[6]arene (WP[6]) and carboxylated-pillar[7]arene (WP[7]) form host-guest complexes with memantine, chlorhexidine hydrochloride, and proflavine by ^{1}H NMR and modelling. Binding is stabilised by hydrophobic effects within the cavities, and hydrogen bonding and electrostatic interactions at the portals. Encapsulation within WP[6] results in the complete and efficient quenching of proflavine fluorescence, giving rise to “on” and “off” states that have potential in biodiagnostics. The toxicity testing of the pillar[*n*]arenes to the OVCAR-3 and HEK293 cell lines showed that they are relatively non-toxic to the cells except at high doses and after prolonged continuous exposure.

Discussion. The pillar[*n*]arenes form a range of host-guest complexes depend on the size of the pillar[*n*]arene and the structure of the guest. Overall, the results show that there could be a potentially large range of medical applications for carboxylated-pillar[*n*]arenes.

Ogoshi T et al (2016) Chem Rev.. 116: 7937-8002
Wheate N et al (2016) J Pharm Sci 105: 3615-3625¶

584 Distribution of therapeutic proteins into thoracic lymph after intravenous administration is protein size-dependent and primarily occurs within the liver and mesentery

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Introduction. The lymphatic system is a primary site for cancer metastases, proliferation of infectious diseases and the immune response to inflammatory diseases and organ transplantation.
Aims. This study aimed to determine, for the first time, the major sites of thoracic lymph access of therapeutic proteins, and the protein properties that enhance lymph access, after intravenous (IV) administration.
Methods. In order to achieve this, novel methods were developed or optimised to collect hepatic, mesenteric or thoracic lymph from male SD rats. Four different sized PEGylated or non-PEGylated therapeutic proteins (native interferon α2b (IFN, 19kDa), PEGylated interferon α2b (IFN-PEG12, 31kDa), PEGylated interferon α2a (IFN-PEG40, 60 kDa) or trastuzumab (150 kDa) were then administered *via* short IV infusion, and plasma and lymph concentrations of the proteins determined *via* ELISA.
Results. The recovery of the therapeutic proteins in the thoracic lymph duct, which collects lymph from most of the body, was significantly greater for trastuzumab, IFN-PEG40 and IFN-PEG12 (all >3% dose over 8 h) when compared to native IFN (0.9% dose). Conversely, the thoracic lymph/plasma (L/P) concentration ratio and thus efficiency of extravasation and transport through the interstitium to lymph was highest for the smaller proteins IFN and IFN-PEG12 (at 90-100% vs 15-30%). The lower total recovery of IFN and IFN-PEG12 in thoracic lymph reflected more rapid systemic clearance and shorter plasma circulation half-life. For all therapeutic proteins, the majority (>80%) of lymph access occurred *via* the hepatic and mesenteric lymphatics
Discussion. Optimising the properties of IV administered therapeutic proteins represents a viable approach to better target and treat pathological states involving the lymphatics, particularly in the liver and mesentery. This includes cancer metastases, infectious and inflammatory diseases. Successful development of the novel technique to collect hepatic lymph will also enable future work to evaluate tissue-specific lymph transport in health and disease.

585 Drug Use Evaluation of Levetiracetam at a Tertiary Teaching Hospital

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School of Pharmacy, Faculty of Health Sciences, Curtin University[1], Bentley, WA[1]

Introduction. The management of seizures in a hospital setting is conventionally governed by site guidelines and the Australian Therapeutic Guidelines. Although guidelines provide a gold standard of evidence based therapy, they are not always adhered to. In Australia, the use of levetiracetam is restricted at multiple points, in hospital guidelines, therapeutic guidelines and by Pharmaceutical Benefits Scheme (PBS) subsidisation, despite its favourable pharmacokinetic and pharmacodynamic characteristics. Aims. To evaluate the use of antiepileptic drugs (AED's) in the treatment of acute seizures and in seizure prophylaxis at a tertiary teaching hospital, with a focus on the use of levetiracetam and its prescribing habits. Methods. In this retrospectives study, 1133 patients were identified as having seizure codes during admission in 2016. Patients with a history of seizures prior to 2016 for which they were receiving drug therapy, were excluded. For patients included in the study, medical records were reviewed to identify new antiepileptic drug therapy during acute treatment, in-hospital prophylaxis and discharge therapy. Results. 153 patients met inclusion criteria and were reviewed with regards to antiepileptic therapy focusing on valproate, phenytoin and levetiracetam, of which 132 patients received AED therapy on discharge. Of these, 59 (44.7%) patients were discharged on levetiracetam but only 11 (8.3%) were diagnosed with partial seizures and 44 (33.3%) had no previous AED therapy. Of those discharged on levetiracetam, 48 (81.6%) were provided with a PBS supply of levetiracetam but only 10 (17.5%) were appropriate. Use of Levetiracetam was higher in undiagnosed and generalised seizures rather than partial seizures (p=0.033). Compliance with site specific seizure guidelines was 53.6±10.7% and compliance with Australian Therapeutic Guidelines was 55.6±7.9%. Discussion. There was a lack of compliance with guidelines for the use of levetiracetam in acute seizure management. The high use of levetiracetam in generalised and undiagnosed seizures as a first line therapy may warrant re-evaluation of the eTG treatment pathway for epilepsy to determine if levetiracetam is an appropriate first line option due to it favourable side effect and interaction profile when compared against other AED's.
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586 Hospital pharmacists' and patients' views about what constitutes effective pharmacist-patient communication

Bernadette AM Chevalier,[1] Bernadette M Watson,[2] Michael A Barras,[1,3] W Neil Cottrell[1]
School of Pharmacy, The University of Queensland,[1]Brisbane, QLD; Department of English, The Hong Kong Polytechnic University,[2] Hong Kong; Pharmacy Department, Princess Alexandra Hospital,[3]Brisbane, QLD

Introduction. Effective conversations between patients and healthcare professionals are necessary for patients to understand and manage their medications. Knowing what patients need from a conversation with a pharmacist about their medications may assist hospital pharmacists in preparing patients for discharge and supporting patients' medication management. There are no published studies investigating hospital pharmacists' and patients' opinions about what constitutes an effective pharmacist-patient conversation.
Aims. To explore hospital pharmacists' and patients' views about what constitutes effective communication exchanges between pharmacists and patients.
Methods. Audio recorded, semi-structured interviews were held separately with pharmacists and patients following their shared medication counselling sessions. Twelve pharmacists engaged four patients each (48 interactions in total) within a large quaternary hospital. Participants were asked questions about what made pharmacist-patient conversations effective. Transcribed recordings were analysed using a process of inductive thematic analysis and then mapped to Communication Accommodation Theory (CAT) strategies.
Results. Ensuring patients were confident in managing their medications was the overall shared goal for participants. Shared themes for effective communication exchanges (*mapped to CAT strategies*) included, *well-explained information (interpretability)*, *engagement* (discourse management), *established rapport* (emotional expression) and *empowerment (interpersonal control)*. Participants offered rich exemplars for these themes.
Discussion. Pharmacists and patients provided valuable insights about what makes pharmacist-patient interactions effective. Patient identified preferences may help guide practitioners to engage patients in effective conversations and assist in the training of pharmacy students.

587 Improving community pharmacy management of non-prescription medicine requests with mystery shopping and feedback

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Introduction. Medicines are a common form of intervention worldwide. In recent years a large number of medicines have moved from prescription-only to non-prescription status. As pharmacies are key locations for the supply of medicines it is important to ensure that pharmacists and their staff are adherent to guidelines and provide optimal care to their patients. Mystery shopping with feedback is a form of audit and coaching that can be employed to improve pharmacy practice.
Aims. To determine if repeated mystery shopping visits with feedback improve pharmacy performance over the course of nine visits, and to determine what factors predict the occurrence of an appropriate outcome.
Methods. Sixty-one Bachelor of Pharmacy students from the University of Sydney acted as mystery shoppers to visit 36 community pharmacies in metropolitan Sydney, Australia. Students underwent theoretical and practical training then presented to an allocated pharmacy each week for nine weeks with a prescribed scenario. Standardised scoresheets were used to score each interaction. Students re-entered the pharmacy within five minutes to provide the staff member involved with feedback and coaching. Data were collated and statistically analysed.
Results. 521 visits were eligible for analysis, 54% of these resulted in an appropriate outcome. Questioning scores and the proportion of interactions resulting in an appropriate outcome significantly improved over time ($P<0.05$). Involvement of a pharmacist, the visit number, increased questioning score, and the prescribed scenario were significant predictors of an appropriate outcome ($P<0.05$).
Discussion. This is the first study to use mystery shopping with feedback across a large number of minor ailment scenarios with multiple repeated visits. The intervention improved pharmacy performance over time across all scenarios, however when examining individual scenarios this was not always the case. This inconsistency may be attributed to the varying difficulty of the scenarios. Consistent with previous work, increased information gathering and involvement of a pharmacist were positive predictors of appropriate outcome. Future work should target scenarios where staff performed poorly and explore means to strengthen the intervention. ¶

588 Surgical antibiotic prophylaxis use and infection prevalence in breast surgery procedures in a major teaching hospital in Western Australia.

Ainslie K Lavers[1], Wai Siong Yip[1], Bruce Sunderland[1], Richard Parsons[1], Jason Seet[2], Sarah Mackenzie[2] Petra Czarniak[1]. School of Pharmacy, Faculty of Health Sciences, Curtin University[1], Bentley, WA; Sir Charles Gairdner Hospital[2], Perth, WA

Introduction. Surgical site infections (SSI's) are a common complication following breast surgery procedures, despite being considered a clean surgery. The prevalence of SSI's can be minimised with the appropriate use of antibiotic prophylaxis such as outlined in the Australian Therapeutic Guidelines (eTG). Aims. The primary objective of this study was to evaluate the level of adherence to the eTG for antibiotic prophylaxis in breast surgery procedures at a Western Australian teaching hospital since the eTG 2014 update. Other aims were examining the impact of prophylactic and post-operative antibiotics on the incidence of SSI's and length of hospital stay. Methods. A retrospective cross-sectional study reviewed medical records from a randomised sample of 250 patients from 973 who underwent a breast surgical procedure between February 2015 and March 2017. Results. Overall adherence to current eTG occurred in 54.4% (123/226) of operations. Pre-operative antibiotics were prescribed in 98.4% (246 of 250) operations. Adherence rates to three specific elements of eTG (drug prescribed, drug dosage and timing of administration) were 91.6% (226/250), 52.8% (132/250) and 96.4% (216/224) respectively. For the 36 of 250 (14.4%) patients with relevant drug allergies, there was a total lack of adherence to the eTG. Post-operative antibiotics were prescribed in 11.2% (28/250) operations. Overall SSI prevalence was low at 5.2% (13/250). No statistical significance was found between SSI's and adherence to eTG. A statistically significant relationship was found between certain procedures, including soft tissue biopsy and hematoma drainage and developing SSI's ($p=0.027$, $p=0.000$ respectively). The mean length of stay in patients was 2.3±1.7 days, with no statistical relationship found between overall level of eTG adherence ($p=0.131$) or SSI's ($p=0.306$). Discussion. Although there has been some improvement in overall appropriateness of surgical antibiotic prophylaxis from 13.3% to 54.4%, further improvement is necessary especially with respect to timing of antibiotic administration and when allergy to the primary recommended antibiotic occurs, that the recommended alternative antibiotic is selected.

589 Psychometric testing of scales measuring perinatal depression literacy and comfort with providing perinatal depression care

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

Introduction. Pharmacists' increased involvement in mental health care, including perinatal depression (PND), warrants research exploring their mental health literacy and comfort with providing care. Despite widespread use in the literature, mental health literacy scales lack psychometric testing.
Aims. To assess the psychometric properties of two scales measuring PND literacy and comfort with providing PND care.
Methods. Bachelor of Pharmacy students completed three PND literacy scales (34 items) at two time points, approximately one month apart. They also completed a 7-item scale measuring their comfort with providing PND care. Test re-test reliability analyses of the literacy scales were conducted using Wilcoxon Signed Rank Test ($p<0.05$). Exploratory factor analysis and Cronbach alpha calculations were conducted to explore the construct validity and internal consistency reliability, respectively, of the comfort scale.
Results. A matched sample of 47 pharmacy students was obtained. Test re-test analyses indicated that 31/34 items were reliable, as demonstrated by non-significant p-values. Principal axis factoring (n=106) with direct oblimin rotation of the comfort scale resulted in a two-factor solution with 64.5% variance explained. Factor One contained four items (0.599-0.910) and pertained to comfort with referring PND patients to external healthcare services. Factor Two contained three items (0.492-0.698) and pertained to comfort with providing care by a pharmacist. One item cross-loaded (<0.2 difference) on both factors. It is recommended that this item is modified prior to the distribution of the scale.
Discussion. There is a lack of psychometrically tested measurement tools when measuring constructs pertaining to PND[1]. By exploring the psychometric properties of the scales, the subsequent reliable and valid measurement of these constructs can be conducted in a standardised and uniform manner across studies and population groups.
El-Den S, O'Reilly CL, & Chen TF. (2015). A systematic review on the acceptability of perinatal depression screening. *Journal of Affective Disorders, 188*, 284-303.¶

590 Communication between community pharmacies and prescribers in New Zealand

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Introduction. Phone calls between pharmacists and prescribers play an important role in resolving potential errors and other issues. Despite their importance in patient care, and at times being a source of frustration for pharmacists, there is very little research on these calls.
Aims. To quantify how often phone calls occur between pharmacists and prescribers, how much time is spent on these calls, who is called, and what are the reasons for these calls.
Methods. An observational study was conducted in 11 pharmacies over 8 weeks in Dunedin, New Zealand. Data captured included information on date, time, length, pharmacy staff involved, health professionals involved, the place being called and the purpose of the call. We also surveyed pharmacists' perceptions of this communication.
Results. Data on 95 phone calls and 63 faxes were captured. The mean length was 110 seconds (95% CI 88-133), at an average of 0.7 calls per hour. Incoming calls were shorter than outgoing calls, at least in part because of delays in getting hold of the prescriber. The most frequent reasons for calling were clarifications and dose inquiries. Pharmacy staff underestimated by half the number of incoming calls, relative to the observed data.
Discussion. Calling prescribers was perceived as a frustrating. The observed frequency of calls was low but some calls were long. Time for single pharmacist interventions may be reduced using alternative communication methods but these need further study.¶

591 Medicine use in early childhood: Which vaccines, branded or generic medicines do parents of children five and under choose?

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Introduction. Despite generic medicines being bioequivalent and cheaper, many people prefer branded medicines to generic medicines. We are not aware of any research that looks at parents' use of branded and generic medicines for their children. We were also interested in linking this vaccination behaviour, as choosing branded medicines for children may be seen as a protective behaviour. In contrast, both generics and vaccines are evidence based, so these could be associated.
Aims. This study aimed to determine the understandings and perceptions of parents of children aged five and under about generic and branded medicines, alongside their medicine preferences for their children and themselves. It also looked at parents' opinions surrounding vaccinations, whether they vaccinated themselves and their children, and whether there was a link between vaccination behaviour and preference for branded/generic medicines.
Methods. Parents of children under five were recruited through an online panel to match the New Zealand demographic profile for parents (by age, gender, ethnicity and region). They completed an online survey about the medicines they use for themselves and their children, the vaccines they and their children have received, and a variety of questions about their perceptions of vaccines, and branded and generic medicines. We also tested their ability to identify branded from generic medicines.
Results. 196 participants met the eligibility criteria and completed the survey. Parents preferred generic medicines (43%) over branded (25%) for themselves but mostly had no preference for their children. Participants more able to identify branded from generics were more likely to choose generics for themselves and their children, $ps < .001$. Parents who got more vaccines for their children were weakly less likely to report having no preference between branded and generics for their children, $p = .02$, but overall there was little link between these choices.
Discussion. Parents' vaccine choices for their young children were not linked to their preferences for branded/generic medicines for their children. Further analyses looking at how demographic variables and perceptions of these products may determine strategies that can be used to improve child health. ¶

592 What is the attitude of Australian pharmacists to the use of medicines for assisted dying at end-of life?

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Introduction. Many Australian State Governments have initiated debate into legalising physician-assisted dying and euthanasia for patients enduring intolerable suffering in end-of-life care. Since it is likely that medications will be utilised to facilitate the patient's death the views of the pharmaceutical profession have not currently been sought. There has been little research undertaken in this area by pharmacists
Aims. The aim of this research was to collect information to obtain the views of pharmacists to physician-assisted dying, euthanasia and palliative care.
Methods. Australian pharmacists were invited to complete an online survey to obtain their views on these topics.
Results. 93 pharmacists from a mixed background completed the survey over the period June - July 2017. 69% identified themselves as community-based; 18% as hospital-based and 3% as academic. Age groups matched Australian pharmacist demographics; 18% < 30 years, 30% 30-39 years, 15% 40-49 years, 25% 50-59 years and 12% >60 years: 63.4% females and 35.5% males. The majority claimed some religious identify (57%) and 34.4% as without. 69.9% of responders supported this legislation, 20.4% did not, 8.6% were unsure and 1.1% were unwilling to answer. 55.9% were concerned that this was a 'slippery slope where vulnerable patients might be put at risk'. 62.4% of respondents would be willing to assist supplying medication on prescription. Many respondents considered that physician-assisted dying already occurred in Australia, with 20.4% believing it to be common practice.
Discussion. Further data evaluation will be presented evaluating respondents confidence in symptom management in palliative care; whereas confidence in the management of pain, constipation, nausea and vomiting were high there was less confidence in the management of delirium and dyspnoea and in the use of non-opioid analgesics such as ketamine. Most respondents thought that palliative interventions were inadequate in the management of intolerable pain and suffering (59% vs 41%) suggesting a lack of confidence in palliative intervention.¶

593 Health professionals' opinion of a brief email format for answering medicine information enquiries.

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Introduction. The quality and timing of responses to medicines information (MI) enquiries can affect their usefulness to the recipient for optimising medicines and improving patient outcomes. At our MI service, an increasing demand for an emailed written answer, prompted us to design a brief email format.
Aims. To assess healthcare professionals' opinion of a brief email format for answering MI enquiries.
Methods. The brief email format was used to answer MI enquiries requiring a written response, for 6 months. Enquiries relating to pregnancy or lactation and those requiring long answers were excluded. An electronic survey was sent to all enquirers receiving a brief email and could be completed anonymously. The survey used a 5-point Likert scale (strongly agree, agree, uncertain, disagree, strongly disagree) to assess the recipient's opinion of the brief email format. Their opinion was sought in five domains: overall satisfaction with the answer to the MI enquiry, whether they thought the answer was too brief, were they happy to contact the service if they needed more detail or further information about their enquiry, whether they would prefer a fully-referenced answer, and if they would like to have copies of key references attached to the email. Primary outcomes were enquirer satisfaction with the quality and length of the answer to their MI enquiry. Secondary outcomes were enquirer preferences with respect to references.
Results. For the first two months of the 6 month period, we have used the brief email format 25 times to answer MI enquiries requiring a written response. To date, we have have received 10 completed surveys (40% response rate). These showed: almost all recipients (9/10) agreed or strongly agreed that they were satisfied with the answer to their MI enquiry, most (8/10) disagreed or strongly disagreed the answer was too brief, 5/10 recipients were uncertain about whether they wanted a fully-referenced answer or whether they wanted copies of key references attached to the email. To follow are the results from the completed surveys received over the next 4 months.
Discussion. The results from the surveys received to date indicate our enquirers consider the brief email format acceptable for answering MI enquiries requiring a written response. There appears to be uncertainty regarding inclusion of references. The results from surveys received over the next 4 months will show whether these trends continue.¶

594 Pharmacy & The Ethical Dilemma of Physician Assisted Suicide (PAS): A Systematic Review

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Introduction. The right to die with dignity has been legalised in certain countries, but to date, not in Australia where it remains illegal. However, this remains an active issue in the community and legislature. Pharmacists need to be prepared and use international experiences to help establish boundaries that protect all those who are involved. While there have been several studies on physicians, nurses and the public's views on PAS little research has been conducted on pharmacists and none on Australian pharmacists. The aim of this study was to systemically review the literature related to PAS and the role of pharmacists.
Methods. A systematic review of articles collected from 6 databases, MEDLINE, PubMed, EMBASE, CINAHL, SciFinder and International Pharmaceutical Abstracts (IPA). Inclusion criteria were limited to articles published in English from January 1987 to June 2017 clinical data on pharmacists' views on PAS and euthanasia. References of the included articles were also reviewed for any additional trials that may have met inclusion criteria.
Results. Eight qualitative studies met our inclusion criteria. The reviewed studies were of registered pharmacists across four countries: Netherlands, Belgium, UK and US, which have legislation enabling euthanasia or physician assisted suicide in some parts of, if not the entire country. The majority of pharmacists within these studies accepted the right of a patient to choose their own death at the end of life. Reports indicate that 45% of those surveyed were unsure of, or against a physicians' assistance in a patient's death. This review identified a willingness from pharmacists to partake in the dispensing of drugs for PAS if legal with the appropriate conditions and protocols for support were made available.
Discussion. This is the first systematic review of the literature pertaining to pharmacists' views on PAS and euthanasia. This systematic review sheds light on the significance of pharmacists' views, in what needs to be an interdisciplinary discussion on the legislative and ethical challenges associated with PAS and euthanasia This systematic review identifies the need for further research and greater pharmacist-based studies in this topical debate. Australian based qualitative studies on pharmacists' attitudes are especially important, in order to help shape new legislative protocols in the dawn of PAS legalisation in Australia.¶

595 Common co-morbidities and polypharmacy in elderly patients in a South Australian tertiary healthcare hospital

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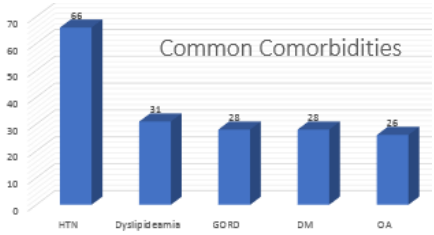

Introduction. The delivery of safe, effective and optimal treatment in an increasingly aged population is a global issue as the older patients have multiple morbidities and are the largest consumer of medicines.
Aims. The aim of this study was to characterise elderly patients who were ≥ 75 years on admission to a tertiary health care hospital in terms of co-morbidities, prescribed medications and related adverse effects.
Methods. The inclusion criteria were patients who received ≥ 5 medications and were aged ≥ 75 years on admission to Royal Adelaide Hospital, Adelaide, SA between September 2015 to September 2016. Each patient's case notes were examined for co-morbidities, prescribed medications and adverse effects as at a recorded date. Medications were classified using ATC (anatomical therapeutic classification) codes and analysed using SPSS software.
Results. A total of 474 patients (42% males) have been identified to date, with a mean age of 84 ± 6 years. Fig. 1 shows the top co-morbidities found. The top five classes of medication taken by these patients were: anti-inflammatory and anti-rheumatic medication (80%), anti-thrombotic (69%), drugs for acid related disorders (53%), agents acting on renin-angiotensin system (50%) and lipid modifying agents (48%). The top five common diseases were; hypertension (65%), dyslipidaemia (31%), GORD (28%), diabetes (28%) and osteoarthritis (26%). Polypharmacy was found to be associated with a higher incidence of adverse drug reactions, drug-drug interactions, inappropriate drug use and non-adherence.
Discussion. Our pilot data on prescribed medications and co-morbidities in elderly patients at South Australia's largest tertiary health care hospital suggests that polypharmacy and its sequelae is an ongoing issue for them.¶

596 Weight loss product usage and advice in community pharmacies in North Queensland

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Introduction. Obesity is currently one of the greatest health challenges in Australia, particularly in North Queensland, where in 2011-12, 75% of people living in the Townsville Mackay Region were either overweight or obese.[1] Community Pharmacies play a significant role in the management of obesity through the provision of weight management programs and products as well as by providing weight loss advice to consumers. However, there is limited information available regarding the weight loss products that are recommended by pharmacies, whether they are providing evidenced based advice and whether consumers are making appropriate weight loss product choices.
Aims. To identify trends in weight loss product recommendations and advice provided by pharmacies to consumers, to ascertain consumer usage patterns and to investigate the reasons for consumer choices of weight loss products.
Methods. This project involved the distribution of a questionnaire to North Queensland Pharmacies. Responding pharmacies were then asked to distribute a brief survey to their consumers on the purchase of a weight loss product.
Results. 78 different products were listed among the top 10 weight loss products sold by the respondent pharmacies. Both the pharmacist and consumer surveys indicated that the most popular weight loss products were meal replacements, with complementary medicines also being used by a significant number of consumers. Consumers were found to be predominantly female between the age of 26 and 45 years. Information sources used by community pharmacies for the provision of weight loss advice showed potential for bias as they were mainly from company weight loss product or program resources.
Discussion. There is a need for more evidence based weight management training resources for community pharmacies. There is also a need for increased consumer awareness of the available evidence or lack of evidence for many weight loss products. Meal replacement products are a popular choice of product with some evidence of short-term benefits, however further studies to determine the long term efficacy of these products may be warranted. Given the higher obesity levels of males compared to females,[1] consideration should also be given to increasing the promotion of weight loss services to the male population.

1. Queensland Health. The health of Queenslanders 2014. Fifth report of the Chief Health Officer Queensland. Queensland Government website. https://www.health.qld.gov.au/publications/research-reports/reports/cho-report/cho-full-report.pdf. Published 2014. Accessed May 16, 2016. ¶

597 Factors associated with pharmacists' perceptions of working conditions in Canada

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Introduction: Previous evidence suggests that pharmacists often experience unsafe working conditions in the provision of patient care in the community pharmacy setting.
Aims: To determine the factors associated with perceived working conditions in five Canadian provinces.
Methods: A survey was administered with questions about demographics, practice, advanced clinical services offered to patients (prescription adaptations, immunizations, and medication reviews), and whether their practice site imposed monthly quotas for each of the advanced practice services. Respondents' satisfaction of their working conditions was assessed using six statements rated with a five point Likert scale. Associations were made using ordinal logistic regression.
Results: Of 11767 registered pharmacists who received an invitation to participate in this study, 2464 (21%) responded, 34% of whom were male. In general, pharmacists were not satisfied with their working conditions. Lack of time to do their jobs, inadequate time to have lunch, as well as inadequate number of staff were commonly reported. Overall, 20% of the pharmacists reported that they need to meet quotas for clinical services. Lack of satisfaction with working conditions was associated with quotas, filling >100 prescriptions per pharmacist per day, >20 minutes prescription wait time and working at chain pharmacy.
Discussion: Pharmacists rate their working conditions to be unsafe. Having corporate enforced quotas for reimbursed clinical services and working in chain pharmacies were associated with lower safety.
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598 What Is Polypharmacy Exactly (WIPE)

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Introduction. There are various definitions of polypharmacy and it is unclear how different clinicians define and assess polypharmacy in practice, which can provide important insight into medication review and rationalisation.
Aims. To develop a website which allows evaluation of different clinicians' assessment of polypharmacy and identification of medication related factors which are considered during medication review and rationalisation.
Methods. A website called What Is Polypharmacy Exactly (WIPE) was developed which presents de-identified patient cases from clinical practice at wipe.logicsquad.net/signup. For each case, the website presents the patient's age and setting, list of comorbidities and medications and asks users to i. rate the degree of polypharmacy ii. rate the potential for harm from medications iii. rate the potential to deprescribe medications and iv. nominate medication classes for deprescribing. WIPE provides users with feedback by expert clinicians after case completion as well as the ability to post comments and engage in clinical discussion regarding each case with other users on the website.
Results. There have been 212 responses on WIPE from 61 users comprising of hospital and community pharmacists, consultant physicians, resident medical officers and medical students. Initial data analysis shows that medication classes such as benzodiazepines, opioids, sedating antihistamines and antipsychotics obtained higher ratings regarding the degree of polypharmacy and the potential for harm compared to statins, inhaled medications and paracetamol. Clinicians were more likely to nominate the medication classes which were associated with higher degree of polypharmacy and potential to cause harm for deprescribing.
Discussion. Clinician ratings reflect important aspects of medication review and rationalisation where medications which are identified as having the potential to cause harm are assessed for the possibility of deprescribing in order to optimise patient outcomes. WIPE can be used as an educational tool and allows a novel platform for users at the national and international level to work together to collectively define polypharmacy, in order to develop clear prescribing guidelines and improve patient outcomes. ¶

599 Do Australian Pharmacists feel prepared to respond to local disasters and emergencies?

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Introduction. During disasters local communities are the first to respond, often working for days before reinforcements from outside agencies can arrive. Health professionals provide essential roles and services during these events. For many health professionals a plethora of literature, competencies, and training support their roles within the disaster space. Despite the important role pharmacists play within the healthcare team, their preparedness to respond to disasters is unknown. Additionally, little is known about what supports pharmacists need to feel more prepared to respond to a disaster.
Aims. To determine how prepared Australian pharmacists feel to fulfil roles in local disasters and what supports they require to become more prepared.
Methods. A collection of semi-structured interviews led to the development of a mixed-methods survey. This survey will be launched at APSA-ASCEPT 2017 with registered pharmacists invited to participate. Contributors will be asked to self-assess their preparedness for playing a variety of roles that may affect their local community in a disaster. Additionally, this project will explore how pharmacists believe they could improve their preparedness, or how they could be supported in disasters.
Results. Results from this survey will feed into a larger research project examining disaster preparedness for pharmacists. The main objective of this research project is to determine how pharmacists can be better prepared to fulfil roles in disasters in Australia. Potential outcomes include competency development, legislative change, and/or short training courses for pharmacists.
Discussion. Pharmacists are essential health professionals during disasters. Unfortunately, little is known about how prepared pharmacists feel to assist in disasters and how they can be supported to play a role. The ultimate goal of this research is to improve local preparedness and professional resilience in Australian disasters and emergencies.
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600 Over-the-counter medicines: the complexity of decision-making for pharmacy students

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Introduction. Community pharmacies are frequently accessed by consumers for minor ailment advice and over-the-counter (OTC) medicines. Various factors influence pharmacist and support staff decision-making in OTC consultations, yet, limited attention has been placed on pharmacy students as pharmacists-in-training.
Aims. To identify how factors affect OTC decision-making by pharmacy students, and to explore the factors influencing OTC medicine choice.
Methods. In-depth data were collected by semi-structured interviews with Queensland pharmacy students and analysed using the Critical Incident Technique. Student stories of OTC experiences were identified as critical incidents if they contained: (i) a description of the situation or trigger which led to the incident; (ii) information about student action/s or behaviours; (iii) an outcome, e.g. referral, medicine supply or refusal. Interview transcripts were coded for incidents, and using thematic analysis, factors were identified within incidents that influenced OTC decision-making.
Results. Ten pharmacy students identified 131 critical incidents, which were mostly pain, dermatology and cold related enquiries. Nine overarching themes influenced student decision-making, with a particular emphasis on customer response, confidence and scope of practice. Product requests were reported as more challenging than symptom requests; this was due to consumer expectations. Negative consumer responses prompted medicine supply against evidence-based guidelines, but only when this was assessed as safe. Real-life practice was suggested to be more effective than university learnings in developing decision-making skills.
Discussion. It became clear that OTC decision-making is a complex process for students. Pharmacy educators should consider learning activities that support students to assertively interact with consumers, and additional opportunities for experiential learning, such as work-based placements and role-plays with simulated patients.

Flanagan JC (1954). Psychol Bull. 51:327-358.¶

601 Insights into consumer use, storage and disposal of unwanted and expired medicines

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Introduction. Consumers can hoard medicines for 'just in case' use.(1) Unwanted medicines are commonly discarded in the rubbish or drain,(2) with health and environmental implications. We have limited insight into these practices.
Aims. To qualitatively explore the quantity and nature of unwanted or when required medicines in the home and self-reported practices related to medicine storage, accumulation, use and disposal.
Methods. Structured telephone interviews were conducted with people who used five or more medicines including prescribed, non-prescription medicines and/or complementary and alternative medicines such as vitamins. Interviews were transcribed verbatim, and integrity of data analysis was assured through research debriefs, quality checking of transcripts and thematic coding by two experienced researchers.
Results. Participating consumers reported 1424 unwanted medicines stored in various locations in 166 households. Although participants did not intentionally stockpile medicines by seeking out early dispensing of repeat prescriptions, a number did keep medicines 'just in case' they were needed in the future, including antibiotics. Some participants reported using expired medicines guided by individual risk assessment strategies. When asked about the risks of storing unwanted medicines, ingestion by children and pets and decreased efficacy of expired medicines were described. However, this knowledge did not always translate to appropriate storage, use or disposal of medicines.
Discussion. Knowledge of the risks of inappropriate medicine storage, use and/or disposal did not guarantee appropriate management of unwanted medicines. Application of variable individualised risk-benefit assessments emerged, with implications for health professionals and the environment. Greater exploration of the underlying basis and significance of these is needed to enable us to identify and address misconceptions.

1.Vellinga A, Cormican S, Driscoll J, Furey M, al e. Public Practice Regarding Disposal of Unused Medicines in Ireland. *Science of the Total Environment*. 2014;478:98-102.
2.Bettington E, Spinks J, Kelly F, Gallardo-Godoy A, Nghiem S, Wheeler AJ. When Is a Medicine Unwanted, How Is It Disposed, and How Might Safe Disposal Be Promoted? Insights from the Australian Population. *Australian Health Review*. 2017;in-press, accepted June 2017.¶

602 Non-prescription sales of antimicrobials in developing countries: a systematic review

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Introduction. Antimicrobial resistance (AMR) is a critical global challenge. Developing countries are more vulnerable to AMR than developed nations due to many complex issues pertaining in the health care system. One contributing factor to inappropriate antimicrobial use is the non-prescription availability of antimicrobials at community pharmacies.
Aims. The aim of this systematic review is to investigate non-prescription sales of antimicrobials in developing countries and assess the contributing factors to non-prescription sales in these countries.
Methods. EMBASE, MEDLINE, SCOPUS, International Pharmaceutical Abstracts and Web of Sciences were searched for articles, published between 1980 and the end of April 2017, that involved studies using simulated patient study designs that evaluated the availability of antimicrobials in community pharmacies in developing countries.
Results. 37 studies from 22 developing countries across Asia, Africa, South America, South-Eastern Europe and the Middle-Eastern regions reported antimicrobial sales without a prescription. The percentage of antimicrobials dispensed without prescription in these countries ranged from 15% to 90%. Poor medicines regulations, lack of available suitably-qualified pharmacy staff, commercial pressure on pharmacy staff, consumer demand, inappropriate prescribing practices and lack of AMR awareness were reported as contributing factors that facilitated non-prescription sales of antimicrobials in developing countries.
Discussion. Non-prescription sales of antimicrobials are substantial in developing countries and a significant contributing reason for overuse and misuse of antimicrobials in the community. Non-prescription sales of antimicrobial agents are associated with inappropriate drug choice, short duration of therapies and wrong dose. Inappropriate prescribing and supply practices contribute to the development of AMR. A multi-faceted approach is required to address the contributing factors facilitating non-prescription sales in order to reduce AMR. ¶

603 Principlism: An approach for determining ethical responsibilities of pharmacists when selling complementary medicines

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Introduction. Principlism is an ethical framework that consists in the application of the four bioethical principles to make decisions in healthcare: respect autonomy, beneficence, non-maleficence and justice. No explicit ethical framework is employed in the pharmacy literature describing the responsibilities of pharmacists when selling complementary medicines. Research regarding the responsibilities of pharmacists when selling complementary medicines consists predominantly of empirical studies. This research tends to focus on the perceptions of pharmacists, pharmacy support staff and consumers regarding pharmacist responsibilities. A number of ethical conflicts for pharmacists are identified in this literature, but little attempt is made to resolve these conflicts.
Aim. To assess principlism as an explicit ethical framework for determining ethical responsibilities of pharmacists when selling complementary medicines.
Methods. The theoretical literature describing principlism and the arguments regarding merits and otherwise of principlism are analysed to explore this approach and its key components.
Discussion. Principlism is typically criticized on the basis of its theoretical foundations and its ability to provide practical guidance when ethical conflicts are identified. It is common in healthcare to accept these criticisms and employ principlism as a form of 'ethics first-aid': a way to identify conflicts without any attempt to resolve them. We argue against this approach. We identify key developments within principlism that clarify its theoretical foundations and provide resources for resolving ethical conflict. We show how developments such as basing the principles in common morality, employing reflective equilibrium and specified principlism provide the necessary theoretical resources for determining pharmacist responsibilities when selling complementary medicines. This work provides the basis for a more informed discussion of pharmacist responsibilities when selling complementary medicines.¶

604 Implementation of the Goal-directed Medication review Electronic Decision Support System (G-MEDSS)

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Introduction. People with dementia in the community setting are prescribed more medications compared to people without dementia, and are particularly vulnerable to the adverse effects of high-risk medications (e.g. anticholinergics, antipsychotics and benzodiazepines). Implementation studies of Computerised Clinical Decision Support systems (CCDSS) interventions have demonstrated effective improvement in appropriate prescribing in older adults. We have developed the Goal-directed Medication review Electronic Decision Support System (G-MEDSS), a CCDSS that incorporates validated deprescribing tools and guides (e.g. the Drug Burden Index (DBI), Patient Attitudes Towards Desprescribing questionnaire and Goals of Care) into pharmacist Home Medicines Review (HMR).
Aims. (1) To test the efficacy and safety of the addition of the GMEDSS in HMR to reduce anticholinergic and sedative medication use in patients with/out dementia; (2) To measure the impact of the medication changes on clinical and functional outcomes.
Methods. This study is a two-arm, parallel group, cluster-randomised trial (ACTRN12617000895381). Accredited Pharmacists (AP) who meet the inclusion criteria will be randomised into the intervention (usual care + CCDSS + G-MEDSS report provided to the patient and patient's referring GP) or control (usual care HMR) group. All AP will undergo training and will be required to pass an online competency MCQ questionnaire. Accredited Pharmacists will collect data (e.g. medication profile, cognitive and physical function) from patients at baseline (during HMR interview) and at 3-months follow up. The primary outcome will be proportion of patients with a reduced DBI. The required total sample size is 500, with 50 pharmacists in each arm of the study to recruit 5 to 10 patients. This will allow us to detect a 10% difference between arms α=0.05 2-sided, 1-β=0.8, intra-cluster correlation = 0.07.
Discussion. We anticipate that the G-MEDSS will reduce anticholinergic and sedative medications, incorporate patient's attitudes towards describing and patients goals in the HMR. This may reduce the proportion of older adults using inappropriate medications and improve clinical outcomes in older adults.¶

605 Pharmacist perceptions of psychotropic monitoring in australian aged care facilities

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Introduction. Current Australian guidelines suggest that the modest benefit of psychotropic medicine use in the geriatric population may be outweighed by associated morbidity and mortality. Psychotropic medication monitoring by Health Care Professionals (HCPs) may be valuable in reducing adverse effects resulting from this class of medicines. The extent to which psychotropic medication monitoring occurs in Aged Care Facilities (ACFs) and the factors which influence monitoring are not well established.
Aim. This qualitative study aimed to explore psychotropic medication monitoring from the perspective of pharmacists and ascertain perceived barriers and enablers to psychotropic monitoring in ACFs.
Methods. A convenience sample of 10 accredited clinical pharmacists who work in ACFs were selected for inclusion. Semi-structured face-to-face interviews were conducted and a range of questions assessing perceptions of monitoring, facilitators, barriers and proposed solutions were included. Interviews were transcribed verbatim and analysed using Nvivo software.
Results. Monitoring is a multi-faceted concept which is influenced by factors at the individual, group, organisation and system level. Thematic analysis revealed 8 primary themes: (i) patient autonomy and characteristics such as medical diagnosis and ability to consent, (ii) education of nurses and general practitioners, (iii) communication channels, (iv) ACF culture, (v) roles and responsibilities (vi) resource allocation such as staffing levels and time constraints (vii) guidelines and protocols and (viii) lack of remuneration.
Discussion. Pharmacist's felt that psychotropic medication monitoring in ACFs is largely suboptimal and recognised a need for significant improvements in practices. Pharmacists saw themselves as enablers to improving psychotropic monitoring and expressed that improved remuneration and resourcing as well as optimised communication channels and education for other HCPs would facilitate this.

606 Do Pharmacists Fit in the Disaster Health Management Team Puzzle?

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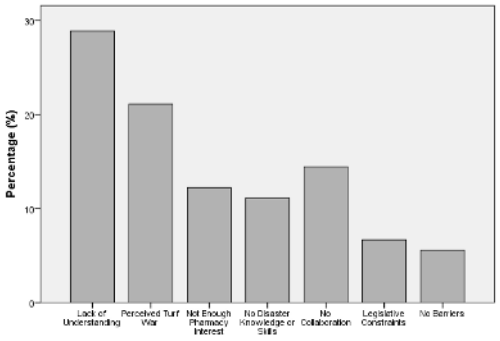

Introduction. Pharmacists have previously been involved in coordinating the logistics and ensuring the supply of medications in the event of a disaster. Over the last two decades, there has appeared in the literature 'poorly documented' new roles that pharmacists have undertaken in disasters. (Ford et al. 2013; Young 2005). However, the acceptance of these roles for pharmacists by the disaster health community is not known.
Aims. To determine the global opinion of the disaster health community as to the roles pharmacists could be undertaking in disasters in addition to logistics and supply chain management.
Methods. Quantitative survey released at the World Association for Disaster and Emergency Medicine (WADEM) 20th Congress from 25th – 28th April 2017, in Toronto, Canada. Data analysed using SPSS software.
Results. 126 surveys were completed out of 222 handed out (56.8%). The majority of respondents (96.7%) believed pharmacists had a role in disasters additional to the logistics and supply chain management. Out of 11 roles provided in a 5-point Likert scale, eight roles received 72.4% or higher 'agree or strongly agree' rating. The other three roles received equal neutral to 'agree or strongly agree' ratings. Lack of understanding of a pharmacist's roles and capabilities was the highest described barrier (28.9%), preventing pharmacists from being included in disaster health teams.
Discussion. The disaster health community agreed pharmacists have roles in disasters in addition to the all-important logistics and supply chain management. When provided with different roles pharmacists have performed in the literature, the disaster health community agreed pharmacists could undertake most of the roles listed. However, also named were several barriers that could be the reason pharmacists aren't currently included in disaster health teams.

607 How do health professionals perceive medicinal cannabis? Results of a systematic review

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Introduction. The number of jurisdictions allowing access to medicinal cannabis has been steadily increasing since the US state of California introduced legislation in 1996. As of 2017, Australian citizens can legally access medicinal cannabis. Unlike jurisdictions such as California, the authorisation and supply medicinal cannabis in Australia is tightly controlled. This uniquely places Australian health professionals at the forefront of therapy.
Aims. To conduct a systematic review exploring the existing primary literature focusing on the perceptions, concerns and knowledge of health professionals regarding medicinal cannabis.
Methods. PubMed, EMBASE and Scopus were searched for articles indexed up to the 31st March 2017 (English language and studies involving humans only). Inclusion criteria were (a) primary research findings; (b) participants were health professionals (c) the study considered 'medicinal' cannabis. Exclusion criteria were: (a) study indexed as an abstract, editorial, commentary or review; (b) the study considered 'recreational' cannabis; (c) participants were students or non-health professionals. Duplicate entries were removed and remaining articles were screened against title, abstract and keywords, followed by full text review of eligible articles.
Results. Of the 2751 articles originally retrieved, 57 underwent full-text review and 18 of these were included. A major similarity among these studies was a high degree of support for medicinal cannabis by health professionals. Irrespective of these attitudes, significant concerns and barriers towards uptake existed. Six subthemes describing barriers and concerns were identified: 1) knowledge, 2) education, 3) availability of information, 4) public health, 5) safety and 6) current legislation. A lack of knowledge was reported by most irrespective of profession or expertise.
Discussion. The literature suggests that although there is a high degree of support for medicinal cannabis, considerable barriers and concerns have the potential to influence health professional decisions, potentially reducing access to treatment for those in need. These results demonstrate that barriers and concerns need to be addressed, in particular, a lack of knowledge, a desire for more education and the availability of knowledge. Subthemes allow us to focus on developing interventions to mitigate these concerns and barriers.¶

608 Community pharmacists' perception of their role in primary mental health care

Mary A Wong, Caroline J Morris, Eileen M McKinlay, Dept Primary Health Care and General Practice, Univ of Otago, Wellington, New Zealand.

Introduction. Rates of mental health-related issues continue to rise in New Zealand, particularly for Maori and Pacific peoples. In recent years there has been an increased focus on primary mental health care to improve access and outcomes for those with mild to moderate conditions. As part of primary health care, community pharmacists can contribute towards this goal. The role of the community pharmacist has evolved over several decades from primarily a supply function to providing clinical pharmacy services including drug information and assisting in medicines optimisation to improve patient outcomes.
Aims. To explore community pharmacists' perception of their role in primary mental health care. Specifically, to identify the services that community pharmacists provide for those with mental health issues and the barriers and facilitators to providing mental health care.
Methods. A qualitative study that involved semi-structured, face-to-face, audio-recorded interviews with 15 practising community pharmacists throughout New Zealand including a broad demographic mix was undertaken. Interviews were transcribed verbatim, coded and analysed using a thematic approach.
Results. Community pharmacists believe that they have an important role to play in primary mental health care. There is, however, a wide range and variation in services provided. These spanned from simply dispensing prescriptions with no patient interaction to all-encompassing patient centred care. Barriers to service provision included lack of time, funding and training; difficulties with privacy and confidentiality in the pharmacy setting; stigma related to mental health.
Discussion. Community pharmacists hold diverse views about their role in primary mental health care. Some describe patient centred care while others describe a limited role and the significant challenges they face. They all endorsed the importance of the long-term relationships community pharmacists hold with patients and other health professionals in delivering effective care to this patient group.

609 Electronic prescribing of insulin with Medications Management, Anaesthetics & Research Support (MARS)

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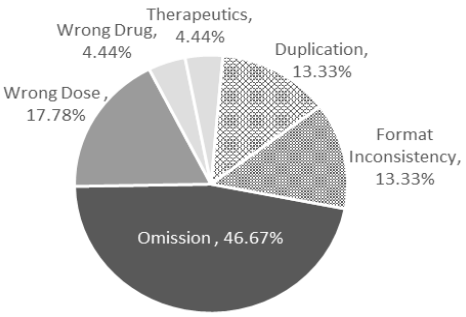
Aim. To identify challenges of insulin prescribing in a new electronic medicines management system at hospital.
Methods. A retrospective analysis of insulin errors during the first 2 months of MARS implementation in March 2017 was conducted. The reported errors were sourced from the hospital incident reporting system and pharmacist interventions. The apparent effectiveness of prescribing support tools such as the 'insulin charting reminder' and 'pharmacist admission note' were evaluated.
Results. The type of insulin charting errors (n=45) were identified. Omission errors (n=21) were largely due to insulin not being prescribed (90%) rather than missed administration (10%). 98% of the not prescribed insulin was due to failure to re-chart after the insulin order expired. New digital specific errors included duplication, format inconsistency, drug selection errors from the drop-down menu and dose typing errors. Duplication arose when more than one prescriber ordered the same dose whereas format inconsistency was reported as a lack of knowledge of how to prescribe insulin in MARS, resulting in suboptimal orders. Anecdotally, difficulties accessing BGL results while prescribing precipitated the failure to intensifying therapy for patients with high blood glucose levels.



The use of the 'insulin charting reminder' and 'pharmacist admission note' was evident in 98% of reported incidents. 54% of cases reviewed had more than one 'insulin charting reminder'. Multiple reminders increased the risk of error due to chart cluttering and reminder fatigue. 83% of 'pharmacist admission notes' recorded precise dosages, however, 17% of dosages lacked accessibility by clinicians as doses were recorded elsewhere.
Discussion. Electronic insulin prescribing can lead to medication errors despite decision support tools. Errors identified are being used to inform clinician education and system re-development to improve patient safety. Safer systems will aid digital conversions at other hospitals.¶

610 Prevalence of potentially inappropriate medicine use in older Australians living in residential aged care facilities. Hosam Bony[1], Renae Lloyd[1], Brianna Kinnear[1], Emilio Petito[1], Vijayaprakash Suppiah[1], Elizabeth Hotham[1] School of Pharmacy and Medical Sciences, University of South Australia[1], Adelaide, SA, Australia.

Introduction. Older Australians living in residential aged care facilities (RACF) may present with coexistence of multiple illnesses leading to complex medical issues. Additionally, physiological changes in the elderly can impact on the homeostasis, pharmacokinetics, pharmacodynamics and the handling of drugs, making this group more vulnerable to harmful effects, especially if polypharmacy is present. Potentially inappropriate medicines (PIMs) are medicines that can cause more harm than benefit. The use of PIMs in this growing population may contribute to further illness or exacerbations of existing medical conditions. Currently, various tools have been developed to aid healthcare professionals to screen for PIMs.
Aim. To assess and characterize the prevalence of potentially inappropriate medications (PIMs) according to the American Geriatric Society (AGS) 2015 updated Beers Criteria in a population of RACF residents.
Methods. Ethics approval was granted to recruit participants from three RACFs in Adelaide, South Australia between June 2015 and February 2016. The study involved the review of charts and documentation of prescribing/medical histories of those 65 years and over. Data analysis was conducted using descriptive statistics.
Results. Three hundred and fifteen charts were reviewed. Participants were taking 9.5 medicines on average with a total of 2946 regular medications for the cohort, 94.5% of them being taken daily. Upon analysing the medication data against the AGS 2015 Beers Criteria, it was determined that there was on average 1.9 PIMs per person, that 52% of the cohort had 2 or more PIMs and that 81% had at least 1 PIM.
Discussion. The AGS 2015 Beers Criteria identified a high frequency of PIMs in our study. The fact that 81% of the cohort had at least 1 PIM suggests that the majority in the cohort are potentially at serious risk of harm and that measures such as routine drug audits should put in place to detect PIMs before they become the source of illness or injury in these patient groups.¶

611 Comparison of the management of medicines in the older-aged living in different leasehold retirement villages
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Introduction. We have previously shown that the older-aged living in a leasehold retirement village have a low adherence to medicines but a reasonable understanding of their medicines/illnesses (Doggrell 2013).
Aims. The aim was to determine whether this was a common finding among leasehold villages by assessing the management of medicines in another leasehold village, and comparing this with our findings in the original village.
Methods. We delivered flyers to individual homes, presented an introduction to the research at a morning tea for the residents. Subsequently, we door knocked at the homes in the new village. This contrasts with the previous study where management would only allow us to interview those who volunteered at the morning tea, not door knock. After we assessed the management of medicines by the older-aged living in the new leasehold village, using semi-structured interviews, we compared the findings with the original leasehold village.
Results. The 68 participants in the new leasehold retirement village were significantly younger than the 22 participants from the original village; 78 vs 82 years old, respectively. Using the Doggrell-Kairuz measurement of adherence, it was shown that more participants were adherent and unlikely to have problems with adherence in the next 6-12 months in the new (75%) than in the original leasehold retirement village (55%). Many other aspects of the management of medicines was similar between the two villages including numbers of prescription or OTC drugs used by individuals, percentage using blister packs, the commonest medicines used by individuals, and the percentage having a good understanding of their medicines/illnesses; with 64% having a good understanding in the new, compared to 59% in the original leasehold village.
Discussion. This comparison shows that the adherence to medicines by the older-aged can vary considerable between leasehold retirement villages. Age may be a factor in this, with the need for assistance in the management of medicines being greater for those with a mean age of 82, compared to 78 years old.

Doggrell SA (2013) Int J Clin Pharmac 35:546-9.
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612 Tablet crushers: The investigation of powder loss using different sizes and brands of atorvastatin tablets

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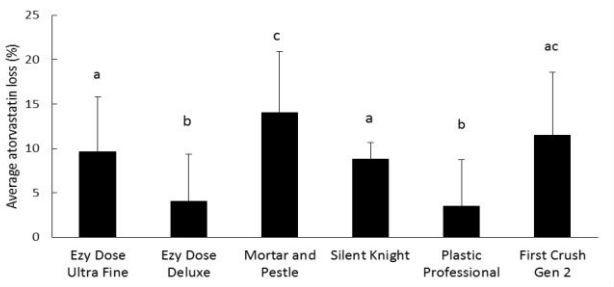
Introduction. Few comparisons have previously been made of tablet crushers in terms of efficacy of dose delivery.
Aims. To compare crusher efficiency in terms of loss of tablet weight and active drug concentration using atorvastatin tablets that vary in strength, size and brand.
Methods. Six tablet crushers were compared: two hand twisting crushers (Ezy Dose Ultra Fine and Ezy Dose Deluxe), one mortar and pestle (porcelain) and three crushers with disposable vessels (Silent Knight, Plastic Professional, and First Crush Generation 2). These were used to crush one tablet of each strength of Lipitor and 20 mg tablets of 5 other brands, and the quantity of atorvastatin recovered from the crusher was quantified using a validated UV-spectroscopy method. The experiment was replicated four times.
Results. Across all 9 tablet brand-strength combinations, the crushers that were consistently better than the others were the EasyDose Deluxe (3.5% loss), which is a hand-held twist-action crusher, and the Plastic Professional (4% loss), which involves pressing the tablet within two paper cups. The mortar and pestle was the worst option, with an average of 14% loss (range 5-28%). Across the four different strengths of Lipitor, losses were significantly higher for lower strength tablets, which were also smaller, with a lower proportion of excipients and lower hardness values than the higher dose tablets.



Discussion. The best crushing devices for atorvastatin tablets were not the same as those for paracetamol tablets determined in a previous study. Losses of atorvastatin ranged from essentially zero to a worrying 28%, and this was dependent on both crusher and tablet characteristics. ¶

613 Study of the 'Hospital Formularies' of different level hospitals based on the 'WHO - Essential Medicines List'

Bharat Gajjar, Barna Ganguly, Nilam Jani, Preyal Panchal. Dept of Pharmacol, Pramukhswami Medical College, Karamsad, Gujarat, India.

Aim: To compare hospital formularies of primary, secondary and tertiary level hospitals based on the 'WHO Essential Medicines List'.
Methods: A cross sectional observational study was conducted in the hospitals from primary, secondary and tertiary health care set ups. In each health care level, one hospital from Government, charitable (except primary) and private sectors was selected. From each of the eight hospitals, the hospital formulary was collected, after the permission of the hospital authority. Formularies were compared within the groups and with 'WHO Essential Medicines List'.
Results: Primary Health Care: Total number of drugs in the formulary of Government sector was 108 and the same of Private sector was 171.
Out of these, 74 (68.52%) drugs are from WHO EML in Government sector, while 81 (47.37%) drugs in Private sector.
Secondary Health Care: Total number of drugs in the formulary of Government sector was 147 and the same of Charitable and Private sectors were 314 and 1160 respectively. Out of these, 103 (70.07%) drugs are from WHO EML in Government sector, 113 (35.99%) drugs in Charitable sector, while 387 (33.36%) drugs in Private sector.
Tertiary Health Care: Total number of drugs in the formulary of Government sector was 209 and the same of Charitable and Private sectors were 944 and 730 respectively.
Out of these, 115 (55.02%) drugs are from WHO EML in Government sector, 287 (30.40%) drugs in Charitable sector, while 157 (21.51%) drugs in Private sector.
Conclusion: Effective management of 'Hospital Formularies' by the means of structuring 'Drugs and Therapeutic Committees', selection of drugs to be included in the formulary on the basis of WHO EML and adherence of clinicians' to the formularies are the mainstays for the rational, effective, safe and affordable health services to the patients.
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614 How does perceived cost influence pharmacy patronage? A scoping review.

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Introduction. Retail business research has identified how customer perceptions of cost, quality and value drive purchase behaviour. In contrast, community pharmacy research lacks insight into how customers perceive cost and value and if this may influence pharmacy choice. An understanding of this relationship can benefit pharmacy owners by helping retain their client base, improving patient health outcomes and ensuring the financial viability of their enterprise in an increasingly competitive marketplace. An investigation into the literature's conceptualisation of this relationship is warranted.
Aim. The aim of this review was to explore what is known about pharmacy customers' perceptions of cost and value, and how these influence patronage patterns.
Methods. A systematic search of 4 databases was conducted with the addition of articles sourced from reference lists using a scoping review framework. The database search was reported in accordance with the PRISMA-P protocol. Thematic analysis was used to identify themes and subthemes relating to cost and value. The results were reported in terms of author name, date of publication, study location, study population, methods and key findings.
Results. Twenty-four studies were yielded which were qualitative and quantitative in nature. Cost and value were found to be key elements influencing pharmacy choice, reasons for switching pharmacies and loyalty intentions. Pharmacy customers perceived costs in terms of monetary, psychological, emotional and convenience-related sacrifices. Value was perceived in two ways. Relating to the perceived worth or utility of a product or service, or in terms of a trade-off between what the consumer receives and what they give up.
Discussion. As a range of perceived costs influence customer behavioural intentions, this literature review helps to inform pharmacies on how they might increase loyalty to their stores. Despite the finding that locational convenience is the greatest cost driver of pharmacy patronage, pharmacies may attempt to influence customer behaviour by minimising unfixed costs to the consumer such as price and time costs, as well as improving patient care.

615 Improving Outcomes in Type 2 Diabetes Patients Using a Pharmacist Diabetes Intervention Tool

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Introduction. Pharmacists' contributions to the improvement of diabetes patients are well documented. However, there is little on pharmacists following a structured approach in the management of diabetes patients.
Aims. The aim of this study was to determine the effectiveness of a multifactorial evidence-based diabetes intervention tool in the delivery of quality diabetes care.
Methods. A tool to facilitate structured diabetes care, the Simpler™ tool, was validated for content, format and design by diabetes experts from Australia and Malaysia through the Delphi method. A two-hour training package to compliment the tool was developed and piloted among 12 pharmacists from Australia and Malaysia. The tool's effectiveness in supporting pharmacists to make interventions was subsequently evaluated during a 6 month, parallel, multi-centre randomized controlled trial among patients in Malaysia comparing those who received Simpler™ interventions with usual care. Pharmacists without formal diabetes qualifications were recruited and upskilled through online training modules on the application of the Simpler™ tool. Patients attending primary care clinics were then randomised to 1) receiving care from the pharmacists who applied the tool (n=55) and 2) patients receiving usual care and dispensing services (n=69).
Results. The Simpler™ intervention arm reduced HbA1c significantly by 1.59% (95%CI: -2.2, -0.9) compared with 0.25% (95%CI: -0.62, 0.11) in the usual care arm, (P=<0.001). In addition, there were significant improvements in systolic blood pressure: (-6.28; 95%CI: -10.5, 2.0; p=0.005) and health related quality of life (-1.75; 95%CI: -2.52, -0.97; p<0.001). The most common medication related problems were patients' *non-adherence* (n=135, 45%) followed by *sub therapeutic dose* (n=65, 22%) and *needs additional therapy* (n=52, 17%). Pharmacists worked in collaboration with doctors to add medications (n=23, 46%) and implement dosage changes (n=17, 34%).
Discussion. The Simpler™ intervention tool facilitated delivery of evidence-based structured diabetes management and improved clinical and quality of life outcomes. This study demonstrates the benefits of the Simpler™ tool to support primary healthcare pharmacists in identifying and conducting evidence based diabetes interventions.
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616 Complementary and alternative medicine (CAM) use in cancer patients commencing new chemotherapy

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Introduction. CAM-chemotherapy drug interactions may result in significantly harmful consequences by, either reducing efficacy or increasing toxicity of the intended chemotherapy regimen.
Aims. To determine CAM use in cancer patients commencing new chemotherapy regimens and whether CAM(s) reported could interact with the prescribed treatment.
Methods. Forty-five patients with a diagnosis of cancer and commencing a new chemotherapy treatment in a large teaching hospital day therapy unit were interviewed regarding current CAM usage. The Natural Medicines Comprehensive Database was utilised to perform an interaction check for each patient reporting CAM use. Study participants were provided with recommendations regarding the safe use of reported CAMs during chemotherapy treatment.
Results. Thirty-six percent of study participants were taking CAMs at the time of commencing chemotherapy, consuming between 1 to 14 products. Furthermore, CAMs that have the potential to interact with chemotherapy treatments were being consumed by 50% of CAM using patients. The majority of this group (69%) were taking CAMs known to have antioxidant properties, which have the potential to oppose the anticancer effect of some chemotherapy agents, such as anthracyclines. Thirty-eight percent of patients reporting CAM use were taking CAMs that could affect CYP450 enzymes that metabolise medications in their treatment protocol. Seventy percent of these CAMs had the potential to either inhibit or induce CYP3A4.
Discussion. Cancer patients being treated with new chemotherapy regimens use CAMs, some of which may interact with chemotherapy regimens and potentially compromise treatment outcomes. It is imperative these patients receive information regarding safe CAM use in chemotherapy. The development of standardised patient education would be beneficial to enable patients to make more informed decisions when deciding to use CAMs during chemotherapy treatment.

617 Are pharmacists' estimates of medication adherence related to HbA1c levels in people with type 2 diabetes?

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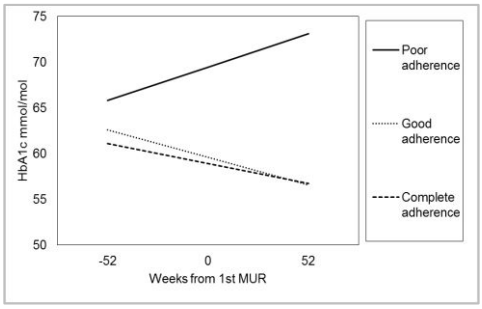
Introduction: Providing individualized adherence support to people receiving oral hypoglycemic medications is expected to enhance clinical outcomes for people with diabetes [Sabate E, 2003]. However, there have been limited studies in real world settings to measure this. This study uses data collected in a natural setting.
Aim: To determine if medication adherence scores (1&2=poor, 3=good & 4=complete adherence) determined during a Pharmacist-led adherence support consultations (Medication Use Review and Adherence Support Service (MUR), New Zealand) are related to measured HbA1c levels over time.



Methods: Adherence support records were obtained from the providers of this service. Patient information was extracted to compile their visit dates, adherence scores, pathology testing date and biomarker (i.e. HbA1c) levels. Data was available for 86 people receiving oral hypoglycemic medications. Data were analyzed descriptively with Microsoft Excel and inferentially using IBM SPSS. Generalized estimating equations were used to explore the change in HbA1c over time, and its relationship to the adherence scores.
Results: People with poor adherence had average HbA1c levels over ~ 1% (11 mmol/mol) higher than those with complete adherence (the reference category), B =11, p = 0.014, but there was no difference between people with good & complete adherence, B = 0.7, p = 0.8. There was a marginal trend for a slight decrease with time of HbA1c levels, B = -0.04, p = 0.08, but this was qualified by an interaction between adherence level and time B= 0.11, p = 0.009 (Figure). Discussion: People who were assessed as having low adherence by pharmacists had significantly higher HbA1c levels, which continued to increase over time. Inadequate monitoring of HbA1c was also observed.

Reference: Sabate E (2003) ed. Pp 71-81, World Health Organization, Geneva, Switzerland¶

618 Developing a screening tool to identify people with swallowing difficulties of solid oral medicines

Simon YM Wong[1], Kathryn J Steadman[1,2], Lisa M Nissen[1], Julie AY Cichero[1,2], Jose Manuel Serrano Santos[1], Esther TL Lau[1]. School of Clinical Sciences, Queensland University of Technology[1], Brisbane, QLD, Australia; School of Pharmacy, University of Queensland[2], Brisbane, QLD, Australia.

Introduction. Swallowing solid oral medicines (e.g. tablets and capsules) is opposing the natural instinct of chewing food before swallowing. People who find it difficult to swallow tablets or capsules may not necessarily have an issue with swallowing food and drink. Existing screening tools for swallowing difficulties do not contain components that are important for identifying people who only have trouble with swallowing tablets and capsules.
Aims. To develop a screening tool for identifying people who require further investigations into their swallowing difficulties (true dysphagia), or those who may only need brief training on how to swallow tablets or capsules safely and effectively.
Methods. A three round modified Delphi with healthcare professionals involved with medicines and/or phobias was used to generate the screening components. Participants then ranked questions that best addressed each screening component. Group consensus for each component and question was analysed quantitatively by percentage of agreement. The importance of question rankings was compared by measuring Kappa values to observe trends in how the Delphi process impacted on the participants' views.
Results. A total of 13 healthcare professionals (pharmacists, general practitioners, speech pathologists, nurses, psychologists, radiographer) participated in the rounds. A screening tool in the form of an 8-item questionnaire was generated. Group consensus was shown by increasing agreement percentages, and stability was demonstrated by a trend of increasing Kappa values.
Discussion. This newly developed screening tool may be useful for identifying people who only have difficulties with swallowing tablets and capsules. Further research is needed to study the feasibility and validity of the screening tool.

619 Dose Administration Aids - How Useful do Patients Think They Are?

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Introduction. Dose administration aids (DAAs) such as a Webster-pak or pill organiser, are frequently recommended to help patients manage their medication regimens, and to improve their medication adherence. Many community pharmacies also provide a DAA packaging service. However, research has suggested that use of DAAs do not encourage health literacy, nor patient self-efficacy in managing their own medicines and medical conditions.
Aims. The aim of this project is to investigate the perspectives and experiences of users of DAAs (i.e. either patients and/or carers), on how useful they find the DAAs to be for managing medicines.
Methods. Participants were recruited from a convenience sample of purposively chosen community pharmacies to achieve variation in pharmacy and consumer demographics, and to ensure that the pharmacies provided a DAA service. The perceptions and experiences of consumers using DAAs were investigated via a questionnaire which consisted of 11 questions, using a 5-point Likert scale with the anchors "Strongly Agree" to "Strongly Disagree". The questions investigated the respondents' opinions on DAAs, the amount and frequency of their medication usage and basic demographic data.
Results. A total of 124 patients or carers from 8 different pharmacies in Brisbane, Australia completed the questionnaire. Approximately 50% of the respondents were over 65 years of age. Almost all respondents found it took little effort to get used to the DAA, and agreed the DAA was helping them to manage their medication. Most participants were also confident in identifying the correct compartment from which to take their medicine and also agreed that the naming, labelling and packaging of the DAA helped them identify each of their medications. However, only two-thirds of the respondents were confident in being able to identify the exact medication in their DAA if a change were to occur.
Discussion. DAAs are useful for helping many patients with managing their medications. However, pharmacists have an important role to play in providing information to patients about the use of DAAs, particularly when any changes occur to any of the medicines being packed. This is important to help improve patient health literacy, and patient self-efficacy in managing their medicines and medical conditions.¶

620 Factors influencing non-adherence among people living with chronic health conditions in Australia

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Introduction. Non-adherence to prescribed medications among people living with chronic health conditions is linked to adverse outcomes at both the individual and societal levels.[1, 2] However, knowledge about who is most at risk of non-adherence is lacking.
Aims. To explore relationships between participants' demographics, health status, prescription and non-prescription medication use and non-adherence to prescribed medications.
Methods. The study utilised data from the 2016 National Survey. Descriptive statistics were used to report on frequencies, generalised linear models used to examine relationships between patient variables and their non-adherence to prescribed medications.
Results. Of the 1217 respondents, the majority (58.7%) reported living with at least one chronic health condition, with88.4% of whom reported using at least one prescribed medications and 82.9% reported using at least one non-prescription medications. People with co-existing chronic health conditions are significantly less adherent to prescribed medication if they were over the age of 45, the likelihood increasing with increasing numbers of non-prescribed medications used, both were significant at the $p<0.001$ level.
Discussion. Initiatives aiming to optimise outcomes for people living with chronic conditions should target those in older age groups and living with co-existing chronic health conditions, and take into consideration their prescription and non-prescription medication use.

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621 Medication information and supply behaviours in elite athletes.

Lily Ngu[1], Bronte Librizzi[1], Michael Ricciardello[1], Amy Street[1], Rhonda Clifford[1], Carmel Goodman[2], Peter Peeling[2,3], Sandra Salter[1]. [1]Pharmacy Division, School of Allied Health, The University of Western Australia, Perth, WA, Australia. [2]Western Australian Institute of Sport, Perth, WA, Australia. [3]School of Human Sciences, The University of Western Australia, Perth, WA, Australia.

Introduction. Sports pharmacy is an emerging area of pharmacy practice. Elite athletes use over-the counter (OTC) medications more often than the general population of the same age. Elite athletes are bound by strict rules around medication and supplement use, with doping and performance issues of equal importance.
Aims. To identify athlete behaviours in obtaining prescription and non-prescription medications, and their use of, and trust in, pharmacists in such processes.
Methods. This was a cross-sectional study of athletes affiliated with a state-based sporting institute. A 39-item electronic survey was developed, validated and disseminated in person at the institute during August-September 2017. The survey examined broad demographics, how athletes obtain prescription and non-prescription medications and information, and the involvement pharmacists may play in their care. Data was analysed descriptively.
Results. Overall 99 athletes aged 18 years and over completed the survey. In the past 6 months, n=91 (91.9%) athletes obtained medications (n=55 (55.6%) obtained both prescription and non-prescription medications; n=15 (16.5%) and n=21 (23.1%) obtained only prescription or non-prescription medications respectively). Of medications obtained, most were sourced from a pharmacy (97.1% of athletes obtained prescription medications and 85.5% non-prescription medications; 31.2% obtained non-prescription medications from a supermarket). Considering medication information, n=12 (12.1%) and n=63 (63.6%) would always or sometimes ask the pharmacist for information. Level of trust in the information provided by the pharmacist was predominately high or moderate (n=33 (33.3%) and n=56 (56.6%) respectively). Forty-one athletes (41.4%) thought that pharmacists could play a role in their medication management.
Discussion. Most athletes obtained prescription or non-prescription medications in the last 6 months, with pharmacy the most common source of supply. Athletes identify and trust pharmacists as sources of information with the potential for them to play a role in their medication management. The suitability of pharmacists for this role must be examined.¶

622 Healthcare and pharmacy service provision for Pakistani migrants residing in developed countries: A systematic review

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Introduction. There has been a growing body of evidence acknowledging that healthcare and pharmacy services provision for migrants, as well as their access of these services, may be influenced by various factors. Understanding the existing dynamics around migrant service access and decision-making may assist health professionals in optimising their service provision in an informed, efficient, and culturally sensitive manner.
Aims. A systematic review of literature to explore the factors influencing healthcare and pharmacy services provision for Pakistani migrants residing in developed countries.
Methods. A comprehensive literature search was conducted using PubMed/Medline, Scopus, EMBASE, Web of Science and CINAHL from the date of inception of databases to search date (15th August 2017), using selective keywords. The initially searched citations were screened to remove duplications. After duplication removal, titles and abstracts of articles were screened to exclude irrelevant articles. The full-texts of remaining articles were retrieved and assessed against the eligibility criteria for inclusion into the review. Two reviewers (AS & JF) independently applied the eligibility criteria and discrepancies were resolved by discussion and consensus of all authors.
Results. The search strategy yielded 2424 articles, of which, 34 studies met the inclusion criteria. The thorough assessment of selected studies revealed that the healthcare and pharmacy services utilisation by Pakistani migrants were influenced by their language proficiency, access and affordability of healthcare, preference for traditional and alternative medicines, cultural and religious beliefs, and support from family and friends.
Discussion. This review highlighted that the healthcare and pharmacy services used by Pakistani migrants tend to be influenced by individual, cultural, as well as health system factors. It is important that healthcare professionals are aware of these characteristics when designing and providing care for migrant populations.¶

623 Piloting a novel observational technique for the administration of medicines to children in paediatric wards

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Introduction: The administration of oral medication to ill children in hospital is well recognised as a challenging process for both nurses and parents. The child is often unaware of the purpose of the medication and may be reluctant to take unpleasant-tasting tablets and syrups, compounding the problem of lack of adherence to treatment in addition to all the well-recognised challenges faced by nurses when administering medication. This puts nurses at the front-line of the medicines management chain when children are admitted to hospital. The aim of this study is to pilot a novel observational technique to identify errors and patient challenges in the administration of oral medication to paediatric patients by nurses.
Methods: This cross-sectional descriptive study applied a novel structured observational technique in which nurses' practices and patients' reactions were explored. The novelty of the tool is supported by the addition of three variables and a scale of acceptability of the medication to a design previously tested in other studies. The single-centre study was conducted in wards of a children's hospital in Brisbane, Australia. The participants were registered and enrolled nurses that administer medication to ill children in the hospital. The number of medicines administered to patients will be recorded along with the number of times that the administration process could be improved. Any deviations from recommended practice will be classified as Medication Administration Errors (MAEs)
Results: The preliminary data collected about the social interaction with the patient will be analysed descriptively and will incorporate new variables to the acceptability of medication. The likely link between MAEs and acceptability of the medication will also be explored.
Discussion: The findings from this study will provide an opportunity to identify acceptability variables that can impact the way that children receive medication in the clinical environment and in their own homes, and to inform the design of educational interventions customised to those practices in a paediatric care.¶

624 Evaluation of antimicrobial use in a tertiary care hospital by using specific indicators: A prospective, observational study

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Introduction. The discovery and usage of antimicrobial drugs is one of the most important and significant contributions to therapeutics in the 20th century. Apart from being very frequently used, they are often misused. The use and misuse of antimicrobial agents needs to be evaluated since misuse of antimicrobials increases the risk of antimicrobial resistance while management and use of antimicrobials have clinical, economic, and environmental implications.
Aims. This study was designed to assess the pattern of antimicrobial prescriptions, to identify the most common problems with antimicrobials prescription and to apply the various antimicrobial use indicators to check the appropriateness of antimicrobial prescribing pattern.
Methods. A hospital-based study was carried out at St. Philomena's hospital located in Bangalore. Ethical committee clearance was obtained from the hospital before starting the study. The research student attended ward rounds on a daily basis and collected the cases, which have been prescribed with antimicrobial agents. Both empirically prescribed antimicrobials as well as the antimicrobials prescribed after culture sensitivity test were included. Specified indicators were applied and the collected data was analyzed
Results. The results of this study indicate that women were slightly more vulnerable than men in developing infectious diseases who were majorly above 60 years of age. In this study, it was found that antibiotics were the most common type of antimicrobials prescribed among which cephalosporins and fluoroquinolones were the most common class of antibiotics used. Antifungals (azoles) and antivirals (anti-influenza) were the next most common type of antimicrobials prescribed. It was also observed that LRTI was the most common infectious disease diagnosed in these patients. In this study, various indicators were also applied to evaluate the use of antimicrobial agents and it was found that the use of antimicrobials was not appropriate.
Discussion. The use of antimicrobials was evaluated and it was found that there is a need to promote rational use of antimicrobials, as irrational use would lead to antimicrobial resistance.¶

625 Does medication increase the risk of infection burden in residential aged care?

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Introduction. Rational use of antibiotics ("antibiotic stewardship") is required to reduce the development of antibiotic resistance. One less-studied focus of antibiotic stewardship is the use of medications that reduce a person's immunity, thus increasing infection risk and antibiotic prescribing. Aged care residents are vulnerable to infection due to their multiple medications, comorbidities, and numerous environmental and physiological factors.
Aims. To determine the association between medication use and infection risk in the elderly.
Methods. A retrospective case-control study was conducted to evaluate medication-related factors associated with antibiotic use by aged care residents. Online records of 726 (375 Case and 351 Control) residents of The Bethanie Group Inc. aged care facilities were evaluated from 1st January 2015 to 31st December 2015. The Case group comprised residents who had at least one incident of infection, as indicated by a documented diagnosis and/or short-term use of antibiotics. Logistic regression determined factors associated with the incidence of at least one infection during 2015; independent variables included medication groups, medical conditions and gender.
Results. The most common infections were urinary tract infection (45.9%) and respiratory tract infection (38.9%). The most commonly prescribed antimicrobials were cephalosporins (33.9%) and penicillins (25.4%). Benzodiazepines (OR:1.78, 95%CI:1.1-2.7), antiepileptics[a] (OR:1.62, 95%CI:1.0-2.5), antidepressants[b] (OR:2.21, 95%CI:1.3-3.5) and tricyclic antidepressants (OR:2.98, 95%CI:1.6-5.5) showed statistically significant association in the increased risk of infections in multivariate analysis.
Discussion. Benzodiazepines and certain classes of antiepileptics and antidepressants were associated with increased risk of infections in aged care residents. This study demonstrated the need for rational prescribing of medications that contribute to increased infection risk, and informs an educational intervention focusing on medication review for at-risk elderly.
[a] Antiepileptics: pregabalin, valproate, carbamazepine, lamotrigine, gabapentin, phenytoin, levetiracetam, lacosamide, levetiracetam
[b] Antidepressants: mirtazapine, moclobemide, agomelatine
OR, Odds Ratio; 95%CI, 95% Confidence Interval ¶

626 Measuring menopause symptoms: a scoping review of existing tools.

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Introduction. Menopause symptoms have a significant impact on women's well-being and quality of life. A variety of menopause symptom tools have been used to investigate the effect of interventions on menopause symptoms limiting the ability to combine results in meta-analyses.

Aims. To identify the tools currently being used to measure menopause symptoms.

Methods. A scoping review was performed to identify tools used to assess menopause symptoms over the last five years. Four databases (EMBASE, Medline, PubMed and Scopus) were searched using the terms (menopause symptoms OR menopause women OR menopausal symptoms OR menopausal women) AND (scale OR questionnaire). All identified studies that used a tool to assess menopause symptoms and assessed more than one menopause symptom were included. The most common tools are discussed and reasons for tool selection explored.

Tool	No. of items	No times used in last 5 years	No of languages
Menopause Rating Scale	11	96	19
Kupperman Menopause Index	11	54	6
Menopause-Specific Quality of Life Questionnaire	29	54	10
Greene Climacteric Scale	21	37	7

Results. Use of a tool to assess menopause symptoms was identified in 295 studies from 43 countries. These studies used 22 named tools and 16 unnamed tools. The 4 most common tools (Table) were used in 81% of studies. Researchers reported modifying the tool prior to use in 18 studies. Validation studies to prove validity and reliability in the language they are administered in was available for 81% of the named tools. Use of invalidated tools was justified because they were considered more suited to the specific population sample due to cultural and language reasons.

Discussion. The majority of studies (⅘) used one of 4 tools because they had been validated in the population and language of administration yet other authors (⅕) considered they needed to create their own tool. It appears that no one tool is adapted to suit all study samples and contexts, consequently there is a need to modify existing tools to improve suitability for specific cultures and languages and to adapt to cultural norms and understanding of symptoms.

627 Completeness of Controlled Drug prescribing in regional NSW

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Introduction. In Australia there are strict requirements for the prescribing of Controlled Drugs. Many of the requirements are consistent from state to state but there are some differences between jurisdictions.

Aims. To assess the level of completeness of prescriptions for controlled drugs in regional NSW.

Methods. Managing pharmacists in pharmacies in the Mid North Coast region of NSW were invited to participate in a prescription audit. All controlled drug duplicate prescriptions that were dispensed from the 1st January to the 30th April 2017 were assessed for completeness in accordance with NSW legislation requirements.

Results. Overall 511 prescriptions for controlled drugs from 3 pharmacies were included in the audit. Only 3% (16) of reviewed prescriptions were found to be complete. Of the Controlled Drug prescriptions requirements in NSW all 5 prescriber elements were included on 80%(411), the 2 patient elements on 93%(476), all 6 medication elements on 7%(38) and all 3 general elements on 89%(454) of the reviewed prescriptions. Only 51 prescriptions (10%) had repeats prescribed and of these only 30% (15) included the repeat interval. There were 24 prescriptions for psychostimulants and 80% (19) of these were endorsed with the appropriate code obtained from NSW Health. There were between 0 and 8 errors (*Md*=2, *IQR*=2-4) on the audited prescriptions. The errors were divided into two groups: omissions (range 0 to 8, *Md*=0, *IQR*=0-1) and medication requirements not handwritten (range 0 to 7, *Md*=2, *IQR*=1-3). Information was missing on 34%(172) prescriptions and medication information was not hand written on 83%(424) of prescriptions.

Discussion. Most audited prescriptions did not contain all elements required by NSW legislation. The most common reason for lack of completeness was that medication requirements were not handwritten on computer-generated scripts. The drug name, strength, form, quantity, directions and repeats (including none) are required to be handwritten to reduce the likelihood of forgery. Handwriting what has been printed on the prescription also provides the prescriber with an opportunity to review the prescription before giving it to the patient. Perhaps it is time to review Controlled Drug prescription requirements as very few prescriptions for Controlled Drugs were complete according to NSW legislation requirements.¶

628 Tablet crusher comparisons: usability testing by people with and without limited hand function

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Introduction. Tablets are crushed using a variety of devices: manual and electronic, exerting a flat press or rotating grinding motion, with or without disposable bags or cups.
Aim. To compare a range of tablet crushers for usability by people with and without limitations in their hand function and hand strength.
Methods. Approval was granted by the UQ Human Research Ethics Committee. 60 people without and 40 people with self-reported limited hand function were recruited. Hand function was assessed using the Arthritis Impact Measurement Scale (AIMS2) and hand strength by dynamometer. For each of 9 different crushers, participants attempted to crush a paracetamol tablet and then completed a Rapid Assessment of Product Usability and Universal Design (RAPUUD) validated questionnaire.
Results. Hand strength was not correlated with hand function, and the AIMS2 score was found to best distinguish participants with and without limited hand function. Two crushers failed during testing – the Minitwist with bags broke after 19 participants, and the electronic grinder stopped working after 58 participants had used it. Consequently 7 crushers were tested by all participants. The hand-held twist-action crushers with ergonomic grip scored highest in terms of usability. The lack of an ergonomic or triangular grip on the twist-action crushers reduced usability to a greater extent for participants with limited hand function. Crushers with cups and bags scored well for usability, and better if they were automatic, but once participants became aware of their high cost they were less likely to score them in their top 3 choices.
Discussion. The usability of different crushers was assessed from the point of view of personal use by people with and without limited hand function, and results may be expected to differ if tested by nurses involved in hospital or aged care medication delivery. The economical twist action crushers without separate bags or cups were generally found to have greater usability and were preferred by these participants. ¶

629 Management of non-healing mouth ulcer presentations in community pharmacies

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Introduction. Oral cancer commonly presents as non-healing lesions/ulcers. Practice guidelines recommend referral to a general/dental practitioner for mouth ulcers persisting longer than 2-3 weeks. This project evaluated management and referral of non-healing mouth ulcer presentations by community pharmacy staff in the Greater Brisbane region.
Methods. Trained simulated patients visited 220 randomly selected community pharmacies within the Greater Brisbane region between March and May 2016. Mystery shoppers presented pharmacy staff with two standardised over-the-counter (OTC) non-healing (> 1 month) mouth ulcer scenarios: A direct product request (DPR) (n=110) or a symptom based request (SBR) (n=110). Results were documented and evaluated against Australian national pharmacy professional practice standards. Referral rates for pharmacy staff (pharmacist, pharmacy assistant or mixed – pharmacist and assistant) handling the interactions were also assessed.
Results. Australian pharmacy practice standards recommend pharmacy staff ask patients six key questions to enable informed decision making regarding appropriate treatment/advice. In the majority of interactions, pharmacy staff identified the patient and their symptoms (76.4%; 168/220 and 68.6%; 151/220 respectively). The remaining four questions relating to symptom duration, treatments tried, other medications and medical conditions were enquired in 32.3%, 52.7%, 30.5% and 27.3% of interactions respectively. Simulated patients were referred to the doctor/dentist in 11.8% (26/220) of all interactions.
Conclusions. Community pharmacy staff handling of non-healing mouth ulcer consultations was suboptimal compared to national professional standards. In particular, infrequent questioning regarding the duration of the non-healing mouth ulcer was likely to have resulted in low referral rates by staff. This study identifies the need for increased oral cancer awareness and education for community pharmacy staff in addition to re-enforcing the importance of practising according to professional standards to effectively screen for potentially neoplastic mouth ulcers/lesions.
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630 Chronic disease, medications & lifestyle: perceptions from a regional Victorian Indigenous Community.

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Introduction. Poor medication management may contribute to the increased morbidity and mortality of Indigenous people in Australia. Yet while there is extensive literature about the perceptions of healthcare providers on this issue, there is limited information on the perceptions of Indigenous people themselves.
Aims. To investigate the perceptions of a group of Indigenous people attending a Victorian regional Aboriginal Health Service (AHS) with diagnosed medical conditions requiring medications, of their lifestyle, disease management and medication usage.
Methods. We used a co-research methodology in which Aboriginal Health Professionals (AHP) who worked at the Health Service were co-researchers with a team from La Trobe University, Victoria. The AHPs conducted individual interviews with a purposive sample of clients participating in chronic disease management programs in a culturally appropriate and competent manner using a semi structured *yarning* process. De-identified verbatim transcripts were coded for thematic analysis and for descriptive statistical analysis by SPSS. Themes were validated by cross check between members of the research group including the AHP research partners.
Results. Our results showed that the majority of participants perceived that changes in lifestyle factors such as diet, exercise, and smoking cessation would help improve their health. Most patients reported having been counselled on their medicines, and while the majority reported adherence and acknowledgement of the efficacy of their medicines, there was a lack of clarity regarding long term maintenance on regimens. Finally, while the majority reported taking OTC products, some did not see the need to inform their doctor about this, or chose not to.
Discussion. Chronic illness was perceived as common in families and community. Patients relied mostly on their health care professionals as sources for their drug information. Patients may have benefited from further counselling in the area of complementary and other OTC medicines, as well as on the necessity of maintenance of regimes for chronic disease management. Finally, lifestyle changes such as dietary improvements and smoking cessation were identified as areas that may assist in improving health.

631 Perceptions of credible drug information sources for Indigenous people attending a regional Aboriginal Health Service

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Introduction. In Australia, there has been scant research into how Indigenous people source information on medications they are taking[1]
Aims. To investigate perceptions of Indigenous patients regarding sources of credible information on the medications they were prescribed.
Methods. Aboriginal Health Professionals (AHP) were co-researchers with the La Trobe University team. The AHPs conducted interviews with patients with chronic diseases in a culturally appropriate and competent manner using a semi structured *yarning* process. De-identified verbatim transcripts were coded for thematic analysis and descriptive statistical analysis by SPSS. Themes were validated by cross check between members of the research group including the AHP research partners.
Results. See table
Discussion. Further research is required to explore (i) whether such perceptions are widespread amongst Indigenous people in other metropolitan, regional and remote communities (ii) whether such perceptions are related to perceived level of expertise of the information provider (iii) whether such perceptions relate to the cultural competence with which counselling was undertaken.

Perceived credible sources of information about medications	% participants
Medical practitioners	65
Pharmacists	60
Aboriginal Health Professionals	35
Internet sites	35
Pharmaceutical Company Product Information Leaflets	20
Nurses	15

1.Tan A, Emmerton L & Hattingh H (2012) A review of the medication pathway in rural Queensland, Australia. *International Journal of Pharmacy Practice* 20:324–339