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Deprescribing feasibility study in New Zealand residential care homes

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Introduction. Deprescribing, the process of safely reducing and/or discontinuing medicines is aimed at reducing inappropriate or harmful medicines to improve health outcomes in older people. To our knowledge, the feasibility of implementing deprescribing in residential care homes has yet to be investigated in New Zealand.
Aims. To assess the feasibility of conducting a deprescribing study in older residents taking multiple medicines in aged care facilities in New Zealand.
Methods. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist was used to develop a study protocol for deprescribing. A RCF provider was approached for consent to participate in the study. General practitioners (GPs) who prescribe for the majority of residents in the RCF will be approached, to consent to their residents taking part in the feasibility study.
Results. A feasibility study protocol has been developed. Drug-specific deprescribing protocols, applicable to older people for twelve medicines, were developed based on an evidence-based literature search, to serve as guidance for
clinical pharmacists and general practitioners, when making decisions regarding deprescribing. Deprescribing protocols were reviewed by a group of national and international experts and modified based on their feedback. Deprescribing recommendations will be developed for each resident by the clinical pharmacist and submitted to the general practitioner for feedback and implementation. An appropriate medication management plan will be formulated for each resident with provision for monitoring to nursing staff to ensure deprescribing is safe and appropriate. Preliminary demographic data collection, assessments on quality of life and cognitive function, will be completed for participants, prior to the commencement of deprescribing. The primary outcome will be the number of residents in whom deprescribing is achieved. Secondary outcomes include quality of life and cognitive function.
Discussion. Deprescribing can reduce polypharmacy, inappropriate medicine use, reduce adverse drug events and improve quality of life. The findings of this deprescribing feasibility study will be used to leverage for a randomised controlled trial to assess important health and economic outcomes in older people.

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Systematic Review of Medication Reconciliation Best Practice

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Introduction. Medication Reconciliation (MR) has been the focus of several national and international patient safety organizations. MR is a part of the medication management process. It should be performed to help minimise medication errors and to improve patient safety at transitions of care. However, whilst the concept is relatively straightforward, there is a little agreement on standardized MR practice.
Aims. To analyze studies which have described medication discrepancies to identify the factors, processes and gaps related to MR practice.
Methods. A systematic review of literature was conducted by searching MEDLINE, EMBASE, CINAHL, PubMed, International Pharmaceutical Abstract (IPA), and Web of Science (WOS), in accordance with the PRISMA statement. The title and abstract of English language articles on “medication reconciliation” published to September 2014 were first screened based on specified eligibility criteria. The full text of articles evaluating MR process that also reported the types and classification of medication discrepancy in their objectives or outcomes were then further assessed for relevance to the review.
Results. A total of 5321 potentially relevant articles were identified and after removal of 2388 duplicates, 2933 titles and abstracts were screened. A total of 345 full-texts were assessed for eligibility and this resulted in the identification of 78 articles for inclusion. The most common transition point was admission to hospital (n=27, 34.6%) and the most common type of discrepancy identified was omission of therapy (n=46, 59%). Among the 78 studies, the mean number of medication discrepancies per patient ranged from 0.07 to 11.61. Most studies (n=55) were performed by pharmacy teams. Fourteen studies utilized the terms intentional and unintentional in describing medication discrepancies. Approximately one third of studies (n=25) involved the gathering of a best possible medication list (BPML) or gold standard list in the MR process.
Discussion. Although the concept of MR is relatively straightforward, we found significant inconsistencies in the operational definition and application of the process in reviewed studies. A clear and consistent approach to report and practice of MR is required and may facilitate medication safety.

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Perspective of pharmacists about their role in religious fasts observed by diabetic patients

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Introduction. Diabetes is a common chronic condition amongst Australians. On-going management requires adherence to medication and diet regimens. Religious practices such as fasting can impact medication use and diabetes control. Pharmacists as medication experts have a key role in helping people observing religious practices such as the Ramadan fast, to maintain good control over their medication regimen.
Aims. This study aimed to explore the perspective of Australian community pharmacists about professional services for patients with diabetes who may opt for observing the Ramadan fast.
Methods. Qualitative, semi-structured interviews with a purposive convenient sample of pharmacists practicing in areas of ethnic diversity in Sydney; with interview data thematically analysed in a constructivist paradigm.
Results. 21 semi-structured interviews (57% male) were conducted, analysis is currently underway. The sample appeared to be dichotomised into those who were experienced in providing services and counselling to fasting diabetic patients versus those who did not appear to be proactive. Most respondents were willing to engage in these services, considering as a clear role for pharmacists. Some respondents highlighted the need for training and skills development in this area. Patient willingness to engage was seen as a decisive factor for service provision.
Discussion. Professional awareness of the effect of religious practices is important and protocols to assist patients in these situations should be developed and disseminated, especially in the case of diabetic patients conforming to the practice of Ramadan.

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What might a community pharmacy triage service look like? A Delphi study

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Introduction. When people become unwell, they often seek advice from a health professional. Many go directly to an accident and emergency department or to their GP. Research, however, indicates that many of these health issues could safely be managed within a community pharmacy setting.
Aims. This study aimed to gain a consensus from experts on what a formal community pharmacy triaging service (CPTS) might look like. The specific objectives were: (i) to identify if a CPTS is feasible; (ii) to ascertain what a CPTS would look like and (iii) to identify what will be required to make a CPTS work.
Methods. The study used a 3 stage Delphi method to build consensus around these three objectives. 20 experts in academia, community pharmacy and general practice from New Zealand, Canada, Australia and the UK were recruited. In Stage 1, each expert was asked to complete an online survey with 10 free text response questions. These questions covered issues such as the structure of a CPTS; benefits, acceptability and viability; pharmacist training; and any obstacles. Once data were received, the free text was coded using thematic analysis and categorised into each of the three objectives. Stage 2 comprised a quantitative online survey consisting of attitude statements developed from Stage 1. Participants were asked to rate the statements from 'strongly disagree' to 'strongly agree'. In Stage 3, participants were sent the same statements in a further online survey (excluding those where consensus was already reached). They were also sent a personalised PDF comprising aggregated results of each statement and their individual response given at Stage 2, and asked to consider and provide their responses again.
Results. Stage 1 results were coded into a number of themes including: signposting patients, confidence in pharmacists current training, views of and impact on other health professionals, after hours/rural CPTS, algorithms, funding and subsidy, pharmacist accreditation, responsibility, advertising, documentation, access to patient medical records, and IT communications between the CPTS and other health professionals. This translated into 54 attitude statements which have been sent to participants. Data from all 3 stages will be presented.
Discussion. A wide range of perspectives and issues were raised about a potential CPTS, and provide an informed starting point for developing this service.

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Culturally competent community pharmacy practice: Exploring the perspectives of those with disabilities

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Introduction. People living with disabilities are an important group of health consumers. On average they have greater healthcare need than those living without disability, and more of this remains unmet.
Aims. To explore the experiences of people living with disabilities when accessing community pharmacy services in New Zealand.
Methods. A qualitative approach investigated the community pharmacy experiences of adults with physical, sensory and intellectual disabilities. The focus group and interviews (people with intellectual impairment only) were recorded and transcribed verbatim. Transcripts from the two participant groups were analysed separately using a general inductive approach to identify key themes related to access. Ten people with a range of impairment types participated in the focus group and 10 people with a range of intellectual and learning impairments were interviewed.
Results. Discourse focussed on access manifested as accessibility of services and of pharmacy premises. Key characteristics of community pharmacy that influenced access included pharmacy staff's: cultural awareness, recognition of patient autonomy, relationship development, assumptions made about disability, communication skills, respect for privacy, and the physical environment. The roles of transport, support staff and personal finances were also highlighted by several participants with an intellectual impairment, as influencing access to services.
Discussion. Additional training has the potential to produce pharmacy staff able to provide an environment which is more accessible to all those who visit pharmacies. Training should include knowledge about common conditions and material tailored to respectful and effective communication with those with physical, intellectual and sensory impairments. Further research is needed to substantiate this recommendation.

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Professionalism of pharmacists on social network sites

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Introduction. Social networking sites (SNS) are a new venue for communication about health and medicines [1]. Health professionals' behaviour on SNS may influence public perceptions.
Aims. We aimed to explore how pharmacists separate professional and personal activities on SNS, their perceptions of professional behaviour on SNS, and opinions on guidelines in this area.
Methods. In-depth interviews were conducted with 31 pharmacists from 9 countries. Interviews were recorded, transcribed verbatim and content analysed.
Results. Analysis of interviews revealed three broad themes: 1) Use of SNS- a majority of participants had a "blended" approach to SNS use (posting personal and professional comments on the same SNS) and about a third had a "dual citizenship" approach (separated professional and personal activities). 2) Professionalism on SNS - perceived unprofessional online behaviours of peers included over exposure of personal life; open complaints about the profession, physicians, and patients; inappropriate descriptions of pharmacists' roles and activities; and breaches of patient confidentiality. Participants displayed positive professional behaviours such as sharing relevant health information with the public and addressing misleading health information. However, posts about compassion for patients and examples of effective patient care were also observed. 3) Guidelines- There was no consensus on having professional social media guidelines available. Some preferred SNS unregulated while most believed certain guidance was needed to keep the profession in high standards in the online environment.
Discussion. A clear-cut strategy to separate professional and personal activities on SNS was not adopted by most pharmacists. Issues associated with pharmacists' online behaviour were reported which could negatively impact people's perceptions of the profession, including of individual practitioners. However, support for guidelines to guide pharmacists on how to behave online was not unanimous.

[1] Moorhead SA *et al*. A new dimension of health care: systematic review of the uses, benefits, and limitations of social media for health communication. *J Med Internet Res.* 2013;15.

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Development and validation of an oral anticoagulant knowledge instrument

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Background: Anticoagulants are a high risk class of medication due to their narrow risk-benefit margin. Knowledge regarding anticoagulant medicines is often poor in patients prescribed them, and improving knowledge has been associated with better clinical outcomes. A validated instrument is the generally accepted method of assessing medication knowledge, but no such tools are available for the novel oral anticoagulants which were recently released in Australia. There is need for a validated instrument that can effectively assess anticoagulation knowledge that caters for the novel oral anticoagulants, as well as the older agents.

Aim: This study aims to develop and validate an anticoagulant knowledge instrument to be used in future studies to assess the medication knowledge of patients taking anticoagulant therapy.

Method: The study will begin by a review of existing literature from which a list of relevant questions will be developed. The questions will be reworded for clarity, and content validity will be undertaken in consultation with anticoagulation experts. The instrument will be administered to a total of 200 subjects from a pool of pharmacists, patients, and the general public. The construct validity of the instrument will be assessed using the contrasted group method. The participants will be retested two months after the initial testing to assess test-retest reliability, while internal consistency reliability will be assessed by calculating the Cronbach's alpha score. Statistical analyses will be conducted using SPSS, 21.0 and Microsoft Excel.

Conclusion: A validated oral anticoagulant knowledge instrument applicable to patients taking warfarin and the novel anticoagulants will reliably identify knowledge gaps in patients. This will assist health care professionals to identify patients at risk and develop strategies to improve the quality use of anticoagulant medications.

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Development of a Score to Predict Hospitalisation due to Adverse Drug Reactions in Older Patients

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Introduction. Adverse drug reactions (ADRs) are a significant cause of hospitalisation in elderly patients.

Aim. To develop a risk score to predict ADR-related hospitalisation in people aged ≥65 years.

Methods. We conducted a prospective cross-sectional study at the Royal Hobart Hospital, Tasmania over a 12-month period to identify the proportion of patients whose admission was caused by an ADR. Identification of ADRs was based on reviewing medical records and patient interview. A wide range of potential predictors of ADR were evaluated in 70 patients admitted due to ADRs that were deemed preventable and 698 controls. Variables included the number of regular medications, number of comorbid conditions, dementia, heart failure, liver failure, renal failure, recent hospital admission and use of potentially inappropriate medications defined by Beer's criteria. The variables associated with ADRs ($p<0.05$) in the bivariate analyses were entered into a multivariate logistic regression model. Variables retained in the final model were used to compute the ADR risk score. A score of 1 was assigned to variables with an odds ratio (OR) between 1.00 and 1.99; and a score of 2, to those with an OR between 2.00 and 2.99. The ADR risk score was computed based on the sum of scores of individual variables. Further analyses were performed to determine the cut-off score, sensitivity, specificity and model performance.

Results. The variables that predicted admission due to an ADR included number of regular medications (≥8), number of comorbid conditions (>6), presence of dementia and hospital admission within the past month. An ADR score cut off of 3 had a sensitivity of 70% and a specificity of 59%. The predictive ability of the risk score was assessed from a calculation of the area under the receiver operator characteristic curve and found to be 0.69 (95% CI, 0.63-0.75), suggesting that the ability of the model to predict ADRs is better than chance alone.

Discussion. The ADR risk score developed, after further validation, may be useful in clinical practice as a tool to identify patients at risk of hospitalisation due to preventable ADRs, and to target a subgroup of elderly that could benefit from interventions aimed to reduce this risk.

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The impact of psycholeptic reduction in residential aged care facilities: Preliminary findings

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Introduction. Psycholeptic medications are commonly prescribed in residential aged care facilities (RACFs). Antipsychotics are often used to treat behavioural symptoms and benzodiazepines are frequently given for sleep disturbances and anxiety. Despite modest efficacy for these symptoms and risk of severe adverse effects, evidence suggests that psycholeptics are not regularly reviewed or reduced. Previous psycholeptic reduction programs have lacked resident outcome monitoring, impacting upon their suitability to detect barriers to psycholeptic reduction.
Aims. To assess the impact that psycholeptic reduction has on residents of RACFs within a multifaceted intervention to improve psycholeptic prescribing (the Reducing the Use of Sedatives Project; RedUSe).
Methods. RedUSe consists of educational sessions supported by benchmarking of psycholeptic prescribing, and multidisciplinary psycholeptic reviews. This prospective cohort study aims to recruit over 200 residents taking regular antipsychotics and/or benzodiazepines from RACFs involved in RedUSe. At the conclusion of the study, residents will be grouped according to whether or not they have had their psycholeptics reduced. Resident behaviour is assessed by staff using the Neuropsychiatric Inventory-Nursing Home version and Cohen-Mansfield Agitation Inventory (CMAI) at baseline and four months of RedUSe. Resident falls are assessed continuously over this time.
Results. Interim results indicate that benzodiazepine and antipsychotic use decreased in 14 of 46 residents prescribed regular benzodiazepines and 7 of 20 residents prescribed regular antipsychotics, respectively. Behavioural measures did not differ significantly besides the total CMAI score which increased in the antipsychotic reduction group 15.43±7.53 ($n=7$, $P<0.05$). The benzodiazepine (n=22 falls, 8 residents) and antipsychotic (n=4 falls, 2 residents) reduction groups had less falls than residents who continued/increased their benzodiazepine (n=31 falls, 11 residents) or antipsychotic medication (n=26 falls, 8 residents).
Discussion. The translation of psycholeptic reduction into resident outcomes will address the absence of monitoring from similar psycholeptic reduction programs. Preliminary results suggest that decreasing psycholeptics are unlikely to affect most resident behaviours. Ongoing data collection and analysis will provide further clarity.

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Purchasing Over-the-counter medicines from the pharmacy: Does a consumer's health status, perceptions and what they value matter?

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Introduction. Over-the-counter medicines (OTCs) are widely available and can be purchased without a prescription from a range of different settings. Although pharmacists are often considered one of the most trusted health professionals, the availability of these OTCs mean that a customer may choose to purchase these OTCs without the involvement of a pharmacist or a pharmacy. It is important to understand what drives the consumers to purchase OTCs from a pharmacy and their expectations in order to better understand the needs and opportunities in this space.
Aims. The aim was to examine the key drivers for consumer OTCs purchasing behaviour and to determine whether their health status, levels of stress, perceived risks and benefits of purchasing OTCs from a pharmacy influence their OTCs shopping behaviour.
Methods. Randomly selected pharmacy customers from two metropolitan pharmacies completed an anonymous, self-administered pre-tested & validated questionnaire examining a broad range of topics such as demographics, self-assessed current level of health/stress, as well as perceived benefits and risks when purchasing their OTCs from a pharmacy, including what they value, trust and expect and how this may influence their OTCs shopping behaviour.
Results. A total of 86 customers participated in this survey. A broad range of customers were captured in this study across both genders (53% males), various age groups (between 18-65+), employment (52% fulltime), income (between <$20,000->$110,000) and family statuses (64% married; 53% no children). 59% & 56% respectively indicated that they were stressed and tense when they arrived at the pharmacy but most were feeling well (60%). Most customers strongly agreed/agreed that trust in the advice from a pharmacy (95%), trust in the products (73%), and altruistic approach of a pharmacy (95%) were critical to them. Further, 82% and 79% respectively disagreed that time pressures or costs were concerns, despite the majority feeling tense and stressed when they came in. 88% of the customers indicated that they intend to buy their future OTCs from a pharmacy instead of a supermarket.
Discussion. Thematic analyses indicates that high levels of trust, confidence and sense of altruism & care were key drivers for buying OTCs from a pharmacy, regardless of time pressures, costs or existing levels of stress & health.

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Can pharmacists in Australia and Switzerland improve access to chlamydia screening?

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Introduction. Much is known about the potential for community pharmacy to provide screening for *Chlamydia trachomatis*. However, few studies have focused on pharmacists as individuals, and the specific influence they may have in providing this service. Consideration of factors that may influence pharmacist involvement in chlamydia screening improves development of screening programs from pharmacy.
Aim. To investigate if pharmacists in Australia and Switzerland may improve access to chlamydia screening based on willingness to provide screening, chlamydia knowledge, and perceived facilitators and barriers to screening.
Methods. A 12-question survey was distributed online using direct email, social media and professional websites (in English for Australia; German for Switzerland). Willingness to provide screening, chlamydia knowledge, and a series of 9 facilitator and barrier statements were assessed Willingness was analysed descriptively. Mean knowledge scores (maximum=8 pts) were compared between countries (independent t-test). The impact of each of the facilitator and barrier statements on willingness to provide screening was assessed using simple binary logistic regression.
Results. Of 162 Australian and 223 Swiss pharmacists, 155 (95.7%) and 179 (80.3%) were willing to provide screening respectively. Australian pharmacists had significantly higher chlamydia knowledge scores than Swiss pharmacists (mean (SD): 7.06$\pm$1.11 v 6.15$\pm$1.42 points; $p<0.001$). The strongest facilitator in Australian and Swiss pharmacists respectively was "in favour of introducing new strategies for treating STI" (odds ratio; OR=32.75, 95%CI (3.77,284.34) and "keen to expand my service" (OR=9.05, 95%CI (4.36,18.82). The strongest barrier in each country was "this process seems complicated" (OR=8.46, 95%CI (1.39,51.5) and OR=9.61, 95%CI (3.32, 27.8) for Australian and Swiss pharmacists respectively).
Discussion. Pharmacists in Australia and Switzerland may improve access to chlamydia screening, as the majority of pharmacists in both countries were willing to provide the service, had high chlamydia knowledge and were focused on the importance of STI. The process for chlamydia screening has an important influence: pharmacists are less likely to provide the service if they perceive the process for this to be complicated.

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Enhancing communication between consumers and community pharmacy staff for over the counter requests: a systematic review.

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Introduction. Appropriate communication between consumers and pharmacy staff is essential to facilitate positive health outcomes when consumers seek care during over the counter (OTC) consultations.
Aim. To identify interventions to improve communication during OTC consultations in community pharmacies.
Methods. Electronic databases were searched from 2000-2014 and reported to comply with the PRISMA statement (Liberati et al, 2009). Inclusion criteria were intervention studies, to improve communication during OTC consultations in community pharmacies with a measurable communication outcome. Independent duplicate abstraction and risk of bias was undertaken using a modified checklist informed by the Cochrane (Higgins, Green, 2011) and Transparent Reporting of Evaluations with Nonrandomized Designs tools (Des Jarlais DC et al, 2004).
Results. Of 5369 records identified, 11 studies complied with inclusion criteria. Studies were RCT (n=6), non-RCT (n=1) and before-and-after (n=4) from Germany (n=3), Australia (n=2), Scotland (n=2) and one each from United States, Switzerland, Vietnam and Thailand. Interventions evaluated were: face to face training sessions (n=10); role-play (n=9); software decision making program (n=1); and simulated patient visits followed by immediate feedback (n=1). Outcomes were measured using: simulated patient methodology (n=8); role-play (n=1); and a survey (n=1) with most (n=10) reporting an improvement in communication behaviours. The quality of reporting was variable.
Discussion. There has been little empirical evaluation of interventions to enhance communication during OTC consultations. All studies targeted pharmacy personnel. No study targeted consumer behaviour. Future interventions should consider all participants in OTC consultations.

Liberati A et al (2009) BMJ 339:b2700.
Higgins PT, Green S eds (2011) Cochrane handbook for systematic reviews of interventions.
Des Jarlais DC et al (2004) Am J Public Health 94(3):361-366

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Barriers to the treatment of gout: a community pharmacy perspective

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Introduction. Gout is an increasingly prevalent form of painful inflammatory arthritis. Effective treatments for gout are well established however management is suboptimal. Community pharmacists can improve outcomes in chronic disease management; however their role in gout management is not well understood.
Aims. To explore community pharmacist experiences with gout patients and their current role in gout management, and to identify any barriers or facilitators to optimal gout management.
Methods. A snowball recruitment strategy was used, with community pharmacists known to the research team initially invited to participate. Semi-structured interviews (one-on-one) were conducted with 16 community pharmacists. Interview questions focused on the pharmacist's experiences of managing gout, providing gout education, and their perceptions of patients' gout management. Interviews were transcribed verbatim and independently analysed by two reviewers to identify themes.
Results. Community pharmacists' current role in gout management includes providing education, monitoring adherence and assisting with any medication questions. The main barriers to optimal management by pharmacists identified were difficulties in monitoring adherence, lack of time in the pharmacy, low prioritisation of gout, and lack of continuing education for both pharmacists and patients. However, pharmacists had a good understanding of medications, and gout associated lifestyle/diet factors. Pharmacists perceived poor patient understanding, incorrect colchicine dosing and patient non-adherence as additional barriers to optimal gout management.
Discussion. Despite displaying a good understanding of gout, barriers within the community pharmacies appeared to prevent pharmacists from providing optimal gout management. Interventions addressing these barriers are necessary to allow the potential benefits of community pharmacist participation in gout management to be realised.

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Preliminary results of Vitamin D supplementation in residents of aged care facilities

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Introduction. Inadequate vitamin D is associated with osteoporosis and fractures. Vitamin D can be obtained in sufficient amounts from adequate sunlight exposure; however, residents of aged care facilities (ACFs) typically have little sunlight exposure. Subsequently, residents may be at an increased risk of premature death or prolonged rehabilitation due to osteoporotic fractures. It is recommended that all residents be supplemented with 1000 IU vitamin D daily and consume an adequate calcium intake.
Aims. To determine the current rate of vitamin D supplementation amongst residents of ACFs in Tasmania.
Methods. Resident details from consenting Tasmanian ACFs were recorded. Information included demographics, daily vitamin D and calcium supplementation, estimated daily calcium intake, estimated sun exposure, diagnosis of osteoporosis, and history of falls and fractures.
Results. Details of 448 mobile, non-palliative residents (309 female and 139 male) from eight ACFs were obtained. Residents were 84±9 years of age. Most (n=281, 63%) had little or no sunlight exposure. One hundred and forty-four residents (32%) were recorded as having osteoporosis or a past fracture. Daily vitamin D supplementation of at least 1000 IU occurred in 87 of these residents (60%) and in 162 of 304 residents with neither (53%). There was no statistical difference in supplementation between the groups (P=0.16).
Discussion. Vitamin D supplementation rates remain below desired levels amongst the residents of Tasmanian ACFs, even in the higher risk group of those with a documented history of osteoporosis or fractures. According to the current guidelines for residents of ACFs, all residents should be taking a vitamin D supplement (combined with adequate calcium intake) to improve bone strength and decrease fracture risk. In this population the consequences of fractures include death or a lengthy rehabilitation. Therefore, all positive fracture avoidance measures, such as adequate vitamin D supplementation, should be implemented. Our results strongly suggest the need for an intervention targeted at improving the rates of vitamin D supplementation within ACFs.

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Postpartum depression screening by pharmacists in primary care: a literature review

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Introduction. Postpartum depression (PPD) is a debilitating condition that affects 10-15%[1] of women globally. Primary-care based PPD screening programs offer a good chance of identifying such people at risk with a view to referral and appropriate treatment. Pharmacists have been successfully involved in screening for varipus health disorders and would be in a a prime position to conduct PPD screening in the course of their professional practice.
Aims. To review the international literature on primary-care based PPD screening programs conducted by pharmacists.
Methods. The search was conducted between April and October 2014 in five databases- Medline, Embase, CINAHL, PsycInfo, International Pharmaceutical Abstracts, and Scopus. Key search terms included: allied health professionals, pharmacist, primary care, postpartum depression and screening. Inclusion criteria for article selection were that a) PPD screening constituted the focus of the research b) research based in a primary-care setting c) focused on the screening outcomes, with/without an intervention and d) screening conducted by an allied health professional. Articles were excluded if they were not in the English language, focused on the antenatal period or based in secondary/tertiary settings. A modified STARD tool was used to assess study quality.
Results. A total of 752 unique articles were obtained with eleven studies meeting the inclusion criteria. All articles selected for review used the Edinburgh Postnatal Depression Scale (EPDS) as the primary screening tool and were led by nurses/midwives to the exclusion of other allied health professionals. Four studies conducted screening only No studies were found which studied the role of pharmacists in primary-care based PPD screening.
Discussion. Wider adoption of screening protocols within all facets of primary care is recommended given the prevalence of PPD. There is little evidence in the literature to suggest that comprehensive research has been initiated to determine the feasibility of PPD screening being conducted by a wide range of allied health professionals, including pharmacists. Further research in the area is timely

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Product recommendations for teething in young children

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Introduction. Parents have a wide range of options for teething treatments, many of which are available through pharmacies but also through stores selling baby products and health foods. Oral medicines such as paracetamol and ibuprofen, and topical gels such as choline salicylate and lignocaine give temporary relief of pain and/or inflammation. Objects such as chewing toys and cold, solid foods can also reduce inflammation and relieve the symptoms of teething by applying pressure to the gums. An array of complementary medicines are available, ranging from homeopathic teething tablets to herbal-based oils and amber necklaces, none of which have any evidence for efficacy to support their use.
Aims. To determine teething product preferences of staff in a position to advise the public about teething treatments.
Methods. Pharmacies, natural health stores and baby shops within a 150-kilometre radius of Brisbane were visited and staff interviewed on their perceptions of products available for use in teething infants, the product that they recommend the most, their view of products most preferred by parents, along with age, education level, gender, and ethnicity.
Results. Across all 150 staff interviewed, the most common first line treatments recommended for teething were ibuprofen (32%) and paracetamol (21%). Other products were recommended as first line treatment by some: chewing on teething toys/rusks/cold foods (15%), SM33 (8%), and Bonjela (6%). Homeopathic remedies or flower essences were preferred by 15% of staff, and amber jewellery by 3% of staff. Naturopaths were most likely to recommend homeopathic medicines, whereas staff working in baby stores preferred the use of teething toys. Oral medicines such as paracetamol and ibuprofen were recommended as first line treatment by 77% of pharmacists and 62% of pharmacy assistants, but 1 pharmacist and 7 pharmacy assistants (7% of pharmacy staff interviewed) selected homeopathic products or amber necklaces as their first-line product choice for pain in teething infants.
Discussion. Staff generally recommend products within in the scope of the market in which they work, presumably due to differences in awareness of or access to information on the evidence supporting their use. 7% of pharmacy staff identified products with no evidence for efficacy as their first choice for teething pain.

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Indonesian Hospital Pharmacists' Roles and Responsibilities

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Introduction. In Indonesian public hospitals, pharmacists provide clinical and inventory services, and are expected to generate revenue from pharmacy department services. Various expectations from different types of hospitals create tensions between pharmacists' roles written in the standards and their real life responsibilities. However, very little is reported about Indonesian pharmacists' responsibilities and roles on the ground. Furthermore, an international perspective is relevant to development of hospital pharmacy in Australia.
Aims. This study was designed to explore the perceptions of Indonesian hospital pharmacists' about their roles and responsibilities.
Methods. The study has HREC approval from the University of Sydney and local hospitals in Indonesia. A purposive and convenience sampling approach was adopted to approach and recruit hospital pharmacists. Face-to-face semi-structured interviews were conducted. The interview guide was developed from literature review and pilot interviews with Australian hospital pharmacy key informants. Where there was consent to audiotape the interviews were transcribed verbatim and translated into English. Extensive field notes were taken for all interviews. Holistic and descriptive coding was used for first cycle coding, with focused coding for second cycle coding to generate themes.
Results. Thirty one Indonesian pharmacists from various types of hospitals and organisations in five provinces in Indonesia were interviewed between February and April 2015. The interview lasted between 15 minutes and 80 minutes, with an average of 45 minutes. Participants were Director of Pharmacy, Deputy Director of Pharmacy, Supervisor of Pharmacy, Clinical pharmacist, and other positions. The ownership of hospitals ranges from public to private non-profit hospital. The type of hospitals consists of general to specialised hospitals.
Discussion. Overall, participants had administrative roles, worked in multidisciplinary team as medicine experts, had onerous responsibilities, self-perceived roles as leaders and managers, and required specific skills and in-depth knowledge. A strong emphasis on pharmacists' administrative roles, led to underutilisation of their roles; and although their responsibilities were of high risk, they lacked in influence to optimise their roles and responsibilities.

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Development of a cognitive framework for pharmaceutical care: ASE-C-POP

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Introduction: Pharmaceutical Care is defined as "the responsible provision of drug therapy to achieving definite outcomes that improve a patient's quality of life"[1]. One of the basic concepts in understanding the needs for Pharmaceutical Care are Drug-Related Problems (DRPs)[2]. As complexity of treatments increase, identification of DRPs by healthcare professionals remains vital for patient safety. DRPs present, however, a list of potential problems not a strategic framework for assessing a medication regime.
Aims: To develop a framework to enable students to construct cognitive strategies for managing complexity in Pharmaceutical Care.
Methodology: A commonly classification of DRPs is that of Strand[2]. We found teaching students to recognise problems using this system did not help intuitive problem solving, and many students struggled to remember and review each element of the classification as presented. We designed a cognitive framework to improve problem identification and problem-solving by pharmacy students. We took an iterative approach in developing the framework, based on interviews with practising pharmacists, and critically compared the approaches taken to review a medication regime. We applied the rhetorical "rule of 3" to chunk the elements of Strand's DRPs into memorable groupings to follow a logical decision tree for therapeutic review.
Discussion: Our framework teaches students to 'screen' a patient's medication, by asking first "is it **A**ppropriate?" that they receive this drug– if so, "is it **S**afe?" and "is it **E**ffective?" **C** in the acronym reminds students to comprehensively look at the whole patient; the 'care' factor. A problem list is built as each medication is screened. Options for resolving DRPs are identified, and if a recommendation for drug therapy is made the 'new' drug is screened as well. This leads to a logical framework for discussing potential DRPs with a prescriber, allowing the prescriber to follow the pharmacists' reasoning.
Outcome: Students quickly embrace the ASE-C-POP approach when in problem-based learning. The framework allows tutors to identify and discuss a student's clinical reasoning. To evaluate this cognitive tool further we plan to conduct a 'Think-aloud' study to examine how health professionals enrolled in an Allied Health prescribing course conduct a comprehensive review, assess safe use of medication and apply this within the NPS Prescribing Competency Framework[3] and then trial this framework in our teaching program.

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Antimicrobial stewardship in paediatric oncology – optimising gentamicin use in febrile neutropenia

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Introduction. Antimicrobial stewardship programs are being developed in hospitals worldwide aiming to decrease inappropriate antimicrobial usage, improve patient outcomes and reduce adverse consequences of antimicrobial use.
Aims. To evaluate the introduction of new guidelines, as an antimicrobial stewardship intervention to improve the use of gentamicin for paediatric febrile neutropenic oncology patients, at a tertiary paediatric teaching hospital.
Methods. On October 1st 2012 gentamicin was removed from empirical antibiotic therapy guidelines for febrile neutropenia. Data on gentamicin usage before and after guideline changes were collected retrospectively from children with febrile neutropenia admitted to the hospital between January 2012 and December 2013 who had at least one gentamicin concentration measurement. Bacterial culture status and duration of gentamicin therapy were compared between admissions before and after guideline change to assess the impact on practice.
Results. Data were collected from 98 children corresponding to 139 separate admissions (81 pre-guideline and 58 post-guideline change). Following guideline change, gentamicin was used in a significantly lower proportion of admissions associated with no cultured bacteria or a gram positive infection; the proportion of admissions in which gentamicin was used that were not associated with a cultured gram negative organism dropped from 79% to 47% (P = 0.001), indicating a change in practice. Following guideline change admissions in which gentamicin was used for >48 hours despite the absence of a confirmed gram negative infection decreased from 50% to 35% (P = 0.18).
Discussion. Guideline change driven through an antimicrobial stewardship initiative involving paediatric infectious disease and oncology clinicians has significantly reduced the inappropriate use of gentamicin in febrile neutropenic children.

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An evaluation of using fish oil among Australian patients with rheumatoid arthritis through medicine review

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Introduction. Rheumatoid arthritis (RA) affects about 2% of the Australian population (Australian Institute of Health and Welfare, 2014). Use of complementary medicines such as fish oil is common among patients with RA. Australian therapeutic guidelines suggest taking at least 2.7g fish oil daily to manage RA symptoms (Therapeutic Guidelines). Australians spend over $200m on fish oil supplements every year, however, use of fish oil supplements can be largely under the influence of media with no supervision from health practitioners. Underdose treatment with fish oil may cause a financial burden to Australian families with RA.
Aims. This project examined the fish oil dosage used among Australian patients with RA using a pilot, representative sample and hypothesised that Medicine Review (a government-funded program in Australia that allows a pharmacist to review patients' medications) can identify those patients taking subtherapeutic dose of fish oil and subsequently optimise the dose and address non-adherence.
Methods. Human ethics approval was obtained. We analysed a sample of 600 de-identified Medicine Review reports from community-dwelling patients. Daily intake of fish oil supplements and the recommendation of pharmacists were extracted and assessed against Australian therapeutic guidelines.
Results. Interestingly, all patients with RA used subtherapeutic doses of fish oil. Only %11 of these patients used regular NSAIDs concomitant with fish oil. Analysing the recommendations of pharmacists in patients with RA using fish oil supplements, showed that in 66% of these cases, pharmacists identified subtherapeutic doses of fish oil, however, only in 33% of the cases, pharmacists made clear recommendations to increase the dose of fish oil.
Discussion. The results indicate of subtherapeutic and ineffective use of fish oil supplements among a sample of Australian patients with RA. Pharmacists have important roles in promoting evidence based use of fish oil supplements through Medicine Review.

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General practitioner-pharmacist collaborations to improve patients' adherence to medication

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Introduction: Pharmacists and general practitioners (GPs) face an increasing expectation to collaborate on a number of healthcare issues, including patient non-adherence to medication.
Objectives: To investigate (i) type of interactions between community pharmacists and GPs aimed at improving patients` adherence (ii) factors influencing inter-professional collaboration; and (iii) opinions about how healthcare professionals might more effectively collaborate in the future to support adherence.
Methods: A qualitative study using focus group discussions (n=6) with GPs (n=22) and community pharmacists (n=23) was undertaken in three distinct geographic areas of Sydney metropolitan. Audio-recordings were transcribed verbatim and content analysed using thematic content analysis. Three themes were identified i) type of interactions ii) factors influencing collaboration and iii) suggested strategies to improve collaboration.
Results: Inter-professional interactions between community pharmacists and GPs do occur, but they are very limited and mostly concern administrative issues. Factors found to influence pharmacist-GP interactions included work environment (time constraints, practitioner accessibility, staffing) and stakeholder attitudes and behaviors (open communication, trust, respect and willingness to work as a team). A range of suggested strategies to improve collaboration were proposed. These included: access to patients' medication histories and clinical information for pharmacists, improved methods for secure electronic communication between parties, and arranging regular meetings.
Conclusions: Collaboration in the context of patient adherence is still undeveloped. There is a need for changes to the current structure of primary care to break down the silos of practice. This may lead to improvement in collaboration between healthcare professionals as a first step in supporting patients' adherence to their therapy.

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What do health consumers want to know about childhood vaccination?

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Introduction. Vaccines are crucial to population health. Nevertheless, there are multiple barriers for parents or carers to vaccinate their children, resulting in lower than required population immunisation coverage.
Aim. This study aimed to identify the information needs and concerns of health consumer regarding childhood vaccination.
Methods. We conducted a retrospective, mixed method study of 1,342 childhood vaccination-related calls to an Australian consumer medicines call centre, NPS Medicines Line (September 2002-June 2010). Call narratives were explored to identify the key themes. Themes were compared for callers from high and low immunisation coverage areas (National Health Performance Authority data linked to caller postcode).
Results. Vaccines that raised the most questions were the measles, mumps, rubella vaccine (29.9%), combined diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis, Haemophilus influenzae type b vaccine (18.5%) and varicella vaccine (17.5%). The most commonly identified theme was safety concerns (60.4%), with questions about vaccine constituents as the predominant issue (31.6%). Other common themes involved adverse drug reactions (12.2%) and general vaccine information (10.4%). The most important difference between low and high immunisation areas was the higher level of concern about vaccine preservatives (mercury and thiomersal) in low immunisation areas.
Conclusion. The consistent number of vaccine-related calls, particularly about safety, demonstrates an information gap that can act as a barrier to vaccination. Improving health professionals' awareness of the immunisation rate in their local area and the concerns that act as barriers to vaccination uptake for their patients can help to fill the information gap and improve immunisation coverage.

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Using the technology: the novel use of a smart phone app to assess the potential redistribution of dispensary tasks in preparation for the introduction of a new checking technician role.

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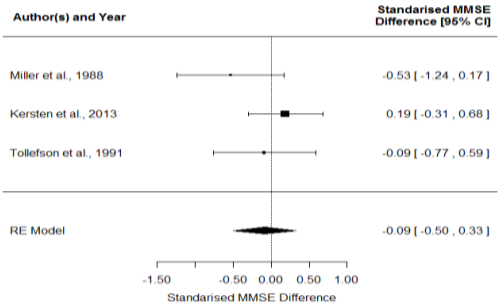
Introduction: Time and motion studies have been used in many workplace settings to determine the amount of time staff spend on specific tasks during their work day. Several techniques have been used previously from shadowing staff to bar-code scanning. This study utilised a smart phone app to allow pharmacists and technicians to report their activities throughout their day.



Aim. To determine if a smart phone app would provide a robust, participant friendly, method to collect data on dispensary staff activities for a time and motion study. .

Methods. A work sampling technique was used in conjunction with a smart phone to collect data at ten minute intervals for five working days. Staff from both hospital and community pharmacies were given seven categories to indicate the task they were currently performing when a reminder beeped at each ten minute interval. The seven categories covered; patient focused activities, dispensing activities and personal time.

Results: The participants reported that this experience was not too intrusive in their day and they found the app quite user friendly. This method produced large data sets, however there were time points missed. Humanistic factors influence the robustness of the data.

Discussion: Shadowing staff or continuous reporting, although the gold standard, is not practical in a pharmacy setting. Space, cost and staffing limitations made phones a practical choice. Smart phones are small, light and portable and most staff are familiar with one so training is simple. Utilising the phone and a free app is a cost effective method of collecting data. Although work sampling does not give a continuous picture of a 'normal' workday it does make it possible to measure how staff spend their time, particularly on repetitive activities.

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The introduction of an advance role for technicians facilitates greater patient focused activities for pharmacists

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Introduction: Checking Technicians exist in the United Kingdom and the potential for this role to be adopted in New Zealand is under discussion. Currently technicians are limited to assembling prescriptions and must await the pharmacist who is required to perform the final accuracy check. This checking requirement can see the pharmacist confined to the dispensary and limited in their contact time with patients.

Aim: To carry out a pilot study to investigate if the introducing a Pharmacy Accuracy Checking Technician(PACT) into the New Zealand pharmacy setting would allow pharmacists to spend more time on patient focused activities.

Method: Twelve pharmacy workplaces were selected, four hospital and eight community, from a variety of locations around New Zealand. A supervising pharmacist and a trainee were identified from current staff. A work sampling technique was utilised to estimate the amount of time dispensary staff spent on dispensary and patient focused activities before and after the introduction of the PACT.

Results: Twelve pharmacies with fifteen technicians were recruited into the pilot study. Initially technicians could spend up to 60% of their day assembling prescriptions and pharmacists could spend up to 30% of their day checking prescriptions with community pharmacists spending twice as much time as hospital pharmacists (mean = 24% vs 13%). After the introduction of the PACT dispensing activities by pharmacists decreased by almost half and the time spent on patient focused activities increased, hospital = 24% and community = 13%. Direct contact with patients increased for hospital pharmacists from 4% to 15% and community from 16% to 23%.

Discussion: This redistribution of roles allowed pharmacists to move into a more patient focused model. There were differences between the two pharmacy settings with hospital pharmacists spending less time in face-to-face contact with patients but both settings increased the time spent overall on patient focused activities.

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A systematic review and meta-analysis on serum anticholinergic activity and adverse outcomes in older people

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Introduction. Studies have reported associations between serum anticholinergic activity (SAA) and decline in cognitive performance, delirium, and functional impairment in older people.
Aims. A systematic review and meta-analyses to quantify associations between SAA and adverse outcomes in older people.
Methods. A literature search in Ovid MEDLINE, EMBASE, PsycINFO and IPA from 1946-2015 was completed. The primary outcomes of interest were cognitive and functional impairments associated with SAA. The Cochrane Risk-Bias tool was used to assess bias in randomised controlled trials (RCTs), and the Newcastle-Ottawa Scale to assess the quality of non-RCTs. Meta-analyses were conducted for RCTs and cohort studies separately.
Results. 5 RCTs, 5 prospective cohort studies, 3 longitudinal studies, 17 cross-sectional studies, and 4 case-control studies met the inclusion criteria. 70% of the studies examined an association between SAA and cognitive outcomes, and 30% examined an association with functional outcomes. The meta-analysis on RCTs showed no association with higher SAA and cognitive performance (I^2 = 27.64%, H^2 = 1.38 and p-value = 0.26), however the pooled data from 4 observational studies showed elevated SAA was associated with reduced cognitive performance (I^2 = 0.00%, H^2 = 1.00 and p-value = 0.34).
Discussion. Larger trials or cohort studies with adequate power and appropriate follow-up periods are required to confirm associations between SAA and adverse outcomes**.**

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Organisational climate related to the use of psychotropic medicines in Australian Nursing Homes: The role of managers

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Introduction. Research suggests that nursing home organisational culture influences the use of psychotropic medicines. Health care research investigating organisational culture often uses culture and climate interchangeably. Schein's theory defines organisational climate as staff perceptions of the work environment, while culture is the shared norms, value and deep seated assumptions of a group of members working together. Managers are identified to influence staff perceptions of the work environment which in turn effect staff practices and resident outcomes.
Aims. This study explores the role of managers and the perceptions of the role of manager in shaping the organisational climate related to the use of psychotropic medicines in nursing homes.
Methods. A qualitative study was conducted with staff from eight nursing homes in Sydney, Australia. Purposive sampling was used to recruit 40 participants representing a broad range of disciplines and roles. The method of constant comparison was used to derive key concepts.
Results. Several managers were mindful of the need to re-inforce organisational goals for resident care to reduce on-site staff preference for psychotropic medicines in residents. Managers' perception of the role of nurse assistants' in providing input into the use of psychotropic medicines in residents varied across facilities. This created different responses from nurse assistants in communicating concerns regarding the use of psychotropic medicines. Many visiting staff perceived the level of manager support for the integration of their services into the nursing home influenced their level of participation for the review of psychotropic medicines.
Discussion. This study highlights that managers are important in creating a positive climate for both on-site and visiting staff which promotes staff to carry out processes necessary to improve the quality use of psychotropic medicines in nursing homes.

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Medicine Procurement in hospital pharmacies in Nepal: A qualitative study based on the Basel Statements

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Introduction. The healthcare system in Nepal, a low-income country in South Asia, has expanded rapidly in recent years, however little is known about common medicine procurement practices. Accessibility and affordability of evidence-based medicines have always been a concern in Nepal. In light of recent natural disasters that devastated Nepal, it has been even more crucial to have readily available access to affordable, good-quality, evidence-based medicines.
Aims. To investigate medicine procurement practices in hospital pharmacies of Nepal within the framework of International Pharmaceutical Federation [FIP] hospital pharmacy guidelines "the Basel Statements".
Methods. We conducted semi-structured interviews with hospital pharmacists or procurement officers in hospital pharmacies of four major regions in Nepal to explore procurement practices. Data were collected until saturation of themes, analysed using the framework approach, and organised around the statements within the procurement theme of the Basel Statements.
Results. Interviews conducted with 53 participants revealed that the procurement guidelines of the Basel Statements were adopted to a certain extent in hospital pharmacies of Nepal. The majority of hospital pharmacies in Nepal reported using an expensive direct-procurement model for procuring medicines. Most had no formulary and procured medicines solely based on doctors' prescribing habits, which were heavily influenced by pharmaceutical companies' marketing strategies. Whilst most procured only registered medicines, a minority reported purchasing unregistered medicines through unauthorised supply-chains. And although the majority of hospital pharmacies had some contingency plans for managing medicine shortages, a few reportedly had none.
Discussion. Procurement guidelines of the Basel Statements were found to be partially adopted. Adoption and regulation of national and international policies is recommended for enhancing medicine accessibility, as well as improving preparedness for health emergencies during natural disasters and health epidemics.

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Exploring the shift in management autonomy between caregivers and children when taking asthma medication: A caregiver's perspective

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Introduction. Even though prevalence of current asthma has declined in children and young adults, in Australia alone, rates are still high by international comparison. Primary responsibility for management of a young child's asthma is assumed by the parent/caregiver, however children take on an increased responsibility over time. Ensuring good practices and quality use of medicines from an early stage is paramount in achieving ongoing optimal health outcomes. The sharing and transfer of autonomy that must occur between parent and child is of particular importance, as the child grows older.
Aims. To understand the development of medicine taking autonomy in children and identify the facilitators and barriers to autonomy from the parent/carer's perspective
Methods. There are three parts to the study. Carers will be assessed on their inhaler technique using a validated error checklist. A semi-structured telephone interview will then be conducted with Carers. Finally they will be asked to complete the Hollingshead index questionnaire and Paediatric carer's quality of life questionnaire (PACQLQ).
Research Questions. When and why do caregivers/parents give children autonomy and what are the factors that impact on these decisions? What does autonomy actually mean and how is it implemented? Once autonomy is established, what is the role of the parent in management?
Data Analysis. Inhaler technique and questionnaire data will be analysed descriptively. Audio data from interviews will be audiotaped, transcribed verbatim and content analysis performed. Researchers will confer to determine emerging themes. A framework of analysis that is both sensitive to empirical and theoretical precedence as well as open to new emerging concepts will be employed.
Potential Significance. We are able to gain insight into the 'norms' that might be established for children when they first start using their inhalers independently. This will allow us to better understand the history behind the experiences of adults and their medication taking behaviours and bridge the gap in knowledge regarding how and why children are given autonomy in managing their own asthma.

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Systematic review of the effect of vitamin D on women's mental health during the perinatal period

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Introduction. The perinatal period is a vulnerable time for depressive disorders with 15-30% of mothers in that time experiencing mood disturbances. Symptoms can be associated with preeclampsia, low birth weight, substance use, risky behaviors and long term adverse effects on the mother and offspring. Investigating a preventive intervention that decreases the number of cases and/or severity of symptoms of mood disturbances is important. Considering that vitamin D deficiency is more prevalent in perinatal women with more than 40% being vitamin D deficient, and that receptors for vitamin D are present not only in bone but also in the brain, recent research has suggested that vitamin D plays a role in addressing these public health issues that affect the local and global population.

Aims. To review the literature to investigate the potential role of vitamin D deficiency on women's mental health particularly in the perinatal period.

Methods. Peer-reviewed RCTs, cohort, case control and cross-sectional studies in English from 2005 onwards were accessed using the AMED, CINAHL, IPA, Maternal and infant care, EBM ALL, Embase, Global health, MEDLINE, PsycINFO and PubMed databases. A comprehensive search strategy was undertaken using variations of following keywords: vitamin D; mental disorders; perinatal depression and women. Studies were included if the sample consisted of at least 50% women; subjects were over 18 years old; serum 25(OH)D was measured; a tool was used to assess symptoms of mood and a quantitative analysis between vitamin D and depression scores was conducted. Authors met on multiple occasions to reach consensus on eligibility of potentially relevant articles found.

Results. The review identified 34 articles matching inclusion criteria; 3 controlled trails, 12 cohort studies, 7 case controls and 12 cross sectional studies. A negative correlation between serum vitamin D levels and depression score was seen in 24 studies with the remaining producing inconclusive results based on vitamin d status and gender.

Discussion. Given that our analysis showed a consensus among studies linking vitamin D deficiency and depression, and that those that failed to produce a result included a majority of males or vitamin D sufficient subjects, further research in the form of studies on vitamin D deficient perinatal women that seek to correct vitamin D levels through supplementation are needed to determine if this relationship is causal. This has the potential to help revise guidelines and change practice in terms of prevention and clinical management of perinatal depression.

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Community pharmacist practices, attitudes, recommendations, information and education needs for herbal and nutrient complementary medicines for weight loss

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Introduction. Over half the Australian population use some form of alternative or complementary medicine, spending more than $2 billion dollars annually. Herbal/nutrient complementary medicines for weight loss (WLCM) are popular among consumers, with pharmacies being a major retail outlet for these products. To date, there are no Australian studies exploring community pharmacists' attitudes and practices regarding WLCMs sold in pharmacies.

Aims. To investigate pharmacists' WLCM practices in the context of other pharmacist weight-management support practices (provision of lifestyle advice, orlistat and meal-replacement treatments); and gain insight into community pharmacist attitudes, recommendations, information and education needs.

Methods. Pharmacists were randomly selected from a sample of 214 community pharmacies located within different socioeconomic areas in the Greater Brisbane region, Australia. Pharmacists completed a survey exploring their weight-management practices, with a specific focus on WLCM practices. Items within the questionnaire were adapted from previously published surveys or developed by the authors. Data collected from the sample group represented pharmacist practices within the Greater Brisbane metropolitan region.

Results. The response rate was 51%. During weight-management consultations, a relatively high proportion of consumers (37%) sought pharmacist advice relating to WLCM compared to other weight-management practices. Only 10% of pharmacists however recommended them. The resources that most pharmacists reported using provide insufficient WLCM information and may not be evidence-based. The majority of community pharmacists (85%) were interested in further education about WLCMs.

Discussion. Results from this study highlight the need for pharmacy professional bodies to develop education programmes for pharmacists that are evidence-based to assist consumers with popular and widely available WLCM products.

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Up close and personal with those experiencing homelessness: Pharmacist involvement in a multidisciplinary healthcare team

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Introduction. The Brisbane City Council holds a biannual *Homeless Connect* event which brings together business and community groups on one day to provide free services to people experiencing or at risk of homelessness. Pharmacists were involved in this initiative and provided health services in a multidisciplinary healthcare environment building on the lessons of previous *Homeless Connect* events (Chan et al, 2015)

Aims. To explore pharmacists reflections on their role in a multidisciplinary healthcare team providing services at a community outreach event for those experiencing homelessness.

Methods. The pharmacists (n=2) documented the types of services provided during the *Homeless Connect* event. A semi-structured interview was conducted post-event to investigate barriers, facilitators and changes that would be recommended for future events. Their perceptions of their role in the multidisciplinary healthcare team were also explored.

Results. Primarily, the services provided included delivery of primary healthcare, advice on accessing cost effective pharmacy services and addressing medication enquiries. The pharmacists also provided moisturiser samples and health information leaflets. Interdisciplinary referrals were primarily between the pharmacists and podiatrists; no pharmacist-medical practitioner referrals occurred. The pharmacists did believe they had a positive role in this health initiative but improvements could be implemented to improve the delivery of these services in future events.

Discussion. Pharmacists can play an important role in providing services to people experiencing or at risk of homelessness and the overall experience was positive for the pharmacists. They were able to integrate into a multidisciplinary healthcare team in this setting but strategies for further collaboration were identified. The possibility of involving pharmacy students in future events was identified.

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Pharmacists' perspectives in optimising statin therapy in older inpatients

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Introduction. Indications for starting statin therapy are guided by established national and international guidelines. However, there is concern about their appropriate use with significant rates of over- and under-treatment with statins in older patients. Optimising statin therapy in this patient group may require multi-disciplinary effort, and in an acute care setting, pharmacists are well-positioned to be involved.

Aims. This study aimed to explore hospital pharmacists' perspectives in optimising statin therapy in older inpatients.

Methods. An anonymous questionnaire using a five-point Likert scale and open-ended questions was developed and piloted to capture pharmacists' attitudes towards their role in optimising statin therapy, compliance of therapy with guidelines, knowledge of statin-related side effects, and making clinical recommendations. An online questionnaire was advertised and distributed through the Society of Hospital Pharmacists' Australia e-newsletter.

Results. In the first two weeks of distribution, 54 practising hospital pharmacists have completed the questionnaire. Ninety-six percent (95% CI 90.7% - 100.0%) of pharmacists agreed that they have an important role in providing advice to physicians regarding the initiation, continuation or cessation of statin therapy. However, 38.9% (25.9% - 51.9%) were unsure that older patients were prescribed statins in accordance with clinical guidelines. In addition, 98.1% (94.4% - 100.0%) agreed that cessation or deprescribing of statins may be appropriate in some patients, but only 51.9% (37.0% - 64.8%) regularly make recommendations about deprescribing in older people, and this was not dependent on years of practice (P=0.123). Interestingly, over 90% (84.6% - 98.1%) identified a need for guidelines for deprescribing statins in older adults.

Discussion. Whilst pharmacists recognise that they have an important role in optimising statin therapy, there is a need for specific guidelines, particularly in regards to when it is appropriate to continue and deprescribe statins in older people.

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Practice Makes Perfect: Adherence a Possible Marker of Inhaler Technique Maintenance

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Introduction: In many patients optimal management of asthma is inadequate, with non-adherence and incorrect inhaler technique being major factors. Interventions have indicated that correction of these issues will lead to improved asthma outcomes[1]. Research has shown that patients can be taught how to achieve good inhaler technique, however maintenance remains problematic. The literature strongly suggests that certain predictors associated with inhaler technique maintenance can also be linked to adherence[1].

Aim: To investigate the relationship between inhaler technique maintenance and adherence and the extent on which they impact on one another.

Method: Data mining of a large community pharmacy database (n=570), Pharmacy Asthma Management Service (PAMS) database. Utilising PAMS data, correlational and regression analyses were undertaken to determine potential predictors of inhaler technique maintenance and the impact of adherence measured by the Beliefs Medication Questionnaire (BMQ).

Results: At baseline, 24% of patients (n=349) demonstrated correct technique (performed all device maneuvers without any errors), which increased to 100% after delivery of pharmacist education. At the first follow up (1 month), correct technique had declined to 49% of patients. A significant relationship was found between inhaler technique maintenance (ability to retain correct technique at follow up) and the BMQ adherence score. There was a statistically significant difference in BMQ adherence score relative to inhaler technique maintenance: $F(2, 230) = 4.5$, $p < 0.05$. Post Hoc comparisons indicated that the mean score for patients who maintained correct technique ($M=1.09$, $SD=1.26$) was statistically different from the mean score for patients who did not maintain correct technique ($M=1.66$, $SD=1.39$).

Discussion: In exploring the predictors of inhaler technique maintenance, our research has identified that those patients who are at greater risk of poor technique maintenance, or also at greater risk of non-adherence to their asthma medications. This finding can help inform pharmacy interventions around inhaler use in the future.

1- Basheti IA, et al (2007) J Allergy Clin Immunol 2007; 119(6):1537–1538.

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An audit of medications across two sites of an aged care organization in South Australia

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Introduction. Multiple morbidities and the concomitant use of multiple medications are known to be common in the elderly and this situation is also reflected in aged care.

Aims. To undertake an audit of medication use by all residents in two residential aged care facilities.

Methods. Following ethics approvals, two researchers (RL and EP) undertook digital recording of the current medications (both regular and PRN) prescribed over one calendar month for approximately 170 residents at Site 1 and 100 residents at Site 2.

Results. Polypharmacy was common, although two residents were not using any medication. A number of issues of concern were identified and included: (1) inappropriate crushing of oral medication (Efexor XR®, Palexia SR®); (2) overuse of paracetamol in an 85 year old woman (two oral dosage forms co-prescribed to a total of 4990mg in a 24 hour period); and (3) use of oral trimethoprim/ sulfamethoxazole topically. All these were highlighted to the RN in charge of the unit involved.

Discussion. Inappropriate crushing of sustained release dosage forms is well recognised (Downey et al 2015) and it is surprising to find this practice still occurring; the potential for hepatic toxicity is heightened in acute paracetamol overdose (Remien et al 2014); and prescribing of topical antibiotics for “non-evidence based indications” risks an increase in antibiotic resistance as well as possible harm to the individual. These few examples emphasise the importance of pharmacist oversight of medication use across health care and suggest that the overall quality of a facility *per se* is no reason for complacency.

Downey CE, Thakerar A, Kirsa S (2015), *Journal of Pharmacy Practice and Research*. 45 (2): 146-51.

Remien CH, Sussman NL, Adler FR (2014), *Mathematical Medicine and Biology* (2014) 31: 302–17.

Lapolla WJ, Levender MM, Dacis SA et al (2011), *Dermatologic Surgery*. 37:1427–33.

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Hospital evaluation of medicine usage……past its DUE date?

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

Introduction. Drug-usage evaluations (DUEs), structured and ongoing audit and feedback processes designed to improve the quality and cost-effectiveness of medicine use, have been essential components of clinical pharmacy practice in Australian hospitals since the 1990s. Resource depletion, altered priorities and lack of a multidisciplinary approach threaten the sustainability of DUE activities.[i]

Aims. To investigate the current support, resource allocation and perceptions towards DUE, the barriers and enablers for delivery in NSW and ACT public hospitals.

Methods. Pharmacy directors, DUE and Antimicrobial Stewardship (AMS) pharmacists at 67 NSW and ACT hospitals with on-site pharmacy services were invited to an online survey requesting a) descriptions of DUE activities; b) recommended knowledge or skills of DUE pharmacists; and c) ways to improve DUE activities.

Results. Less than one-third of respondents (8/25) had a dedicated DUE position in their hospital, compared to 19 AMS-dedicated positions. Allocations ranged from 0.2 to 1.0 full-time equivalent pharmacist. Emergent themes were the lack of resources, competing priorities and compulsory audits; challenges associated with data collection; the skillset of available pharmacists; the onerous process of ethics approvals and engagement of all disciplines and executive in DUE practice. There was a strong desire to improve the situation. A number of enablers were identified including the National Safety and Quality Health Service Standards.

Discussion. Many 'traditional' DUE activities have been replaced by activities promoting appropriate use of antimicrobials, and mandatory benchmarking audits; albeit utilising DUE principles as a framework. The capacity of many hospitals to undertake targeted individual hospital-focused improvement has diminished. While the implementation of electronic medication management may facilitate patient identification and data collection, change management strategies are required to translate DUE findings into clinical practice. For the valuable outcomes to the patient and healthcare system that DUE activities provide, a 'fresher' more collaborative approach to clinical practice research, training, promotion and funding models with support from executive and medicines governance is now required.

[i]Avent M et al (2010) JPPR 40:15-18.

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An analysis of prescribing and supply of medicines for ophthalmic surgery in NSW hospitals.

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Introduction. .The appropriate supply of medicines before and after eye surgery is a resource-intensive activity with apparent variations in hospital prescribing and practices.

Aims. To describe a) current prescribing and dispensing practices of medicines for patients undergoing routine eye surgery in NSW hospitals; and b) compliance with current Australian guidelines[i].

Methods. 65 NSW public hospitals were invited to participate in an on-line survey requesting information regarding routine eye surgery; medicines used and their methods of supply; and any Drug and Therapeutics Committee (DTC)-approved clinical pathways or standing orders. The responses were analysed for variations in practice and concordance with available guidelines.

Results. Pharmacists from 11 hospitals completed the survey. Cataract surgery was the most frequently performed surgery. All respondents reported that a DTC was responsible for the formulary, oversight of standing orders and policies or protocols concerning medicines used in eye surgery. The survey identified wide variations in current practice with an apparent lack of adherence to Australian guidelines for antibiotic prophylaxis. Prescribing of both corticosteroid and non-steroidal anti-inflammatory eye preparations also varied. Supply of medicines was reported as labour-intensive for hospital pharmacies. Respondents also reported concerns with inappropriate prescribing, frequent wastage and inadequate patient counselling.

Discussion. Recently published evidence and international guidelines provide an evidence-based approach to prescribing of medicines for the prevention of endophalmitis and inflammation post cataract surgery [ii, iii] The development of professionally-accepted Australian guidelines and medication counselling resources, have the potential to reduce unnecessary medicine dispensing; decrease workload, wastage and costs; and improve patient outcomes.

i. Therapeutic Guidelines Ltd eTG45 July 2o15

ii. Endophthalmitis Study Group, European Society of Cataract & Refractive Surgeons (2007) J Cataract Refract Surg 33: 978-88

iii. Kessel Line et al. (2014) Ophthalmology121: 1915-1924.

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Evaluation of the physical, chemical and microbiological stability of compounded minoxidil suspension

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Introduction. Many medicines available in adult dose forms are required for use in paediatrics. The dearth of commercial paediatric oral liquid preparations has led to the requirement for pharmacists to prepare extemporaneous mixtures. Many of these products lack scientific evidence and have arbitrarily been assigned short shelf lives, leading to patient inconvenience and increased costs. Minoxidil is an antihypertensive used in children for the treatment of severe hypertension resistant to other drugs and requires an extemporaneous suspension to be prepared from the tablets. There are no published studies to support this formulation and so a default one week shelf life is currently assigned.
Aims. To generate data to support an extended shelf-life of extemporaneously compounded minoxidil suspension.
Methods. A validated HPLC assay method was used for determination of minoxidil in an extemporaneously compounded oral suspension. The stability studies were conducted at 4 ˚C, 25 ˚C, and 4 ˚C with 3 days at 25 ˚C after 7 days; the latter to simulate daily dispensing of the product and the possibility of the sample being left out of the refrigerator over the weekend. Duplicate samples were taken from each of triplicate bottles at each selected time point throughout the 24 week stability study. Physical properties such as colour, dispersibility and pH were evaluated at each time point. Microbiological testing was performed.
Results. The minoxidil suspension was stable at 24 weeks and 12 weeks with more than 90% of potency at 4 ˚C and 25 ˚C, respectively. No colour or pH changes were seen at 4 ˚C, but white to yellow colour changed at 25 ˚C on week 4. The suspensions were easily dispersed when shaken. Microbial tests were within the required limits.
Discussion. The shelf-life of this formulation of minoxidil oral suspension (1 mg/mL) manufactured under the code of Good Manufacturing Practice can be extended to 24 weeks at the storage conditions of 4 ˚C and 4 weeks 25 ˚C due to colour changes.

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Evaluation of the physical, chemical and microbiological stability of compounded carbimazole suspension

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Introduction. Many medicines available in adult dose forms are required for use in paediatrics. The dearth of commercial paediatric oral liquid preparations has led to the requirement for pharmacists to prepare extemporaneous mixtures. Many of these products lack scientific evidence and have arbitrarily been assigned short shelf lives, leading to patient inconvenience and increased costs. Carbimazole is used in children for the treatment of hyperthyroidism and requires an extemporaneous suspension to be prepared from the tablets. There are no published studies to support this formulation and so a default one week shelf life is assigned.
Aims. To generate data to support an extended shelf-life of extemporaneously compounded carbimazole suspension.
Methods. A validated HPLC assay method was used for determination of carbimazole in an extemporaneously compounded oral suspension. The stability studies were conducted at 4 ˚C, 25 ˚C, and 4 ˚C with 3 days at 25 ˚C after 7 days; the latter to simulate daily dispensing of the product and the possibility of the sample being left out of the refrigerator over the weekend. Duplicate samples were taken from each of triplicate bottles at each selected time point. The study length was 24 weeks or until chemical stability was below 90% for all temperature conditions. Physical properties such as colour, dispersibility and pH were evaluated at each time point. Microbiological testing was performed.
Results. The carbimazole suspension maintained greater than 90% potency for 3 weeks at 4 °C. The formulation was unstable at 25 ˚C with maintenance of greater than 90% potency of 3 days. No colour or pH changes were seen. The suspensions were easily dispersed when shaken. Microbial tests were within the required limits.
Discussion. The shelf-life of this formulation of carbimazole oral suspension (2 mg/mL) is limited to three weeks at the storage condition of 4 ˚C.

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Management of cardiovascular risk in clozapine patients: how can we optimise it?

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Introduction. Clozapine therapy is associated with the development of several cardio metabolic conditions that contribute to an increase in morbidity and mortality. Patients diagnosed with schizophrenia are at increased risk of cardiovascular disease with contributing factors such as inappropriate medication prescribing, insufficient monitoring and a lack of primary intervention.
Aims. To identify ways of optimising the management of cardiovascular risk in clozapine patients.
Methods. Retrospective information on weight, BMI, waist circumference and cardiovascular medication was collected in a sample of 148 outpatients and 55 inpatients from two large tertiary metropolitan hospitals in Queensland.
Results. The average number of antipsychotics prescribed to patients were 1.9 (2.1for inpatients, 1.4 for outpatients) with a high prevalence of quetiapine, olanzapine and paliperidone. Lithium and antidepressants were more often prescribed for inpatients than for outpatients, being selective serotonin reuptake inhibitors and mirtazapine the most common ones amongst all patients. Metformin prescribing rates were higher in metal health patients than those in those patients with Type 2 Diabetes. Waist circumference and BMI figures showed that over 75% of patients were at 'substantially increased risk' of cardiovascular events.
Discussion. Prescribing of medications that may increase cardiovascular risk is prevalent in both inpatients and outpatients settings. The appropriateness of drug selection at the time of prescribing could be optimised by pharmacists and prescribers reviewing prophylactic measures that avoid adverse effects experienced by clozapine patients. With the transition of the access to clozapine from “hospital only” to being available in community pharmacy, further research is also needed on the role of the community pharmacist on the management of cardiovascular risk of clozapine patients.

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The role of community pharmacists on the assessment of swallowing difficulties of medications: consumers’ perceptions

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Introduction. Pharmacists are in the prime position to promote the quality use of medicines and to provide clinical assessment for people with swallowing difficulties of medications. However, the views of the general public towards the role of pharmacists in providing swallowing assessment have rarely been explored.
Aims. To gain a greater understanding of the opinions that the general public hold towards the role of pharmacists in assessing people with swallowing difficulties of medications as a pharmacy service intervention.
Methods. Consumers from three community pharmacies in Brisbane, Australia were recruited over 4 weeks and completed a 7-item closed question survey.
Results. A total of 564 consumers participated in the study. 6.6% (37/564) of people reported experiencing swallowing difficulties with medications. Overall, 44% (248/564) of consumers, regardless of their ages, would seek advice from pharmacists if they experienced swallowing difficulties of medications, in which half of these (50.2%; 150/299) were females. 32.4% (12/37) of people with difficulties swallowing medications were aged 66 or over (χ^2=4.7, p<0.05), with the majority of them (85%; 91/107) stating that they would not discuss their medication swallowing problems with pharmacists (χ^2=45.1, p<0.01). 38.8% (38/98) of males who would approach pharmacists for advice regarding difficulties in swallowing medications would also like pharmacists to assess swallowing difficulties of medications (χ^2=9.8, p<0.05).
Discussion. These findings suggest the strong value of community pharmacists playing a more active role in assessing swallowing difficulties of medications. With respondents aged 66 or over indicating a clear preference to seeking advice from other healthcare professionals (e.g. doctors) regarding such complications, future research should focus on exploring new approaches that promote the role of community pharmacists in identifying and assessing patients not only in the community but also in areas where patients are at high risk of swallowing difficulties of medications such as aged care facilities.

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Information seeking behaviors of breastfeeding women when considering the use of over-the-counter medicines

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Introduction. Breastfeeding women is a population that will often require an over-the-counter medicine. Safety of medications during breastfeeding can be concerning to both breastfeeding women and health professionals. Information regarding the safety of medications during breastfeeding is often limited and/or conflicting which may lead to confusion or inappropriate health advice.
Aim. To understand the information seeking behaviors of breastfeeding women, the type of information sought, trusted information sources, preferred form(s) to receive information and how easily this information is understood.
Methods. A pilot study was implemented within an Australian community pharmacy setting with women attending in-pharmacy baby care clinics being invited to participate in a survey.
Results. This study found breastfeeding women actively seek information about over-the-counter medicines prior to their use during breastfeeding. Information is often sought from various healthcare professionals and multiple sources are used prior to the decision to use a medication. Network analysis showed that women preferred to source information (in descending order) from a pharmacist, general practitioner, the internet, and a nurse or lactation consultant. Peer groups were less frequently used to source drug safety information. The majority of women reported that verbal information was easier to understand than written information.
Discussion. Understanding information seeking behaviors of breastfeeding women can drive improvements in provision of advice and support. For example, this pilot study suggests that improvements could be made in written drug safety information intended for breastfeeding consumers. Extension of this study can provide a greater understanding of these behaviors in the wider population with the ultimate goal of improving health literacy for breastfeeding women.

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Sustainable Me – A Pharmacist's Role in Reducing the Carbon Footprint of Healthcare Delivery

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Introduction. Climate change has been described as the most significant global health threat of the 21st century. Already, negative impacts on human health and wellbeing are being observed. These impacts present enormous challenges for the healthcare sector and the time has come for healthcare professionals to demonstrate leadership in addressing these challenges. Since any unsustainable organizational practices of healthcare organisations may ultimately have a negative impact on human health, there is an implicit moral obligation for these organisations and the people who work in them, to deliver healthcare more sustainably. If one considers that in 2010 pharmaceuticals comprised 22% of the carbon footprint of the NHS England (equating to 4.4 million tonnes of CO_2 emissions) and 3% of England's total carbon footprint (NHS Sustainable Development Unit, 2012), by reducing the carbon footprint of pharmaceuticals used in their healthcare organisations, pharmacists can have a significant impact on reducing the organisation's total carbon footprint and ultimately on the public's health.
Aims. The engagement of pharmacists with sustainability initiatives in the workplace has been largely unreported in international and national pharmacy journals. This paper aims to highlight the important role that pharmacists can play in helping to reduce the carbon footprint of healthcare delivery.
Methods. Literature was reviewed to identify areas where pharmacists could influence the more sustainable use of pharmaceuticals in their organisations.
Discussion. Much of the carbon footprint of pharmaceuticals is embedded carbon from their manufacture and delivery. Through efficient inventory management practices, pharmacists can reduce the number of orders and potentially reduce the number of deliveries required. Pharmacists can also help to reduce the amount of pharmaceutical waste generated. Of the waste that is generated, they can help improve the segregation of waste streams to increase the amount of non-contaminated packaging waste that is recycled and reduce the amount of pharmaceutical waste being incinerated or ending up in landfill.

NHS Sustainable Development Unit. (2012). Sustainability in the NHS Health Check 2012. NHS Sustainable Development Unit. Cambridge, UK: NHS Sustainable Devlopment Unit.

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Stability evaluation of piperacillin and tazobactam in syringes

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Introduction: For testing an allergy, a combination of piperacillin and tazobactam (P and T) (Tazocin®), its dilutions are prepared within 24 hours after reconstitution. Due to the lack of stability data of diluted drugs in polycarbonate syringes. Hospitals are unable to pre-prepare dilutions in syringes which results in extra cost and difficulty in workflow management.
Aims: To test the stability of P and T in 1 mL B&D® polycarbonate syringes and to establish the validated shelf life. Two concentrations of P and T (20 and 2.5 mg/mL / 2 and 0.25 mg/mL, respectively) syringes were investigated.
Methods: Analytical methods to test P and T using HPLC were developed and validated. The stability testing protocol was designed to simulate conditions to which dilutions of the drugs are exposed in the hospitals.
Results: The robust, precise and accurate analytical methods were developed to test P and T. The stability studies demonstrated that P and T dilutions, in 1 mL B&D® polycarbonate syringes, retained over 90% of their initial concentrations for seven days at both 4°C and 25°C.
Discussion: Dilutions of P and T in polycarbonate syringes remained chemically and physically stable for seven days. The syringes retained close to 100% of the initial concentrations at the end of the seven day period at 4°C, confirming the shelf life of 7 days, provided the sterility can be ensured.

Choi JS et al (1994) Am J Hosp Pharm, 51(18): p. 2273-2276.
Park TW et al (1994) Am J Health Syst Pharm, 52(18): p. 2022-2024.

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Antimicrobial prescribing for urinary tract infections in a residential aged care setting.

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Introduction. Drug resistant pathogens have been linked to inappropriate, suboptimal and prolonged antimicrobial therapy. Residents of aged care facilities are predisposed to developing a drug resistant urinary tract infection. Protocols that restrict antimicrobial prescribing aim to reduce the occurrence of such infections.
Aims. To determine the effectiveness of an antimicrobial prescribing restriction protocol for the treatment of urinary tract infections in the Australian residential aged care setting.
Methods. This was a retrospective, quasi-experimental study in a regional Victorian 153-bed residential aged care facility. The analysed sample was determined by all urinary tract infection cases recorded between 1st April 2014 and 31st July 2015. Compliance with the Australian Antibiotic Therapeutic Guidelines was segmented across time to illustrate the effect of a prescribing protocol on antimicrobial management.
Results. A total of 116 cases for 72 patients were reported. Cases where treatment was withheld or data missing were excluded from the analysis. Antimicrobial prescribing for 103 (95.4%) of the 108 analysed cases for 67 patients (74.6% female) were deemed non-compliant with the Australian Antibiotic Therapeutic Guideline, version 15. Non-compliance was attributed to prolonged duration of antimicrobial therapy (80.5%), inappropriate frequency of administration (30%) and inappropriate dosing (0.07%). The was no evidence to suggest a difference in compliance as a result of protocol implementation.
Discussion. Antimicrobial management of urinary tract infections was not influenced by the implementation of a prescribing restriction protocol in this setting. With the exception of one case, the prescribed antibiotics were not restricted by the protocol that was implemented. The proportion of inappropriate prescribing highlighted the incidence of prolonged therapy duration and inappropriate prescribed frequency of administration. Further, this study emphasised the need for more stringent documentation and pathology review. To improve infection management in this setting, future implementation of interventions restricting antimicrobial therapy for urinary tract infections are required.

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Effectiveness of implementation strategies for clinical guidelines to community pharmacy: A systematic review

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Introduction. The clinical role of community pharmacists is expanding, as is the use of clinical guidelines in this setting. However it is unclear which strategies are successful in implementing clinical guidelines and what outcomes can be achieved.
Aims. The aim of this systematic review is to synthesise the literature on the implementation of clinical guidelines to community pharmacy.
Methods. A systematic search was performed in six electronic databases for relevant articles. Studies were included if they reported on clinical guideline implementation strategies in the community pharmacy setting. Two researchers completed the full search strategy, data abstraction and quality assessments, independently. Quality assessments were completed with three validated tools. A narrative synthesis was performed to analyse results.
Results. A total of 1,937 articles were retrieved and screened. Nineteen articles (reporting on 22 studies) were included for review. Educational interventions were the most commonly utilised strategy (n=20) and computerised decision support systems demonstrated the greatest effect (n=4). Most studies were multifaceted and used more than one implementation strategy (n=18). Overall outcomes were moderately positive (n=17) but focused on process (n=22) rather than patient (n=3) or economic outcomes (n=3). Most studies (n=20) were rated as being of low methodological quality and having low or very low quality of evidence for outcomes.
Discussion. Studies in this review did not generally have a clear rationale for the choice of implementation strategy. Most utilised educational strategies but the greatest effect on outcomes was demonstrated using computerised clinical decision support systems. Poor methodology, in the majority of the research, provided insufficient evidence to be conclusive about the best implementation strategies or the benefit of clinical guidelines in this setting. However the generally positive outcomes across studies and strategies indicate that implementing clinical guidelines to community pharmacy might be beneficial. Improved methodological rigour in future research is required to strengthen the evidence for this hypothesis.

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A qualitative evaluation of the implementation of guidelines and a support tool for asthma management in primary care

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Introduction. Asthma management in Australia is suboptimal. The "Guidelines for provision of a *Pharmacist Only* medicine: short acting beta agonists" (SABA guidelines) and a novel West Australian "Asthma Action Plan card" (AAP card) were concurrently developed to improve asthma management.
Aims. The aim of this qualitative research was to evaluate the implementation of these asthma resources.
Methods. Feedback was sought about the implementation of the SABA guidelines and the AAP card using focus groups with key stakeholders. Audio recordings were transcribed verbatim. Data were analysed thematically using constant comparison. Themes identified provided an understanding of the determinants of the use of the resources.
Results. Seven focus group sessions were held with 57 participants. Pharmacists had good awareness of the SABA guidelines unlike pharmacy assistants. There was a lack of awareness of the AAP card where passive implementation strategies were used. Pharmacy assistants were extensively involved in the provision of SABAs, despite legislative requirements for pharmacist involvement. Barriers to use of the card included: Difficulties with patient engagement and collaboration, unsuitability of the format, inappropriate content and organisational barriers. Stakeholders felt that mandatory recording of non-prescription SABAs would improve asthma management.
Discussion. Greater consideration needs to be given to implementation of resources. Passive educational implementation strategies did not produce adequate awareness. The role of pharmacy assistants in the supply of SABAs was overlooked and this may have had an impact on guideline-based practice in community pharmacy. While the AAP card is a hypothetically useful resource it did not fulfil its function. Difficulties with patient engagement are a significant barrier to resource implementation and optimal asthma management and consequently interventions directed toward health professionals should focus on skills needs related to achieving patient behaviour change. Organisational barriers also require consideration.

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Comparison of sample preparation methods used for vitamin D LC-MS assay

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Introduction. Vitamin D compounds are tightly bound to proteins. Protein precipitation and saponification methods have been commonly used to extract vitamin D from samples. Traditionally, saponification has been used for the extraction of vitamin D from foodstuffs including milk, and protein precipitation is widely used for the same process in plasma and serum. There have been only a limited number of instances where saponification has been used for serum and plasma.
Aims. Prior to comparison of the two major extraction methods, a major aim of method development was to extract the maximum amount of each vitamin D from the blood matrix to enhance the possible in-use sensitivity.
Methods. Protein precipitation and optimised saponification with extraction were compared in order to study the best approach to release vitamin D compounds from lipids and proteins in serum. Serum samples from ten individuals were used in this comparison. Peak areas obtained following LC-MS analysis were used to assess the efficiency of each set of conditions.
Results. Protein precipitation extracted significantly higher amounts of vitamin D when compared to saponification.
Discussion. To our knowledge, these two methods have not been compared for their performance in vitamin D assay methods. Significant losses of vitamins during saponification have been observed, consistent with previous reports. The results illustrate the importance of optimising the extraction technique for the measurement of all analogues of vitamin D in human serum.

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Glucosamine in Osteoarthritis- Why do trials differ?

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Introduction. Glucosamine is used for the management of osteoarthritis (OA). Many studies have suggested that glucosamine can delay the disease progression and can improve the joint functions. However, some studies have reported contrary results. There could be a number of reasons for the observed conflicting outcomes including the use of different dosage regimen, different salt forms of glucosamine as well as use of prescription glucosamine or use of glucosamine dietary supplements. Unlike prescription glucosamine, the quality of dietary supplements is not closely monitored. Therefore, there is a possibility that the actual amount of glucosamine present in dietary supplements is different than what is claimed on the label.
Aims. To develop a novel high-performance liquid-chromatography (HPLC) method for the quantification of glucosamine and to determine the amount of glucosamine present in five commercially available dietary supplements using the newly developed HPLC method.
Methods. Chromatographic separation of glucosamine was achieved using a hypercarb column with a mobile phase gradient consisting of 0.1% ammonium formate in acetonitrile as well as in water at a flow rate of 0.5ml/min. The developed method was validated for intra- and inter-day linearity, accuracy, precision and reproducibility. It was then used to determine the amount of glucosamine present in five different glucosamine products.
Results. The developed method was selective for glucosamine and the retention time of glucosamine was 7.9 min. The method was linear with the correlation coefficient (r^2) of more than 0.99 over the range of 10-400 µg/ml for glucosamine. The % relative standard deviation for intra- and inter-day accuracy, precision and reproducibility were less than 5%. The amount of glucosamine determined in five glucosamine supplements ranged between 97-105%.
Discussion. Unlike previous HPLC methods, the newly developed HPLC technique does not require pre-derivatisation as well as it can separate glucosamine from other excipients present in the supplements. This simple and effective technique can be employed by the analytical laboratories for the quality control of glucosamine dietary supplements.

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Investigation of amyloid plaques and enoxaparin brain penetration using Raman and infrared spectroscopy

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Introduction. Alzheimer's disease (AD) is a neurodegenerative disease associated with the deposition of β-amyloid (Aβ) dense core plaques in brain tissue. Peripheral administration of enoxaparin has been linked to decreased Aβ burden although the ability of enoxaparin fractions to enter the brain is unclear. Furthermore, Raman spectral mapping is a non-destructive tool which may have applications for dense-core plaque characterisation.
Aims. Our primary aim was to detect Fourier transform infrared (FTIR) and Raman spectral changes in the brain tissue of AD mouse models (Tg2576) associated with the administration of enoxaparin. Secondly, we aimed to investigate differences between plaque and non-plaque tissue using FTIR and Raman mapping.
Methods. Tg2576 mice were administered ip injections of enoxaparin (n=10) or water for injection (n=5), three times a week for five months. Cerebral cortex sections were prepared and capillaries were identified using differential interference contrast (DIC) microscopy. Raman spectra was collected at 0, 1, 2, 5, 10 and 20 µm from the capillary walls. FTIR spectra was acquired using 10 µm steps to a position 70 µm from the capillary walls. Differences in spectra were analysed using partial least squares (PLS) regression. FTIR and Raman spectral maps of dense core plaques were also assessed for spectral differences using clustering and principal component analysis.
Results. Spectral differences were detected between the enoxaparin-treated and control mice. Using PLS regression, a 17-factor model for the FTIR spectra and an 11-factor system for the Raman spectra explained these differences. FTIR and Raman mapping also detected spectral differences between non-plaque and plaque tissue at 2931, 2862 and 1685-1615cm^{-1} in the FTIR and 1667, 1433, 1124, 1082 and 1000cm^{-1} Raman spectral bands.
Discussion. This study detected differences in the brain chemistry of enoxaparin-treated and control mice suggesting that enoxaparin fractions can either cross the blood brain barrier or have peripheral effects which affect the brain. The FTIR and Raman mapping supports findings from previous studies regarding the distribution of phospholipids and changes in protein structure. Importantly, this study shows a shift in the phenylalanine band within the plaque core. Further studies are required to assess the association between phospholipid content, conformational protein changes and phenylalanine binding within Aβ dense core plaques.

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Exploring buccal mucosal and pulmonary delivery of the scorpion peptide HsTX1[R14A] for the treatment of autoimmune diseases

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Introduction. HsTX1[R14A] is a potent and selective Kv1.3 channel blocker peptide, with the potential to treat autoimmune diseases without compromising the physiological function of other potassium channels. To overcome poor oral absorption of HsTX1[R14A] and regular self-injections, alternative dosing routes need to be explored.
Aims. To assess the systemic delivery of HsTX1[R14A] via the buccal mucosa and pulmonary routes.
Methods. For buccal mucosal permeation studies, freshly isolated porcine buccal mucosa was mounted into Ussing chambers, and 1.5 mL of FITC-HsTX1[R14A] (100 µg/mL) with or without cetrimide (5% w/v) or a formulation of FITC-HsTX1[R14A] in a chitosan mucoadhesive gel (3%, w/v) with or without cetrimide (5%, w/w) was placed in the donor chamber. Samples were collected from both donor and receptor chambers and the concentrations of FITC-HsTX1[R14A] were measured by HPLC. To assess the pulmonary absorption, SD rats were intra-tracheally dosed with HsTX1[R14A] either in solution (1-4 mg/kg) or mannitol-based dry powder (1 mg/kg), and plasma concentrations determined by LCMS and compared those achieved following intravenous administration (1 mg/kg).
Results. While FITC-HsTX1[R14A] did not permeate the buccal mucosa when administered alone, addition of cetrimide and incorporation into a cetrimide-containing chitosan gel led to 0.32% and 1.8% of the applied dose, respectively, appearing in the receptor chamber over 5 h. Following intra-tracheal administration, HsTX1[R14A] in solution and dry powder was rapidly absorbed with absolute bioavailability values of 39.2 ± 5.2% and 40.4 ± 17.0%.
Discussion. These studies have shown that buccal mucosal and pulmonary delivery of HsTX1[R14A] are potential routes which can lead to therapeutically-relevant concentrations of this immunomodulatory peptide. Further studies in relevant disease models will identify if the systemically delivered HsTX1[R14A] improves the disease conditions.

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Selection of pharmaceutical vehicles for development of liquid dosage form of Omeprazole

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Introduction. Omeprazole, a proton pump inhibitor (PPI) and one of the highest selling drug is available only as a solid dosage form due to stability issues such as high sensitivity to pH, light, humidity, temperature, and organic and aqueous vehicles. Extemporaneously compounded formulations of omeprazole are assigned a short shelf life of seven days. Stable liquid formulations of omeprazole are required for paediatric, terminally ill and geriatric patients. Identification of liquid vehicles in which omeprazole can be stable is critical for development of these formulations.
Aims. To select appropriate pharmaceutical vehicles for the development of liquid dosage form of Omeprazole.
Methods. We screened 20 different aqueous and non-aqueous vehicles and surfactants (Glycerol, Propylene Glycol, PEG 600, PEG 200, Tween 20, Tween 80, Cremophor EL, NMP, Capmul MCM, Capmul MCM C8, Capmul PG 8, Capmul PG 12, Caproyl 90, PEG 300, Castor oil, Corn oil, Peanut oil, Captex 355, Sunflower oil, Oleic Acid) qualitatively (by observing colour change) at room temperature for 7 days. The selected excipients were evaluated by performing stability study (by qualitative and quantitative analysis) at different storage condition as per ICH guideline for 3 months.
Results. From preliminary screening, we selected 4 vehicles, i.e. Captex 355, Tween 20, Tween 80 and Cremophor EL, with which omeprazole was stable for 7 days. Table 1 summarizes stability data for 3 months.

Table 1: Results of extensive stability study at different storage Condition

Storage condition	Duration of Stability of Omeprazole with selected vehicles			
	Captex 355	**Tween 20**	**Tween 80**	**Cremophor EL**
4°C	3 Month	3 Month	<3 Month	3 Month
25°C and 60% RH	<3 Month	<15 days	<7 days	<3 Month
40°C and 75% RH	<3 Month	<7 days	<7 days	<7 days

Discussion. From the stability study, we identified some excipients for developing liquid dosage form with stability up to 3 months.

Cristina I Marius B (2010) Stability study of omeprazole, Farmacia, 58(2): 203-210

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Surface characterization and morphological analysis of gliclazide microcapsules incorporating a tertiary bile acid and potential application in diabetes treatment

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Introduction. We have shown that a mixture of gliclazide (G) and a primary bile acid exerts hypoglycemic effects in a rat model of Type 1 diabetes.
Aims. This study aimed to develop and characterize novel microcapsules incorporating G with the anti-inflammatory bile acid, ursodeoxycholic acid (UDCA), and investigate the effects of UDCA incorporation on the microcapsules in terms of morphology, stability, and release properties.
Methods. Microcapsules were prepared using sodium alginate (SA) without UDCA (G-SA; control) and with UDCA (G-UDCA-SA), and analyzed in terms of shape, size, morphology, surface structure and composition, thermal and chemical compatibilities, drug contents, Zeta-potential, stability, mechanical strength, release, and swelling characteristics at different pH and temperatures.
Results. Results showed that both microcapsules (control and test) maintained a similar spherical shape, morphology, and surface composition had good stability, release, and compatibility profiles, and the incorporation of UDCA resulted in less G-content per microcapsule ($p<0.05$) and also resulted in stronger microcapsules that were more resistant to swelling and mechanical stress ($p<0.01$) and showed better release profile ($p<0.01$). The production yield and the microencapsulation efficiency were high and remained consistent with and without UDCA.
Discussion. The results demonstrated that G- microcapsules showed uniform structure and UDCA incorporation optimized their release profile and their rheological parameters, which suggests potential application of UDCA in G oral delivery and effect in diabetes.

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Investigation of the antibacterial properties of the bracket fungus *Ganoderma lucidum*

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Introduction: The wound healing properties of aboriginal medicinal plants is well established amongst native Australians. *Ganoderma lucidum,* a bracket fungus indigenous to Queensland's tropical rainforests, is also common to Japan (known as Red Reishi) and China (Lingzhi). Traditionally, *G. lucidum* was used to heal wounds and ensure smooth tissue regeneration. In traditional Asian medicine, the fungus has been utilised for centuries as a universal panacea for wellbeing, restoration, longevity, wisdom and happiness. Further, the medicinal effects were mythicised as a cure all remedy and claimed to cover a range of diseases such as hypertension, diabetes, rheumatism, chronic wounds and bacterial and viral infections. As such, we aim to evaluate the bactericidal properties of *G. lucidum* and scientifically validate traditional accounts.
Aims: To evaluate the antibacterial role of *G. lucidum* with regards to reducing microbial load in a chronic wound.
Methods: Bioactive compounds were isolated in 95% v/v ethanol, absolute methanol or deionised (d.i.) water. The extracts were dissolved in d.i. water to various concentrations (5, 10, 25, 50 mg/mL). Using the well diffusion assay, extracts were assessed for their antimicrobial activity against a range of common wound-colonising bacteria. All assays were performed in triplicate (n=3). Zones of inhibition were measured (mm) and expressed as ±SEM. Positive controls used: trimethoprim+sulfamethoxazole (25 µg) for Methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin G (10 µg) for Methicillin-sensitive *Staphylococcus aureus* (MSSA), gentamicin (10 µg) for *Escherichia coli*, erythromycin (15 µg) for *Streptococcus pyogenes* and *Bacillus cereus*.
Results: After 24 hours and at a concentration of 50 mg/mL, primary compounds, extracted *via* d.i. water, exhibited bactericidal activity against *B. cereus* (9.7±0.3; +ve control=17.3±0.3), MRSA (13±0.6; +ve control=26.7±0.3), MSSA (11±0.6; +ve control=40.7±0.7), *S. pyogenes* (14±1; +ve control=30.5±0.5) and *E. coli* (9.6±0.7; +ve control=18±0.0).
Discussion: The results clearly demonstrate that primary extracts obtained from *G. lucidum* at a concentration of 50 mg/mL, elicit bactericidal activity against Gram positive and Gram negative bacteria.

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Enantiomeric separation and quantitation of Warfarin and metabolites in an overdose patient by LC-MS/MS

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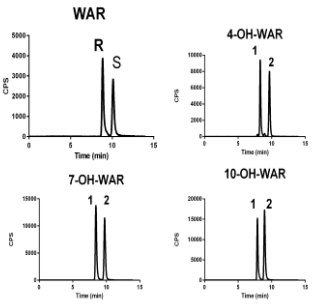

Introduction: Warfarin (WAR) is administrated orally as a racemic mixture of R- and S-WAR for treatment and prophylaxis of venous thromboembolisms. WAR overdose leads to significant toxicity with acute, life-threatening bleeding. S-WAR is preferentially metabolized through the CYP2C9 pathway to the inactive 7-OH-WAR, while 10-OH-WAR results from CYP3A4 metabolism of R-WAR. Reported LC/MS methods for WAR use either achiral separation or chiral separation with only limited consideration of metabolites.
Aim: To develop and validate a simple and rapid method for the simultaneous quantitation of enantiomers of WAR and its metabolites in clinical samples.
Method: Chromatographic separation was performed on a chiral Astec ChirobioticV column (100mm X 4.6 mm, 5µm particle size) with an Astec Cyclobond I guard column (20 mm X 4.0 mm,5 µm). Gradient flow of a 5:95 mixture of 0.1% aqueous formic acid and acetonitrile elutes the analytes with good chromatographic separation. The method was applied to clinical samples from a WAR overdose patient.
Results. The method was validated for matrix effect, intra- and inter-day precision, freeze / thaw and storage stability. Accuracy and precision were within acceptable range (<15%). Chromatograms of WAR & metabolites are shown in the above Fig. (1 & 2 represent the order of elution of the metabolite enantiomers).
Discussion. R-10-OH-warfarin is the major metabolite in clinical samples from patients undergoing warfarin therapy, via the CYP3A4 pathway. (Peak 1 was the dominant peak).Hydroxylation at the 7- position is catalysed by CYP2C19 and CYP2C9, but CYP2C9 predominates with an almost 1000-fold higher affinity for S-WAR than R-WAR. (Peak 2 was the dominant peak).Identification of the two 4'-OH-WAR peaks is less clear-cut, as multiple enzymes (e.g. CYP2C8 and CYP3A4) are likely to contribute to this enzymatic pathway.

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Development of Ritonavir Solid Lipid Nanoparticles

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Introduction: HIV-infection causes decreased immunity by destruction of immune cells such as T-helper cells, dendritic cells, macrophages related with cell-mediated immunity. Antiretroviral drugs are used for effective management of HIV-infection however, development of drug resistance leads to failure to attain sustained drug levels in reservoir sites such as lymphatic systems, causing ineffective viral suppression. Solid lipid nanoparticles (SLNs) having fine particle size and posing high resistance in gastric environment, are highly efficient in carrying the lipophilic drugs like protease inhibitors such as ritonavir through lymphatic region.
Aim: To formulation and evaluation of solid lipid nanoparticles of anti-HIV drug ritonavir for lymphatic targeting.
Methods: In the present study SLN were prepared by solvent evaporation and high pressure homogenization methods followed by ultrasonication using Compritol 888 and sodium lauryl sulphate (SLS). SLN were characterized for size, zeta potential, entrapment efficiency, in vitro release and stability studies.
Results: The average particle sizes for all optimized formulations were well within 300 nm and PDI (0.361) and Zeta potential (-32.4 mV) were also found to be in acceptable ranges. About 60 % drug was encapsulated in nanoparticles. SEM results analysis revealed that solid lipid and drug lead to matrix type structure which indicates that drug molecules are completely dispersed in the lipid structures leading to formation of nanoparticles. XRD pattern of ritonavir had all characteristic peaks showing hydrated crystalline polymorphic form. Besides that, the intense crystalline peaks at 2θ of 21.03° and 18.86° were observed in the diffraction spectrum of drug substance (ritonavir) and less intense and broadened peaks were observed with solid lipid nanoparticles as compared to the drug indicate transformation into an amorphous form. The in vitro release studies showed that the optimized batch containing 6.25:1, lipid to drug ratio and 4% SLS has maximum release at the end of 12 h, followed Korsemeyer Peppas model and showed diffusion controlled release.
Conclusion: Solid lipid nanoparticles of anti-HIV drug ritonavir for lymphatic targeting can be used to treat AIDS. Further in vivo studies need to be performed to prove the effectiveness of the developed formulations for lymphatic delivery.

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Cafeteria Diet Induced Insulin Resistance in Wistar Rats and its Prevention by Quercetin

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Introduction: Diet and the life style are said to be the major reasons for insulin resistance, obesity and metabolic abnormalities. But, there are very few explanation and animal modelling to explain this hypothesis. Hence we thought to develop a diet induced model to mimic human insulin resistance in rats and we used quercetin as standard as it is a known anti-diabetic plant-flavonoid with multimodal action.
Aim: To develop an animal model to cafeteria diet (CFD) induced insulin resistance and to investigate the role of quercetin.
Methods: Three types of CFD were prepared mainly based on different composition of cheese, condensed-milk, chocolate, biscuit, bread, potato, coconut. These three diets were fed in cyclical manner for 63-days. Control rats received normal pallet-diet and another CFD-fed group was administered quercetin (10 mg/kg/day, p.o). Fasting blood glucose (FBG) was determined on 29th and 63rd day. On 63rd day, oral glucose tolerance test (OGTT), insulin tolerance test (ITT), serum insulin, lipid profile and liver antioxidants were determined. Homeostatic model assessment (HOMA-IR) and insulin tolerance index (K-ITT) were calculated.
Results: Rats showed marked food-intake by first week and then remained steady. Body-weight steadily increased and it was significant by 4 to 9 weeks. CFD-rats showed increase in FBG and a significant change in glucose intolerance on 63rd day (by OGTT). K-ITT decreased and HOMA-IR increased significantly, indicates insulin resistance. Triglycerides and total cholesterol increased significantly, indicates dyslipidemia in CFD-rats. Liver antioxidant-activity such as glutathione, total-thiols, catalase, superoxide-dismutase and glutathione-peroxidase significantly decreased indicates oxidative stress with metabolic abnormalities. Quercetin treatment normalizes K-ITT and antioxidants in liver significantly, though glucose tolerance was not significantly normalized. These finding shows that quercetin enhances insulin action in CFD-rats along with antioxidant activity.
Conclusion: Cafeteria-diet induces insulin resistance in Wistar rats and it can be prevented by consumption of quercetin. Quercetin was found to enhance the actions of insulin in CFD-rats.

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Stability of amorphous indomethacin suspensions in *in vitro* media

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Introduction. Amorphous suspensions may be used to improve oral bioavailability of poorly water-soluble drugs, particularly in pre-clinical testing. Recent work (Surwase et al, 2015) has shown polymer incorporated with indomethacin (IMC), forming a solid dispersion (SD), or incorporation of polymer into the suspension vehicle allows good dissolution at pH 6.8 (intestinal pH) without recrystallisation.
Aims. To determine whether pre-exposure of the amorphous suspensions to acidic conditions (reflecting time spent in the stomach before gastric emptying) will result in IMC recrystallisation.
Methods. Amorphous IMC & IMC-PVP SDs were prepared by quench cooling. Physical mixes (PM) of amorphous IMC with PVP & also crystalline IMC (both alpha & gamma forms) with PVP (Controls) were prepared. Each solid was suspended in pH 1.2 aqueous solution & in water at 37^0C. At various times up to 8 h samples were removed and centrifuged; the supernatant was analysed for dissolved IMC using UV spectroscopy while th`e recovered solid was evaluated for solid state form using FT Infra Red.
Results. The PM of amorphous IMC with PVP showed slower recrystallisation (approx 120 min) than had been found for amorphous IMC alone (previous work) & the concentration in solution (initially very high) gradually dropped with time. The SD showed no signs of recrystallisation in the 8 h period & achieved much higher concentrations in solution than amorphous IMC or the crystal forms of IMC in the PMs.
Discussion. Thus it is expected the SD would inhibit recrystallisation while the suspension remained in the stomach even for several hours so that high concentrations would result once the suspension reached the intestine to give good bioavailability.

Surwase SA et al (2015) Eur J Pharmaceut Biopharm, 96, 32-43

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***In vitro* evaluation of nicotine release from nicotine gum, Swedish snus and Pituri**

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Introduction. Oral smokeless tobacco products that may be chewed, sucked or held in the mouth are used as alternatives to smoking. Pituri is the smokeless tobacco that is used by indigenous Australians, primarily in Central Australia, which is prepared using Australian *Nicotiana* leaves and wood ash. Pituri is initially masticated during preparation of the quid, and then held in the mouth and occasionally chewed or sucked. Snus is commonly used as a comparison product for oral smokeless tobacco research; it consists of powdered tobacco that is placed between the lip and gums for extended periods.
Aims. To investigate the release of nicotine from Pituri in comparison to Swedish snus and nicotine chewing gum.
Methods. Each tobacco product was immersed in 50 ml of formulated artificial saliva at 37^0C within a dissolution tester. Chewing action was manually performed using a spatula to press on the tobacco product at the same rate. Three different chewing actions were tested: without pressing, initial pressing for 30 seconds, and pressing for 30 seconds at 15 minute intervals over a two-hour interval. All experiments were replicated three times. Nicotine levels were assayed using HPLC with caffeine as internal standard.
Results. The maximum cumulative release of nicotine over a two-hour interval, produced by pressing every 15 minutes, ranged from 42% in nicotine gum, 53% in Pituri and 66% in Swedish snus. Nicotine release from Pituri and Swedish snus were similar and fast, and contrasted with the slow release from nicotine gum. Pressing had a large effect on the release of nicotine from nicotine gum but minimal effect in Pituri and Swedish snus.
Discussion. Nicotine gum is commercially manufactured and has uniform distribution of nicotine content within the formulation. In contrast, Swedish snus and Pituri originate from processed tobacco leaves with variable nicotine distribution, as nicotine content in tobacco leaves varies according to section of the leaf as well as coarseness and size of tobacco leaf fragments. Swedish snus showed the highest cumulative nicotine levels as a result of its fine texture whereas Pituri was comprised of leaf fragments mixed with ash.

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Development of an assay for quantification of inulin in tissue samples

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Introduction: Inulin, a natural polysaccharide, was approved for human use by the US Food and Drug Administration in 1992. Inulin has been widely used in food and pharmaceutical industry as low calorie bulking agent, texture modifier and dietary fiber, binding agent and solubility enhancer for poorly soluble drugs. One of the most important uses of inulin in medical practice is for kidney function (glomerular filtration rate or GFR) testing. With the increasing need for quantification of inulin in vivo to better assess therapeutic doses and for accurate and convenient measurement of GFR, an analytical method for quantitative measurement of nanomolar concentrations of inulin in biological fluids was developed through this study.
Aims: The objective of this study was to develop a reliable assay for quantification of low levels of inulin in tissue and urine samples for use in pharmacokinetic studies.
Methods: Moisture content was measured with an automatic moisture analyzer (MB35 moisture analyzer, Ohaus Corporation, USA). Inulin labelled with fluorescein-5-thiosemicabizide was analysed by HPLC (RF-10AXL Fluorescence detector, LC-20AD pump, Shimadzu, Japan) and Fluorescence spectrophotometer (CARY Eclipse).
Results: Moisture content of inulin was 1.89% using the moisture analyser. HPLC gave inconsistent results for inulin quantification even after optimizing conditions e.g. column, solvent systems, flow speeds, inulin concentrations. Fluorescence spectrophotometer (FS) was then employed and reliably measured inulin content with a detection limit for inulin in water of 0.01ng/ml. Method was validated for selected parameters. A good linearity was obtained for inulin from 2 ng/ml to 50 ng/ml when dispersed in plasma. Inulin was found to be stable in spiked urine samples (6 ng/ml) at both room and frozen conditions for at least 5 days.
Discussion: HPLC was not useful for inulin quantification because of the inconsistent results. In contrast, Fluorescence spectrophotometry was an ideal analytical approach for measurement of fluorescent inulin samples in water and biological samples.

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Detecting drug in saliva – a tool to determine medication compliance

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Introduction. Over half of patients are not fully compliance to long-term medication regime. For example, type 2 diabetes therapies. Currently, there are limited tools available to accurately determine a patient's compliance. Therefore, doctors cannot be certain that treatment failure is due to treatment selection, or patient non-compliance.
Aims. Determine if metformin can be detected in saliva sample from patient taking the medication
Methods. Potential subjects are individuals who are currently taking metformin as part of their treatment regime. Subjects are recruited from community pharmacies. Saliva samples are collected from each subject, treated with 5% SDS, and stored up to 72 hour before analysis. Solid phase extraction is performed before the sample is analysed using HPLC. Conditions of isocratic HPLC analysis include mobile phase of 5% v/v acetonitrile in phosphate buffer (pH5.8), at a flow rate of 0.9 mL/min. The stationary phase consists of a Gemini C18 column. Metformin is detected by absorbance at λ = 232 nm with retention time around 5.5 minutes.
Results. Metformin is detected in saliva samples from patients taking metformin dose as low as 1g twice a day. The lower limit of detection is 1 ug/mL. As all subjects declare good compliance, detection of metformin in saliva >12 hours after dosing is not determined.
Discussion. These results suggest that metformin is present in saliva after oral dosing. Further studies will determine the quantity and duration in which the metformin can remain detectable in saliva. This result provides a good platform for further work, which will aim to develop a device that can be employed to detect metformin at a doctor's clinic. In addition, previous study has suggested that perindopril can also be detected in saliva sample. If more commonly prescribed medication can be detected in saliva sample, multi-drug detection kits can be implemented.

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Crushing tablets: comparison of tablet crushing devices

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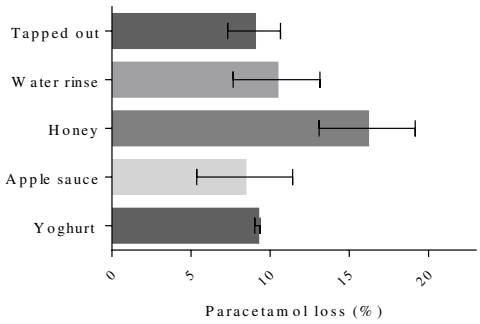
Introduction. Conventional mortar and pestle and commercial tablet crushing devices are commonly used to crush tablets, yet there is no standard protocol for crushing and transferring.

Aims. To determine the percentage of drug losses from crushing with different tablet crushing devices and transferring by different methods, measured by weight and by drug concentration.

Methods. 24 tablet crushing devices were used: 3 with disposable cups, 6 with disposable bags, 12 without separate vessels and 3 types of mortar and pestle. One paracetamol tablet was crushed and then transferred by tapping the powder out. Certain devices were also used to crush and transfer by mixing with water (with and without an additional rinse) or mixing with food, selected for inclusion according to manufacturer information and device size and construction. Paracetamol recovery (the quantity that could be taken by the patient) and leftover (remaining in the device) were measured by a validated UV method.



Results. Drug losses, assessed in terms of paracetamol concentration, were 1.9-13.3% when the powder was tapped out, with most of this remaining in the crushing device. Transferring with water did not improve recovery unless a second rinse was included. One device was tested with food based on manufacturer recommendation but losses were not reduced with yoghurt or apple sauce, and powder loss was significantly greater when honey was used (see Figure).

Discussion. As a significant amount of drug loss may occur during crushing and transferring to the patient, an alternative dosage form or route of administration should be considered before altering medication. If crushing is unavoidable, tablet crushing devices with minimum drug loss should be adopted. To increase dosing accuracies, transferring crushed tablet with water and an additional rinse should be recommended as the standard procedure by the manufacturers and health professionals.

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Impact of an evidence-based program on pharmacy students' communication confidence and education practices

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Introduction. Communication skills in asthma and the teaching thereof, have been identified as an area of weakness in pharmacy students, therefore may require an alternative approach. The Practitioner Asthma Communication and Education (PACE) for pharmacy program is an evidence-based program that has shown utility in improving communication skills in physicians and pharmacists (Shah *et al., 2011)(*Elaro *et al., 2013)*.

Aims. To determine the impact of the PACE program on pharmacy students' behaviours and self perceived ability to communicate with/educate paediatric asthma patients and their carers.

PACE for Pharmacy Domains:	Baseline (n=209)	Follow-up (n=209)	Difference	P-value*
1. **Confidence** in using communication strategies (1=not at all confident to 5=very confident)	3.5	3.9	0.4	0.000
2. **Beliefs of the helpfulness** of communication strategies (1=not helpful to 5=very helpful)	4.3	4.4	0.1	0.020
3. **Frequency** of use of communication strategies (1=never to 5=very often)	3.4	3.8	0.4	0.001
4. Ability to **self-reflect** on the use/effectiveness of strategies (1=never to 6=always)	3.7	4.3	0.6	0.000

*Paired Samples Student's *T* test.

Methods. This study took the form of a pre-post study design. The PACE Australia program for pharmacy was condensed and adapted into the Pharmacy coursework retaining a prominent focus on the pedagogical communication framework of PACE. Pharmacy students were trained in the program and were asked to reflect on their encounters with paediatric asthma patients/carers during their clinical placements and to report on 4 domains that related to their behaviours, communication confidence and education practices pre and 1-month post PACE program completion.

Results. We collected data from 209 Pharmacy students pre and post participation in the PACE program. Pharmacy student self-reported data showed a statistically significant increase in confidence and frequency of use of evidence-based communication strategies, beliefs of the helpfulness of strategies and in their ability to self-reflect on the use/effectiveness of strategies (See figure).

Discussion/Conclusion. The findings of this study support the value of integrating the evidence-based PACE Australia program into the Pharmacy coursework. This may lead to an improvement in pharmacy students' communication confidence levels and education practices around paediatric asthma when they enter the workforce.

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Teaching approaches in Health courses: a reflective study for curriculum redesign

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Introduction. The planning and implementation of contemporary curricula that provides an authentic real-world learning experience for students presents the opportunity to empower academics to participate in conversations about pedagogy. The quality of student learning is directly related to the teaching conceptions of academics involved in providing the student learning experience. Therefore a critical reflection of the teachers' own beliefs about learning, knowledge, and the social role of "teacher" would assist with the transition from traditional to contemporary curricula that provide an authentic real-world learning experience for the students. That transition to new curricula needs to involve considerations around the implications, applications, and limitations of learning theories, and the need for continuing curricula refinement.
Aims: To explore the appropriateness of current pedagogical approaches in the implementation of a contemporary pharmacy curriculum for Clinical Sciences.
Methods. Academics from five different disciplines (Pharmacy, Medical Imaging, Radiation therapy, Podiatry and paramedic science) in the School of Clinical Sciences were invited to complete a six multiple choice question survey to best describe their current and desired teaching activities. Those activities in the survey were directly related to pedagogical theories.
Results. The results will present a descriptive analysis of the pedagogical theories applied in different activities (e.g. lectures, tutorials, etc.) in different stages (e.g. foundational, developmental, etc.) and within different disciplines (e.g. pharmacy, medical imaging). Unexpected results will be analysed in a systematic manner.
Discussion. The findings from this study will inform the discussion of the validity of the questionnaire towards application for curriculum review in different disciplines, faculties and universities. These results will also help to identify likely strategies needed in the implementation of ideal teaching approaches for curricula in Clinical Sciences disciplines. Additionally, this study supports a culture of critical reflection informing continuous review of teaching approaches and the curricula provided.

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Assessment of prescribing competence: perceptions of students and teaching staff

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Introduction. The prescribing of medicines is a complex and often difficult task, requiring the integration of specific knowledge and skills within the context of a unique patient. Demonstration of competence to prescribe represents a challenge for all professions and for the higher education sector. The most appropriate process to effectively assess prescribing competence remains difficult to define.
Aim. To explore the perceptions of final year students and academic staff regarding the teaching and assessment of prescribing.
Methods. An anonymous on-line survey was distributed to all final year students and appropriate teaching staff within five healthcare professions (nurse practitioner, optometry, paramedic science, pharmacy and podiatry).
Results. A total of 107 complete survey responses were received (26 staff, 81 student). The response rate was 25% and 70% for students and staff respectively. Overall, 94% of students felt either confident or very confident to take a medical history; 96% felt confident or very confident to take a medication history. Students felt less confident to assess the factors which may impact adherence to prescribed medicines. Students identified most aspects of prescribing, as articulated in the NPS Competencies Required to Prescribe Medicines[1] as having been taught during their course; 82% felt they had received comprehensive teaching and/or exposure to Element 2 (Understands the treatment options and how they support the patient's clinical needs). Staff perceptions differed to students, with approximately one third of academic staff considering Element 2 to have been taught in a limited capacity only. Students favoured direct observation as a method of assessing many elements of prescribing. Teaching staff considered the preparation and resources required to use this as an assessment method a negative consideration.
Discussion. The majority of students felt their course had prepared them for the task of prescribing within their professional role and scope of practice. Inter-professional differences in both the teaching and assessment of prescribing are identified. Further work is required to develop consistency in the assessment of prescribing competence across health professions.

1. National Prescribing Service (2012) Competencies required to prescribe medicines. Sydney. NPS Ltd.

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The development of mental health vignettes to assess the mental health first aid skills of final year BPharm students

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Introduction. Mental Health First Aid (MHFA) is "the help offered to a person developing a mental health problem or experiencing a crisis"(Kitchener et al., 2013). MHFA training can reduce stigma towards and improve recognition of mental disorders and increase confidence in providing services among pharmacy students (O'Reilly et al., 2011).
Aims. To test final year BPharm students' ability to recognise a person at risk of mental illness and provide appropriate first aid.
Methods. BPharm students completed MHFA training and self-evaluated their confidence in recognising mental health problems and providing support. Pharmacy and mental health researchers developed two mental health vignettes for an audio-recorded simulated patient role-play. Using a purpose-designed assessment rubric, students were assessed on their ability to recognise the risk of mental illness, ask critical questions, provide help, refer appropriately and support empathetically. Students also self-assessed their own performance and on-the-spot feedback was provided.
Results. The majority of students self-reported they felt confident in their abilities after the training. Thirty-six out of 163 students who completed MHFA training were randomly assigned to one of two vignettes. The majority of students identified the risk of mental illness and referred to an appropriate health professional. However, over 25% of students struggled to ask critical questions about suicide and alcohol/drug use, indicating a lack of confidence in addressing these issues.
Discussion. Despite completing MHFA training within 3-6 weeks of the simulated patient role-play, over 25% of students were unable to ask critical questions. Nonetheless, most students recognized the early warning signs of mental illness, recommended appropriate professional help and empathetically interacted with the simulated patient.

Kitchener B, et al. 2013 Mental health first aid manual. 3rd ed. 12 Melbourne: Mental Health First Aid Australia.
O'Reilly, CL, et al. (2011). Impact of mental health first aid training on pharmacy students' knowledge, attitudes and self-reported behaviour: a controlled trial. Aust N Z J Psychiatry. 45:(7)549-557.

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Evaluating the effectiveness of a patient safety educational intervention for intern pharmacists

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Introduction. At the commencement of their internships, students transition into clinical roles, increasing their rate of socialisation into the profession. Interns working in environments with suboptimal practices may be influenced to develop poor practices, which have the potential to place the safety of their patients at risk. Therefore, it is important that pharmacy intern training programs have the capability to foster good practices among interns and enable interns to identify poor practices.
Aim. To evaluate the effectiveness of a face-to-face educational intervention in improving intern pharmacists' patient safety attitudes.
Methods. A patient safety education program was delivered to intern pharmacists undertaking The University of Sydney Intern Training Program in 2014. Their patient safety attitudes were evaluated immediately prior, immediately after, and three-months post-intervention. Underlying attitudinal domains were identified using exploratory factor analysis (EFA). Changes in factor scores were examined using ANOVA.
Results. Of the 115 interns enrolled, 95 (78.7%) completed all three surveys. Four underlying attitudinal domains were identified: attitudes towards addressing errors, questioning behaviours, blaming individuals and reporting errors. Improvements in all attitudinal domains were evident immediately after the intervention. However, only improvements in attitudes towards blaming individuals involved in errors were sustained at three-months.
Discussion. Whilst the educational intervention was associated with short-term improvements in interns' patient safety attitudes, other factors are likely to be influencing attitudes in the longer-term. Professional socialisation theory suggests that these factors may most likely be related to poor work environments. It is therefore necessary that interns are provided with additional mechanisms through their intern training programs to enhance attitudinal sustainability and enable generational change.

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Pharmacy and the Nasal Symptoms (PHANS)

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Introduction. People with nasal symptoms often present to community pharmacy and self-select their treatment. Research suggests that when patients self-select allergic rhinitis (AR) products they do so inappropriately.

Aims. To map the current status of nasal symptom management in community pharmacies and to identify opportunities for increased pharmacist engagement.

Methods. The sampling frame was customers who present to Sydney Metropolitan community pharmacies with nasal symptoms. All customers who purchased a nasal-related product or speak to the pharmacist with regards to nasal symptoms were approached to participate in this study. The following data was collected: demographics, symptoms, treatments used and advice received. To date, data has been collected by a researcher in real time from 3 pharmacies and will continue for one week at a time in several pharmacies in the Sydney Metropolitan area.

Results. Data has been collected from 83 participants over a total of 12 days with 74% (61/83) of participants self-selecting their treatment. Of the 49 participants who reported symptoms of sneezing, itching, blocked and runny nose, 40% (19/49) selected antihistamine (antiH), 23% (11/49) selected intranasal corticosteroids (INCS), 19% (9/49) selected decongestants and 19% (9/48) selected a combination of antiH and INCS. For the 24 participants who reported blocked and runny nose only, 17% (4/24) of the participants selected antiH, 25% (6/24) selected INCS, 50% (12/24) selected decongestants and 9% (2/24) selected a combination of antiH and CS. 11 other participants purchased nasal-products, 5 cold and flu medication for fever and 6 nasal products for disorders not relating to AR or cold and flu.

Discussion. The data collected describes the current status of nasal-related medication purchased within the community pharmacy setting. The preliminary data indicates that only a quarter of the nasal-symptoms sufferers are treating their nasal-symptoms with appropriate medication. This identifies the needs for pharmacists to increase their level of engagement with patients seeking treatment for their nasal symptoms. This is particularly important, as the consequence of undertreated AR remain under-recognised.

Walker S, Durham S, Till S, Roberts G, Corrigan C, Leech S*, et al.* (2011). Immunotherapy for allergic rhinitis. *Clinical & Experimental Allergy* **41:** 1177-1200

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Evaluation of second year oral assessment in pharmacy at Charles Darwin University

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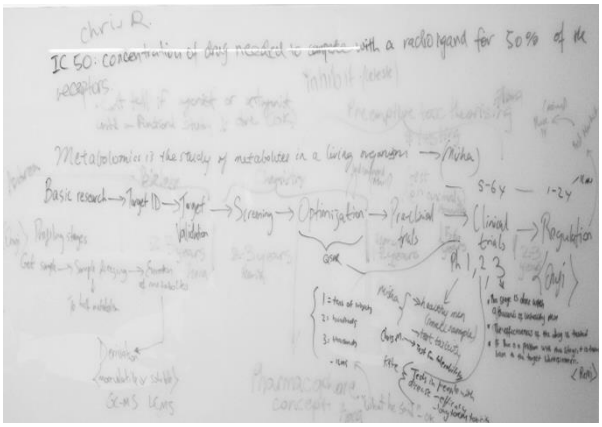
Introduction. Oral Assessment (OA) strategies vary within the second year pharmacy curriculum at Charles Darwin University (CDU). Implementation of OA strategies early in the course prepares students for counselling and case presentation in later practice units.



Aims. To compare the reliability and reproducibility of various OA strategies within the second year pharmacy curriculum.

Methods. Curriculum mapping including comparisons of assessment strategies, rubrics, student results, challenges and opportunities occurred. A review of the literature and analysis of student performances across and within units of study, informed later robust discussions, with a focus on second year students. Limitations of this method of evaluation include the absence of qualitative input from students.

Results. There are several different OA strategies implemented in second year in pharmacy education at CDU. These include group work activities involving mind-map preparation on the whiteboard as shown in the image, panel interviews, and YouTube® clip authoring. Each of these assessment items has been developed to align with unit learning outcomes, course learning outcomes and CDU graduate attributes. These assessments prepare second year students for the more rigorous counselling and clinical case presentation assessments in the capstone units and professional practice.

Discussion. Students develop metacognition of their capacity to handle situational-induced stress during second year OAs. These assessment items are opportunities for formative feedback to determine the progression of students and iron out any misconceptions, and also assist the lecturers in identifying students with underdeveloped oral communication skills early in their study.

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Evaluation of oral assessment in pharmacy education at Charles Darwin University

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Introduction. Oral Assessment (OA) strategies vary within the pharmacy curriculum at Charles Darwin University (CDU). All OA provide opportunities for students to demonstrate deep learning and communication skills.
Aims. To compare the reliability of various OA strategies to identify gaps in student knowledge. To identify the capacity for OA to promote students' metacognition identifying the relevance of their own learning.
Methods. Curriculum mapping, including assessment strategies, rubrics, results, challenges and opportunities occurred. Literature review and analysis of student performances across and within units of study, informed later robust discussions. Limitations of this method of evaluation include the absence of qualitative input from students.
Results. There are many OA strategies implemented from second year onwards in pharmacy education at CDU. These include group work activities, panel interviews, YouTube® clip authoring, counselling assessment, clinical case presentations and a business plan presentation. Each of these assessment items has been developed to align with unit learning outcomes, course learning outcomes and CDU graduate attributes.
Discussion. Opportunities presented by OA strategies are real time feedback (formative assessment); allowing students to demonstrate deeper understanding of materials; theoretical concepts are applied to cases; more dialogue provides an opportunity to clarify; and promoting healthy collaboration, without collusion. Challenges include the students' learning styles influencing their performance; exam conditions inspiring situational anxiety; high requirement for staff interaction, the technology involved can be daunting; students may seek non-verbal guidance; the assessors may be familiar with previous student performance; rubrics contain limited criteria; and according to Medina et al (2008) OA may not translate to work ready skills. Providing a list of alternate questions to answer is perceived to be fair and still determines learning outcome attainment, empowering students to practise and improve their confidence.

Medina, M S, et al. (2008). Evaluating the impact of a pre-rotation workshop on student preparation for clinical advanced pharmacy practice experiences. *Pharmacy Practice (18863655), 6*(4), 219-223.

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Do we have the right formula? Chemistry retention in pharmacy students

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Introduction. We know that the pharmacist is the chemistry expert of the health care team, but do pharmacy students understand this or even care?
Aims: We asked 1st year pharmacy students if they saw the relevance in learning chemistry in "The molecular basis of therapeutics"; a new unit aimed at grounding chemistry in the pharmacy curriculum from 1st year, within the new pharmacy curriculum being rolled out at QUT.
Methods: We surveyed the first cohort of students, about their perceptions of the relevance of chemistry to pharmacists, and their preparedness to study a pharmacy-focused chemistry unit in their first year. Students also completed a multi-choice quiz to gauge their understanding of relevant concepts before and after completing the unit.
Results: Two groups of students were surveyed at different times and showed different perceptions of their own preparedness for study. Group 1, surveyed before they completed the quiz and before seeing the first lecture, portrayed high levels of self-confidence, while group 2, surveyed after the first lecture and after they had completed the pre-knowledge quiz, portrayed lower confidence in their level of preparedness. There were also differences observed in quiz results between the two groups.
Discussion: Is this difference due to a lack of chemical concept retention or something else? We will discuss this, while continuing to analyse the retention of chemical concepts throughout the roll-out of the new curriculum.

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Teaching Cancer Therapeutics in a Postgraduate Masters Program in Pharmaceutical Medicine

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The Pharmaceutical Medicine Masters Program at UNSW Australia aims to develop graduates with the skills and knowledge to contribute to the global biopharmaceutical industry. Students are introduced to all stages of the medicines development pathway from early stage discovery and development, through pre-clinical research, clinical trials, registration, reimbursement to post-market pharmacovigilance and compliance. The program is delivered online through the UNSW Learning Management System, Moodle and students attend regular evening-based online tutorials using the Blackboard Collaborate webinar system.

In 2014, 22 % of novel medicines approved by the FDA were indicated for the treatment of cancer (1). With this growth in oncology medicines entering the market and the focus on oncology in pharmaceutical pipelines it was considered an opportune time to develop and deliver a new course focussing on cancer therapeutics.

The course covers seven sections: how does cancer arise; principles of cancer treatment; what is in the treatment cupboard; how do we assess targets and response to cancer treatment; cancer-omics; cancer medicines development – past, present and future; and who pays? Each section of the course is supported by key primary papers, video lectures and adaptive tutorials as well as having students participate in a discussion forum based on course topics.

Students undertake an individual in-depth research assignment on an area of cancer therapeutics development of their interest where they explore the landscape of current development in their target area, choose a particular pathway to target an hypothetical new therapeutic and describe their chosen clinical trial designs as well as their registration and reimbursement strategies to ensure a successful launch of their novel therapeutic. As well, they make a video presentation on their topic and peer review each other's videos. Students are also expected to maintain a reflective journal based on the Kolb Learning Cycle as a way of enhancing their learning and connecting it to their work in the pharmaceutical industry. Further information on the results of the course will be provided.

References.

1. FDA (2015) Novel new drugs 2014 summary.
http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugInnovation/UCM430299.pdf

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What constitutes an appropriate dose of thiamine: a quality use of thiamine study at Footscray Hospital

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Introduction. Despite thiamine fortification of bakers flour, thiamine deficiency and Wernicke-Korsakoff Syndrome still is prevalent in Australia. Currently, there is an emerging trend to provide high dose thiamine to patients deemed at risk for Wernicke's Encephalopathy amongst some clinicians and at some hospitals. At our hospital, we have noted wide variation in thiamine utilisation typically ranging from high dose thiamine (100-300mg tds) to a lower dose range (50 - 100mg/day). The practise of providing high dose thiamine appears to be based upon one set of guidelines which have been endorsed by RCP. Little is known about clinician knowledge of these guidelines and related practise.

Aims. To investigate the use of thiamine in the upper GI surgical unit and Addiction Medicine unit at Footscray Hospital, both units likely having patients "at risk" for Wernicke's Encephalopathy; thereafter, to survey a sample of general medical staff about thiamine knowledge and utilization.

Methods. A retrospective chart audit of thiamine utilisation amongst the past 100 patients admitted under the upper GI surgical unit (prior January 2014) was conducted to identify thiamine utilisation. Later, an education session was provided to the Department of Surgery (February 2014) about screening for risk of thiamine deficiency and treatment. Thereafter, a prospective inpatient audit (clinical interview) was conducted amongst admissions to Addiction Medicine and the upper GI surgery (February - April 2014), looking at screening for risk of thiamine deficiency. Finally, a survey of a broad sample of hospital medical staff about their utilisation of thiamine was also conducted looking at their overall knowledge.

Results. In the retrospective audit, 11% of upper GI surgical patients received thiamine; the average dose was 182mg oral/day; alcohol use, cancer and surgical procedure were the most common scenarios when thiamine was given. In the prospective audit, 34 Addiction patients and 27 upper GI surgical patients were screened. Indicators of malnutrition were found in 32% Addiction and 41% upper GI surgical patients. Most Addiction patients received thiamine in contrast to surgical patients.

Discussion. Thiamine use appeared to be determined by the clinical detection of alcoholism, cancer and only some GI surgical procedures. The dose and route of administration was mostly determined by past medical education.

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Flipping the classroom in pharmacology for first year graduate entry MBBS students

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Introduction. The flipped classroom is not a new teaching and learning approach. In its standard form it is a mix of on-line and face-to-face learning activities that create a more active experience for students compared to traditional lecture-based programs.
Aim. To provide an account of the experience of implementing a flipped classroom mode of delivering pharmacology into the pre-clinical year of a graduate entry medical course.
Methods. Using the flipped classroom, the pharmacology content was taught using pre-class and face-to-face components. The pre-class component comprised of on-line lectures, while face-to-face activities involve short quizzes, and content review, application and analysis in collaborative student group activities. An evaluation of student perceptions of their program experience was undertaken using an on-line survey and focus group interviews.
Discussion. The main points emerging from the evaluation will be discussed. Students were initially unsettled in, and resistant to, the flipped classroom. However, this perception did change. I found that as students became more settled in this mode my role in class evolved from the usual 'sage on the stage' to being a 'guide on the side'. Students responded well to a consistent program structure, timely upload of on-line lectures and short quizzes in class of knowledge acquisition against the learning objectives and relevance to clinical practice.

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Adenosine A_1 receptor biased agonism in ventricular cardiomyocytes

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Introduction. Adenosine A_1 receptor (A_1AR) stimulation is a powerful protective mechanism in cardiac ischemia-reperfusion injury. Despite this, therapeutic targeting of the A_1AR has been largely unsuccessful due to on-target adverse effects, including pronounced bradycardia, atrioventricular block and hypotension. Biased agonism has the potential to overcome these limitations by enabling the separation of therapeutic from adverse effects. Recently, the A_1AR agonists, VCP746 and capadenoson, have been shown to promote cardioprotective signalling in the absence of bradycardia, a profile suggestive of ligand bias.
Aims. This study aimed to investigate the signalling profile of prototypical and novel A_1AR ligands in cardiomyocytes, and identify the potential for signal divergence between the ligands that lower heart rate and those that do not.
Methods. Rat neonatal ventricular cardiomyocytes were utilized to investigate a number of signalling pathways, including inhibition of cAMP accumulation, phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK 1/2), cardiomyocyte cell survival and coupling to G protein-coupled inwardly-rectifying potassium (GIRK) channels in the presence and absence of pharmacological inhibitors.
Results. All A_1AR agonists increased ERK1/2 phosphorylation and inhibited cAMP accumulation. Prototypical agonists reduced cardiomyocyte beat rate frequency via a GIRK predominant signalling mechanism. VCP746 exhibited a reduced potency in cardiomyocyte beat rate reduction, suggesting the potential for signal divergence at the level of GIRK channel activation.
Discussion. Collectively, these studies provide preliminary insights into the signalling profile of both prototypical and novel A_1AR ligands in native cardiomyocyte settings.

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Cardiac-specific insulin-like growth factor-1 receptor over-expression attenuates diabetes-induced upregulation of hexosamine biosynthesis pathway and oxidative stress in diabetic mouse myocardium.

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Introduction. Diabetes-induced cardiac complications include left ventricular (LV) dysfunction and remodelling. Progression of this "diabetic cardiomyopathy" is linked to increased reactive oxygen species (ROS) generation and altered hexosamine biosynthesis pathway (HBP) signalling. Cardiac-specific activation of the insulin-like growth factor-1 receptor (IGF-1R) in transgenic (Tg) mice is protective in several cardiac pathologies, however its impact on diabetes-induced LV HBP expression and activity (protein O-GlcNAcylation) and ROS had not been sought.

Aim. We tested the hypothesis that cardiac-specific IGF-1R Tg expression protects against diabetes-induced upregulation of LV HBP signalling and oxidative stress, LV remodelling and dysfunction in a diabetic mouse model

Methods. Diabetes was induced in 6 week old non-transgenic (Ntg) and IGF-1R Tg male FVB/N mice via streptozotocin (55mg/kg i.p. for 5 d; controls received citrate vehicle). After 12 weeks of untreated diabetes, LV function was determined in anaesthetised mice (KXA, 85:8.5:1.0 mg/kg i.p.). Cardiomyocyte hypertrophy, LV collagen deposition and TGF-β expression, LV NADPH oxidase, LV HBP and oxidative stress were determined.

Results. Diabetes-induced upregulation of LV NADPH oxidase (superoxide generation, Nox2 expression), LV oxidative stress (protein 3-nitrotyrosination) and LV HBP were attenuated in IGF-1R Tg mice. The latter comprised HBP expression of the GFAT1 and GFAT2 isoforms of the HBP rate-limiting enzyme, as well as HBP activity of O-GlcNAc-transferase and O-GlcNAcase (which add and remove the O-GlcNAc sugar moiety respectively). We also show that IGF-1R Tg mice were protected from diabetes-induced LV diastolic dysfunction (Doppler echocardiography) and LV remodelling.

Discussion. The protective mechanisms of targeting the cardiac IGF-1R in the context of diabetes may involve suppression of key triggers of diabetic cardiomyopathy, such as upregulation of the ROS-HBP axis.

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Decreased vascular hydrogen sulfide production is associated with vascular oxidative stress in rats fed a high-fat western diet.

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Introduction. A Western-style, high fat diet is known to cause vascular dysfunction and oxidative stress (Matsuda 2013) The gaseous mediator, hydrogen sulfide (H_2S) contributes to regulation of vascular function and acts as a vasoprotective molecule (Streeter 2013). However, the effects of high fat diet on vascular H_2S production and function are not known.

Aim. The aim of this study was to investigate the effects of high fat diet on vascular function and H_2S production.

Methods. Wistar hooded rats were fed a western diet (WD, 21% fat) or control rat chow (6% fat) for 12 weeks. At the end of the experiment aortae were collected for assessing vascular function. Vascular superoxide anion production was quantitated by lucigenin-enhanced chemiluminescence and vascular expression of the NADPH oxidase subunit NOX2 and the H_2S producing protein cystathionine-γ-lyase (CSE) were examined by Western blotting.

Results. WD rats had significantly higher body weight and body fat than control (P<0.001). Both endothelial function and NO bioavailability were significantly reduced in the WD group (P<0.05), but vascular smooth muscle cell function was not different. Vascular superoxide production and NOX2 expression were significantly increased in aorta from WD rats (P<0.05). L-cysteine-induced vasorelaxation was reduced in the fat-fed group (P<0.05) and insensitive to inhibition of CSE. In addition, vascular H_2S bioavailability and CSE expression were significantly reduced in aorta from WD rats (P<0.01).

Discussion. Fat-feeding induces vascular oxidative stress and a reduction in endothelial function and bioavailability of NO. Furthermore, there is both a reduced capacity for vascular H_2S production and a change in the mechanism of vascular H_2S production in fat fed rats. These data show that increased vascular oxidative stress has a negative impact on endogenous H_2S production and activity that may contribute to vascular dysfunction.

Matsuda & Shimomura (2013) Obesity Research and Clinical Practice 7:e330-341
Streeter, Ng & Hart (2013) Medical Gas Research 3:9

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Initial clinical experience with pharmacological conditioning of brain dead donor hearts with glyceryl trinitrate and erythropoietin.

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Introduction: With the success of the transplant procedure, older and sicker individuals are now being accepted as recipients for heart transplant resulting in a shrinking pool of 'standard criteria' donor hearts. This has necessitated increased consideration and acceptance of 'marginal' donor hearts for transplantation. We have instigated a 'pharmacological conditioning' strategy given at arrest and storage to maximize donor heart protection.

Aim: To examine the effect of glyceryl trinitrate (GTN) and erythropoietin (EPO) on graft recovery, primary graft dysfunction (PGD) and survival in heart transplants performed at St Vincent's Hospital.

Methods: Donor hearts retrieved between August 2010 and November, 2013 were arrested and stored in Celsior supplemented with 0.1mg/ml GTN and 5 U/ml EPO (n=61). Historical comparisons were made between the supplemented group and hearts stored in Celsior alone (April 2005 to July 2010; n=104) and modified St Thomas' solution (STS) (January 2000 to March 2005; n=100). Donor, recipient and procedural risk factors for PGD were determined for each group, and post-transplant use of mechanical circulatory support (MCS), length of hospital stay and survival out to 12 months were compared between groups.

Results: Hearts stored in Celsior (± GTN+EPO) were retrieved from a higher proportion of donors aged > 50yr (25%; $p<0.01$). Increased use of MCS pre-transplant (36%, $p<0.0001$) was observed in the Celsior supplemented group only. Implantation of these hearts required increased cross clamp times (111min, $p<0.0001$). Use of MCS within 24 hours post-transplant was 32.0%, 31.7% and 24.6% in STS, Celsior and supplemented celsior groups respectively. There were no differences in the length of hospital stay between groups. Survival at 1-month was 92%, 95% and 98%; at 3-months 89%, 91% and 93%; and at 12-months 86%, 89% and 90% respectively.

Conclusions: Pharmacological conditioning with EPO and GTN allowed comparable or reduced use of MCS post-transplant and maintained survival in higher risk recipients receiving hearts from a pool of older donors.

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Hyperglycaemia and systemic inflammation precede the cardiac complications of type 1 diabetes in mice

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Introduction. Evidence suggests that increased levels of inflammatory mediators, and infiltrating inflammatory cells are associated with diabetic complications. Whether inflammation precedes diabetic cardiomyopathy has however has not been determined. We tested the hypothesis that systemic inflammation precedes the cardiac complications of diabetes, using a time-course based experimental approach. Methods. Streptozotocin (55mg/kg, i.p. per day, over 5 days)-induced diabetic mice were followed for 2, 4, 8 or 16 weeks of diabetes; sham mice received citrate vehicle. Left ventricular (LV) function via echocardiography was performed in anaesthetised mice (85/8.5/0.8mg/kg ketamine/xylazine/atropine, i.p.) prior to tissue collection. Results. Hyperglycaemia and systemic inflammation were elevated after 2 weeks (Table); LV remodelling and diastolic dysfunction (echocardiography-derived E/A ratio; results not shown) were not however evident until after 8 weeks of diabetes. Conclusion. These findings reveal that systemic inflammation clearly precedes cardiac complications of diabetes in mice.

Results (mean±SEM)	2 weeks		4 weeks		8 weeks		16 weeks	
	Sham	Diabetes	Sham	Diabetes	Sham	Diabetes	Sham	Diabetes
Final bodyweight (g)	27.3±0.8	26.7±0.9	27.1±0.6	26.2±0.4	26.3±0.6	25.6±0.5	30.1±1.0	28.9±0.7
Blood glucose (mM)	11.0±0.8	29.4±1.2*	12.5±0.4	32.4±0.6*	11.8±0.2	31.3±0.8*	10.9±0.6	32.6±0.5*
GHB (mM)	21.3±0.7	38.2±4.1*	20.1±0.1	58.6±4.3*	22.3±0.3	76.3±1.5*	21.7±0.7	73.6±4.3*
Monocytes (% WBC)	4.26±0.5	5.64±0.4	3.64±0.6	5.95±1.1*	3.63±0.7	5.64±0.7	4.00±0.5	6.77±1.0*
Plasma TNFα (pg/mL)	57.8±1.0	71.0±4.3*	62.6±1.5	77.5±4.1*	63.1±2.0	72.6±6.4	65.7±3.9	76.0±5.9
β-MHC mRNA (fold)	1.00±0.2	3.29±0.4	1.00±0.3	1.08±0.3	1.00±0.6	3.90±1.9	1.00±0.3	4.21±1.5*
CTGF mRNA (fold)	1.00±0.2	1.84±0.7	1.00±0.2	0.59±0.1	1.00±0.6	2.35±0.9	1.00±0.3	2.60±0.6
CD68 mRNA (fold)	1.00±0.1	1.53±0.9	1.00±0.3	0.67±0.2	1.00±0.2	2.70±1.7	1.00±0.3	3.09±1.3*

β-MHC, β-myosin heavy chain; CTGF, connective tissue growth factor, GHB, glycated haemoglobin;WBC, white blood cells; *$p<0.05$ diabetes vs age-matched sham (n=8-11/group, 2-way ANOVA, Fisher's LSD post-hoc test).

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MDM2 E3 ligase-mediated ubiquitination of HDAC1 in vascular calcification

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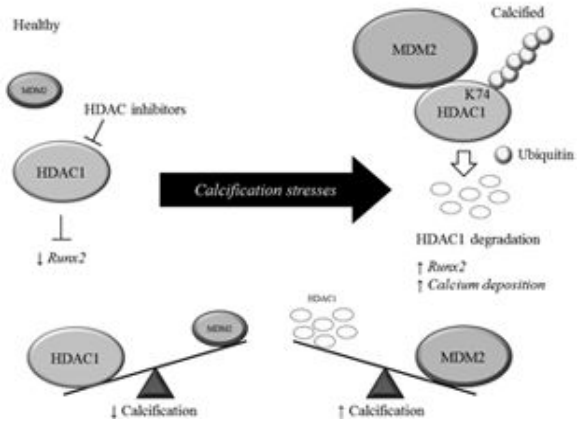

Vascular calcification (VC) often associates with many cardiovascular and metabolic diseases. Although VC is the cause of high morbidity and mortality, molecular mechanisms have yet to be elucidated. Here we report that MDM2 E3 ligase-induced ubiquitination of histone deacetylase 1 (HDAC1) mediates VC. Both non class-selective and HDAC1-selective HDAC inhibitor potentiated VC in primarily cultured rat vascular smooth muscle cells (RVSMCs). HDAC inhibitor potentiated vitamin D3-induced VC in mice. Loss of HDAC1 enhanced VC in vitro. Vascular smooth muscle-specific knockout of HDAC1 caused the potentiation of vitamin D3-induced VC in the mice. HDAC1 protein, but not its mRNA, was reduced in cell, calcification animal models in ApoE knockout mice (both atherosclerosis-associated and high calcium-associated models), and in human calcified coronary artery. Vascular calcification-provoking condition induced polyubiquitination of HDAC1 as determined by treatment with MG132, immunoprecipitation-based ubiquitination assay, and far-Western blot analysis with tandem ubiquitination binding entities (TUBEs) as a probe. Mapping study revealed that HDAC1 K74 was responsible for the ubiquitination. Inhibition of proteasomal degradation attenuated VC in vitro and in vivo. By utilizing bioinformatics, literatures, and cDNA microarray analysis, we found that MDM2 E3 ligase was induced in the calcification-provoking conditions in RVSMCs. We also confirmed that MDM2 expression was significantly enhanced in vascular calcification models of ApoE knockout mice and human coronary intimal and medial calcification samples. Forced expression of MDM2 enhanced VC, whereas loss of MDM2 blunted it. These results demonstrate a previously unknown ubiquitination pathway as well as the involvement of HDAC1 in VC.

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Roles of orphan nuclear receptors, estrogen-related receptor gamma and small heterodimer partner, in cardiac hypertrophy

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Orphan nuclear receptors are atypical orphan nuclear receptors that lack a conventional DNA binding domain and they regulate diverse biological events including glucose metabolism in diverse organs. However, their roles in cardiac hypertrophy as well as heart failure have not been fully evaluated. Recently, we have investigated that both estrogen-related receptor gamma (ERR□) and small heterodimer partner (SHP, NR0B2) play roles in the regulation of cardiac hypertrophy. Thus, we aimed to characterize the role of ERRγ and SHP in cardiac hypertrophy. ERRγ expression was increased in hearts from human hypertrophic cardiomyopathy patients and in both cellular and animal models of cardiac hypertrophy. Transgenic overexpression in mouse heart as well as forced expression of ERRγ in cardiomyocytes induced hypertrophic phenotypes. Knock-down of ERRγ blocked agonist-induced hypertrophic phenotypes. ERRγ bound directly to the proximal ERR-responsive element in the GATA4 promoter in a sequence-specific manner and thereby induced transcription. ERRγ-induced hypertrophy was blocked by inhibition of GATA4. GSK-5182, an inverse agonist of ERRγ, completely blocked cardiac hypertrophy in cardiomyocytes. It also prevented aortic banding-induced cardiac hypertrophy and fibrosis in mouse heart. Next, we aimed to investigate the role of SHP in cardiac hypertrophy. The roles of SHP in cardiac hypertrophy were tested in primary cultured cardiomyocytes and in animal models. SHP null mice showed a hypertrophic phenotype. Hypertrophic stresses repressed the expression of SHP, whereas forced expression of SHP blocked the development of cardiomyocyte hypertrophy. SHP reduced the protein amount of Gata6. By direct physical interaction with Gata6, SHP interfered with the binding of Gata6 to GATA binding elements in the promoter regions of natriuretic peptide precursor type A. Metformin, an anti-diabetic agent, induced SHP and suppressed cardiac hypertrophy. The metformin-induced anti-hypertrophic effect was attenuated either by SHP siRNA in cardiomyocytes or in SHP null mice. These results establish SHP as a novel anti-hypertrophic regulator that acts by interfering with GATA6 signaling. SHP may participate in the metformin-induced anti-hypertrophic response. These findings demonstrate novel ERRγ/GATA4 and SHP/GATA6 signal cascades in the development of cardiac hypertrophy.

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Ligand-directed signalling and beta3-adrenoceptors in the rat cremaster muscle artery

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Introduction. Vascular β_1 and β_2-adrenoceptors cause smooth muscle relaxation and subsequent vasodilatation. The presence and functional role of vascular β_3-adrenoceptors is yet to be established (Michel et al., 2010).
Aims. This study examined the interactions between pressure-induced myogenic tone, isoprenaline-induced vasodilation and β_3-adrenoceptor ligands in the rat isolated cremaster muscle artery.
Methods. Male Sprague-Dawley rats (6 wks, ~200 g) were anesthetized (sodium thiopentone, 100 mg/kg i.p.), the cremaster muscles excised and the main artery removed. Functional responses were examined in pressurized (50 or 120 mmHg) cremaster muscle arteries using video microscopy.
Results. Arteries maintained at 120 mmHg were more constricted than those at 50 mmHg, indicating increased myogenic tone at the higher pressure. Vasodilatation induced by the non-selective β-adrenoceptor agonist isoprenaline was not altered by the degree of myogenic tone and inhibited by the selective β_2-adrenoceptor antagonist ICI 118,551 (0.1 μM). Two β_3-adrenoceptor agonists, CL 316,243 and BRL 37344, had no effect on artery diameter at either pressure. A selective β_3-adrenoceptor antagonist, L 748,337 (0.1 μM) had no effect on isoprenaline-induced relaxation, but another β_3-blocker, SR 59230A (0.3 μM) significantly inhibited responses. The effect of SR 59230A was pressure-sensitive, with a greater inhibitory effect on isoprenaline-induced vasodilatation at 120 mmHg. SR 59230A did not inhibit vasorelaxation induced by the adenylate cyclase activator forskolin. SR 59230A's effect was inhibited by a p38 MAP kinase inhibitor, SB 202190 (10 μM).
Discussion. These observations suggest ligand-directed signalling through β_3-adrenoceptors (Sato et al., 2007) occurs in arteries, perhaps not altering smooth muscle tone directly, but modulating the response to activation of β_2- or other receptors in the vessel.

Michel et al (2010) Naunyn-Schmiedeb. Arch Pharmacol. 382: 103-108.
Sato et al (2007) Mol. Pharmacol. 72: 1359-1368.

494

Australian native stingless bee (*Tetragonula carbonaria*) cerumen modulates dermal fibroblast proliferation, migration and differentiation *in vitro*

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Introduction. Cerumen produced by *Tetragonula carbonaria* represents a novel Australian source of this plant-derived bee product. Following bioactivity-guided fractionation, we isolated a fraction of *T. carbonaria* cerumen that scavenged free radicals and inhibited the pro-inflammatory 5-lipoxygenase signalling pathway *in vitro*.
Aims. Using cultured human fibroblasts derived from healthy dermis (NF) and chronic wounds (CWF), we investigated the effect of the fraction on cellular responses implicated in normal and pathological wound healing.
Methods. The bioactive fraction was obtained from a methanolic extract of cerumen, collected from 40 *T. carbonaria* hives in South-East Queensland. The effects of the fraction on NF and CWF proliferation were investigated using MTT assays. *In vitro* scratch assays and automated time-lapse microscopy were used to examine NF migration over 48 h. Gene and protein expression of α-smooth muscle actin (α-SMA) in transforming growth factor (TGF)-β_1-stimulated NFs (10 ng/mL; 72 h) were measured by quantitative reverse transcription polymerase chain reaction and immunocytochemistry, respectively.
Results. The cerumen fraction time- and dose-dependently stimulated NF (214.6±26.4% vs. dimethyl sulfoxide control; 3 μg/mL) and CWF proliferation (134.8±5.7% vs. dimethyl sulfoxide control; 3 μg/mL) over 120 h ($P<0.05$). NF migration was significantly increased after 48 h exposure to 1 μg/mL fraction ($P<0.05$). However, fraction concentrations of 3-5 μg/mL inhibited NF migration; and TGF-β_1-induced α-SMA gene and protein expression ($P<0.05$).
Discussion. The bioactive fraction of *T. carbonaria* cerumen may promote the closure of acute and chronic wounds, by stimulating fibroblast proliferation during the early phases of wound healing. Its inhibitory effects on fibroblast-myofibroblast differentiation may additionally resolve the late, wound maturation phase of healing and prevent pathological scarring. Chemical analyses of the bioactive fraction, to elucidate the structures of its constituents, are ongoing.

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Modulation of the β_2-adrenergic receptor pathway during the onset of myocardial sustained ligand-activated preconditioning

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Introduction. Novel sustained ligand-activated preconditioning (SLP) significantly improves tolerance to ischaemia-reperfusion (I-R) injury in young to aged hearts. Opioidergic SLP is induced via 5 days δ-opioid receptor (OR) agonism and subsequent protection is mediated via a β_2-adrenergic receptor (β_2-AR) pathway.
Aims. To characterise time-dependent shifts in the β_2-AR signalling axis unique to the SLP 'induction' phase.
Methods. Young (12 week) C57/Bl6 male mice were implanted with vehicle or slow release morphine (75 mg) pellets for 24 h or 5 days. A subset of mice had pellets removed and wounds closed after 5 days, were administered naloxone (7 mg/kg, ip), and hearts excised after a further 10 days to determine changes after stimulus withdrawal. Myocardial proteomic expression, phosphorylation and translocation of β_2-AR pathway components were determined via western blot. Concentration-response curves were obtained in 5 day vehicle vs. SLP hearts exposed to increasing concentrations of formoterol (long-acting β_2-AR agonist), fenoterol (selective β_2-AR-coupled G_s signalling agonist), and NKH 477 (AC activator).
Results. Significant alterations in expression, phosphorylation and translocation of β_2-AR, G stimulatory protein (G_s), and protein kinase A (PKA) were evident during SLP induction, and predominantly dissipated after stimulus withdrawal. These proteomic changes did not translate to altered sensitivity to β_2-AR agonists (formoterol and fenoterol) or the adenylyl cyclase (AC) activator NKH477.
Discussion. This study further characterises the novel SLP response during the induction phase, providing a molecular profile that implicates significant alterations in the β_2-AR/G_s/PKA signalling axis. The absence of altered β_2-AR pathway functionality may reflect rearrangement of cellular machinery to prime the heart for stress tolerance or other unidentified mechanisms. This unique signalling phenotype requires further delineation to determine the importance of these findings.

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Post-Ischaemic Mitochondrial Bioenergetics in a Murine Model of Type II Diabetes following Inhibition of Mitochondrial Fission.

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Introduction. Modifications in mitochondrial biogenesis may contribute to the metabolic alterations and insulin resistance that characterise obesity-induced type II diabetes (T2D). Mitochondrial fission is regulated by fission proteins including dynamin-related protein-1 (DRP-1), and may be a key determinant of cardiac resistance to ischemia-reperfusion (I-R) in a T2D model.
Aims. To determine the contribution of mitochondrial fission to ischaemic tolerance in the T2D mouse heart.
Methods. Eight week old C57Bl/6 mice were fed an obesogenic diet for 14 weeks prior to mdivi-1 administration (mitochondrial fission inhibitor of DRP-1, 5mg/kg/day, ip) for 7 days. Hearts were langendorff-perfused and exposed to 25 min ischaemia/45 min reperfusion. Post-ischaemic mitochondrial function was evaluated in isolated mitochondria on an Oroborous Oxygraph- 2k.
Results. Post-ischaemic contractile function and LDH efflux was worsened by T2D compared to healthy controls. Mdivi-1 treatment partially restored post-ischaemic contractile function in T2D hearts with no change in LDH efflux. Mitochondrial complex I activity reduced by ~50% in T2D hearts vs. healthy controls. Inhibition of mitochondrial fission failed to improve complex 1 activity, however mild improvements in complex II activity were evident. Interestingly, mdivi-1 reduced mitochondrial DRP-1 protein expression and increased expression of mitochondrial fission protein OPA1 in all hearts. Additionally, mdivi-1 also elevated stress-kinase signalling in all hearts.
Discussion. Mitochondrial complex I activity is shifted in T2D post-ischaemic hearts, suggesting that metabolic alterations alter the mitochondrial bioenergetics of the myocardium following ischemia. Mitochondrial fission inhibition showed a compensatory increase in complex II respiration in T2D, with associated changes in stress-kinase signaling. Thus, fission inhibition is a potential therapeutic tool in preserving mitochondrial integrity in post-ischaemic hearts.

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Comparison of the anti-fibrotic effects of Serelaxin with an angiotensin receptor blocker and an AT_2 receptor agonist in a high salt-induced model of kidney disease.

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Introduction. Fibrosis (organ scarring) is a hallmark of cardiovascular and renal disease, for which there is no effective cure. Current treatments such as angiotensin receptor blockers (ARBs) only have limited anti-fibrotic efficacy, thus highlighting the need for improved therapies.
Aim. To compare and combine the anti-fibrotic efficacy of Serelaxin (RLX; recombinant form of the naturally-occurring relaxin hormone) to CGP42112 (AT2 receptor agonist) or candesartan cilexetil (ARB), in a high salt-induced model of kidney disease.
Methods. Male C57B6J mice were subjected to 8-weeks of a high salt (HS; 5% NaCl) diet. During the last 4 weeks, sub-groups of mice (n=8-9/group) were treated with either candersartan (2mg/kg/day via drinking water), RLX (0.5mg/kg/day via mini-pumps), CGP42112 (1.44mg/kg/day via mini-pumps), candesartan+RLX or CGP+RLX. Untreated/HS diet-fed and mice given a normal diet (NS) were included as controls. Changes in blood pressure were assessed every 2-weeks, while several measures of renal fibrosis were evaluated at week-8.
Results. HS-fed mice underwent increased kidney TGF-β1 expression, myofibroblast differentiation, glomerulosclerosis, interstitial and total fibrosis, but decreased blood vessel density (as assessed by morphometry of histological stains or hydroxproline analysis), compared to their NS counterparts (all $p<0.001$ vs NS); in the absence of any changes in blood pressure (tail-cuff) or gelatinase activity (gelatin zymography). Candesartan significantly lowered blood pressure but not the adverse remodeling effects of HS. CGP42112 partially lowered aberrant TGF-β1 levels and restored blood vessel density (both $p<0.05$ vs HS) without significantly affecting HS-induced kidney fibrosis. Alternatively, RLX significantly ameliorated all these adverse effects of HS while increasing angiogenesis and gelatinase activity (all $p<0.01$ vs HS). Candesartan+RLX blocked the renoprotective effects of RLX, while CGP+RLX did not result in any added effects over that of RLX alone.
Discussion. These findings suggest that RLX has superior anti-fibrotic efficacy/renoprotection over candesartan or CGP42112 in experimental HS-induced kidney disease.

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Inhibition of vanilloid-induced platelet aggregation is independent of thromboxane biosynthesis and granule release

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Introduction. The mechanism by which vanilloids, including plant-derived vanilloids (capsaicin (CAP) and dihydrocapsaicin (DHC)), and endogenous vanilloids (N-arachidonoyl-dopamine (NADA) and N-oleoyldopamine (OLDA)), inhibit *in vitro* platelet aggregation is not known.
Aims. To determine whether vanilloids inhibit *in vitro* platelet aggregation by interfering with: 1) the arachidonic acid (AA) metabolic pathway and subsequent thromboxane B2 formation (TXB2), and/or 2) adenosine diphosphate (ADP)-induced dense (5-hydroxytryptamine (5-HT)) and/or α-granule (platelet factor 4 (PF4) and β-thromboglobulin (β-TG)) release.
Methods. Platelets obtained from venous blood of healthy volunteers (n=4) were treated with AA (250 μg/mL) in the absence and presence of 3.125 and 50 μM CAP, DHC, NADA and OLDA to determine TXB2 formation (measured by ELISA). The effects of 3.125, 25 and 100 μM of each of the vanilloids on 5-HT, PF4 and β-TG release from dense and α-granules of platelets treated with 10 and 5 μmol/L ADP, were also determined using ELISA.
Results. Vanilloids did not significantly affect TXB2 from AA-activated platelets, or PF4, β-TG and 5-HT release from ADP-activated platelets.
Discussion. The inhibitory effects of CAP, DHC, NADA and OLDA on *in vitro* platelet aggregation does not appear to be mediated through inhibition of the AA pathway, as TXB2 was not affected. Furthermore, as dense- and α-granule release were not affected, inhibition of platelet aggregation by vanilloids is not due to interference with ADP receptor activation.

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Reversing the effect of cancer-related inflammation on hepatic drug-metabolising enzymes and transporters

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Introduction. Effective cancer treatment can be undermined by the substantial PK variability displayed by anti-cancer drugs both between and within individuals. Cancer-related inflammation has been demonstrated to reduce hepatic drug-metabolising enzyme and transporter (DMET) expression and activity, reduce PK of anti-cancer drugs, and reduce survival in cancer patients. Currently there is no pharmacological strategy to reverse cancer-related inflammation, and thereby improve chemotherapeutic PK and clinical outcomes.

Aims. This study aimed to identify possible anti-inflammatory drugs that could be useful in reversing the effect of cancer-related inflammation on hepatic DMETs.

Methods. To achieve this aim, the HepaRG hepatic cell line was used as an *in vitro* liver model due to its stable expression of DMETs and proven response to inflammatory mediators. Quantitative reverse transcription PCR was used to measure changes in inflammatory mediator (C-reactive protein, Suppressor of cytokine signalling 3) and DMET expression levels following culture in the presence of tumour-cell conditioned medium or interleukin-6 (positive control, 10 ng/mL) alone and with clinically-relevant concentrations of selected anti-inflammatory drugs (interleukin-6 receptor antibody, ruxolitinib, simvastatin, aspirin, dexamethasone).

Results. Both interleukin-6 and the tumour cell-conditioned medium elicited an inflammatory response in the HepaRG cells and decreased the expression of several relevant enzymes (e.g. *CYP3A4*) and transporters (e.g. *SLCO1B1*) by as much as 100-fold and 2-fold, respectively. This effect was successfully reversed by an interleukin-6 receptor antibody and ruxolitinib, a Janus-activated kinase 1 and 2 inhibitor, but not by the other drugs tested.

Discussion. This study identified two anti-inflammatory drugs that were able to reverse the effect of cancer-derived inflammatory mediators on hepatic DMET gene expression *in vitro*. Further inhibition studies are warranted to identify the minimum concentration required to inhibit the effects of cancer-related inflammation. These results raise the possibility of pharmacologically reducing the large PK variability that impedes successful cancer treatment through the use of anti-inflammatory drugs as adjuvant therapy.

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The interactions of P-glycoprotein with antimalarial drugs, including substrate affinity and inhibition.

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Introduction. The combination of passive drug permeability, affinity for uptake and efflux transporters, as well as gastrointestinal metabolism defines net drug absorption. Efflux mechanisms are often overlooked when examining the absorption phase of drug bioavailability.

Aims. Knowing the affinity of antimalarials for efflux transporters such as P-glycoprotein (P-gp) may assist in the determination of drug absorption and pharmacokinetic drug interactions during oral absorption in drug combination therapies. Concurrent administration of P-gp inhibitors and P-gp substrate drugs may also result in alterations in the bioavailability of some antimalarials.

Methods. *In-vitro* Caco-2 cell monolayers were used here as a model for potential drug absorption related problems and P-gp mediated drug transport of drugs. Rhodamine 123 (Rh123) was used to detect potential efflux inhibition using 96 well plate fluorescence, while drug detection was done using either HPLC-UV or LC/MS detection methods.

Results. Artemisone had the highest permeability at around 50 x 10^{-6} cm/sec, followed by amodiaquine around 20 x 10^{-6} cm/sec, both mefloquine and artesunate were around 10 x 10^{-6} cm/sec and methylene blue between 2 and 6 x 10^{-6} cm/sec depending on the direction of transport. This three fold difference was able to be halved by use of P-gp inhibition. MRP inhibition also assisted the consolidation of the methylene blue transport. Mefloquine was shown to be a P-gp inhibitor affecting transport of our P-gp substrate, Rh123, although none of the other drugs impacted upon Rh123 transport rates.

Discussion. As mefloquine is a P-gp inhibitor and methylene blue is a partial substrate, methylene blue may have increased absorption if co-administered with such P-gp inhibitors.

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Thiopurine and Allopurinol: Together at last, in Sickness and in Pregnancy.

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Introduction. Although allopurinol was first trialled to improve the response of acute lymphoblastic leukemia to mercaptopurine therapy it did not enhance survival, and the drug later became known for its urate-lowering properties. A decade later, both mercaptopurine and its pro-drug azathioprine were found to provide steroid-sparing properties for treating inflammatory bowel disease (IBD). At that time it was discovered that allopurinol/thiopurine co-therapy produced a 'metabolic overdose' effect, leading to recommendations that allopurinol be contraindicated for thiopurine therapy (although the combination continued to be used for renal transplantation). More recently, it has been shown that allopurinol/low-dose thiopurine co-therapy greatly improves IBD response while reducing adverse reactions. However, data on allopurinol in pregnancy are scarce, and even rarer for the co-therapy.
Aim: Allopurinol/thiopurine co-therapy during pregnancy was retrospectively assessed for 13 cases of IBD.
Methods: Patients were identified at our two hospitals, one in the UK and one in Australia, using local IBD databases. Patient notes were used to collect data regarding pregnancy and fetal outcomes, including in utero fetal ultrasound scans, APGAR scores, fetal birth weights and neonate checks.
Results: 12 women with a total of 13 pregnancies [14 live births] were identified as being treated with co-therapy before conception and during pregnancy. There were no miscarriages or spontaneous pre-term deliveries [seven vaginal deliveries; six caesarean sections]. No congenital malformations were identified. A primagravid twin pregnancy complicated by pre-eclampsia and twin-to-twin transfusion syndrome required caesarean section at 25 weeks. Otherwise, there were no low birthweight [< 2.5 kg] babies and APGAR scores were normal.
Discussion: The co-therapy was not associated in our case series with adverse pregnancy outcomes. This reassures patients and clinicians that the co-therapy can be utilised to maintain remission of IBD, both before conception and during pregnancy. Co-therapy also lowers the dose of cytotoxic thiopurine and reduces undesirable metabolites.
- Sheikh M et al (2015) J Crohns Colitis 9:680-4.

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Human Oligopeptide transporter 2 (PEPT2) mediates cellular uptake of polymyxins

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Introduction and aims. Polymyxins are a last-line therapy to treat multidrug-resistant Gram-negative bacterial infections. Nephrotoxicity is the dose-limiting factor for polymyxins and recent studies demonstrated significant accumulation of polymyxins in renal tubular cells. However, little is known about the mechanism of polymyxin uptake into these cells. Oligopeptide transporter 2 (PEPT2), an important Solute Carrier transporters (SLCs), is expressed at the apical (urine facing) membrane of renal proximal tubular cells and facilitates drug reabsorption in the kidney. In this study, we examined the role of PEPT2 in polymyxin uptake into renal tubular cells.
Methods. We investigated the inhibitory effects of colistin and polymyxin B on the substrate uptake mediated through 15 essential SLC transporters in the over-expressing HEK293 cells. The inhibitory potency of both polymyxins on PEPT2-mediated substrate uptake was also derived. Transport assay and fluorescence imaging were employed to investigate PEPT2-mediated uptake of MIPS-9541, the representative fluorescent probe of polymyxins.
Results. Both colistin and polymyxin B potently inhibited the PEPT2-mediated ^{3}H-glycosarcosine uptake with IC_{50} values of 11.4 ± 3.1 and 18.3 ± 4.2 μM, respectively. In contrast, both polymyxins had no or only mild inhibitory effects on the transport activity of the other 14 SLC transporters evaluated in this study. The representative polymyxin probe MIPS-9541 potently inhibited the PEPT2-mediated ^{3}H-glycosarcosine uptake (IC_{50} = 15.9 μM) and also a substrate of this transporter (K_m = 74.9 μM). The PEPT2-mediated uptake of MIPS-9541 was time-dependent, which was also impaired in the presence of glycosarcosine, colistin and polymyxin B.
Conclusions. Our study provided the first evidence of PEPT2-mediated uptake of polymyxins and greatly contributes to better understanding the accumulation of polymyxins in renal tubular cells.

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The inhibition of human UDP-glucuronosyltransferase 2B10 (UGT2B10) by antidepressant and antipsychotic drugs: Implications for drug-drug interactions

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Introduction. UGT2B10 catalyses the N-glucuronidation of drugs and other xenobiotics containing an aliphatic amine or aromatic N-heterocyclic group. Although the inhibition selectivity of UGT2B10 is poorly understood, it would be anticipated that compounds containing an amine functional group would inhibit this enzyme. Antidepressant and antipsychotic drugs typically incorporate a primary, secondary or tertiary amine group.
Aims. To characterise the effects of structurally diverse antidepressant and antipsychotics drugs from various therapeutic classes on UGT2B10 activity, and assess the potential of selected compounds to precipitate inhibitory drug-drug interactions (DDIs) with UGT2B10 substrates.
Methods. Twenty three antidepressant and 10 antipsychotic drugs were screened for inhibition of recombinant UGT2B10 using cotinine as the probe substrate. Subsequent experiments determined the inhibitor constants for the most potent inhibitors identified in the inhibition screening studies using human liver microsomes (HLM) as the enzyme source. Incubations contained cotinine (at a concentration that corresponded to the K_m), enzyme (UGT2B10 or HLM), and UDP-glucuronic acid (co-factor; 5 mmol/L) in phosphate buffer (0.1 mol/L, pH 7.4). Cotinine N-glucuronide formation by incubations was quantified by high performance liquid chromatography.
Results. Initial studies using a panel of recombinant human UGT enzymes and UGT enzyme-selective inhibitors demonstrated that cotinine is a highly selective substrate for UGT2B10. The inhibition of cotinine N-glucuronidation by 33 antidepressant/antipsychotic drugs was subsequently assessed at four inhibitor concentrations; 1, 10, 100 and 500 µmol/L. The three most potent inhibitors were amitriptyline, doxepin and mianserin, with IC_{50} values ranging from 2.2 to 7.6 µmol/L. IC_{50} values for the remaining compounds ranged from 26 to > 500 µmol/L. Amitriptyline, doxepin and mianserin competitively inhibited human liver microsomal cotinine N-glucuronidation with K_i values < 2 µmol/L.
Discussion. Most potent inhibition of UGT2B10 was associated with tri/tetra-cyclic tertiary amine antidepressant drugs. The K_i values observed for amitriptyline, doxepin and mianserin suggest the possibility of DDIs.

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Pharmacokinetics of transfer of azithromycin into the breast milk of African mothers

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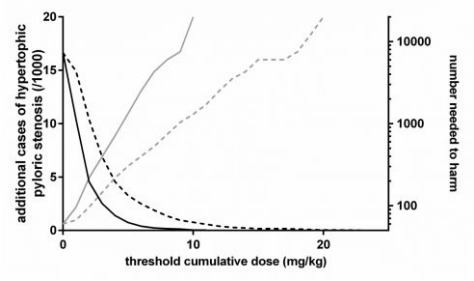

Introduction. Azithromycin (AZI) is a semi-synthetic macrolide antibiotic used to treat a number of bacterial and parasitic infections. Currently there is limited data on transfer of AZI into breastmilk making accurate assessment of infant dose exposure difficult.[a,b] This is particularly important given the association of neonatal exposure to AZI with infantile hypertrophic pyloric stenosis (IHPS).[c]
Aims. i) Develop a population pharmacokinetic (PPK) model for AZI in breastmilk ii) Estimate infant exposure of AZI and risk of IHPS using the developed PPK model through simulation.
Methods. Breastmilk concentrations from 40 Gambian women after a 2g dose of AZI at labour were modelling using NONMEM. A previous model of plasma AZI in pregnant women was used to allow for estimation of milk:plasma ratio (MPR) and changes of this ratio over time were investigated. 1,000 simulated data sets were used for infant dose estimation and IHPS risk for several AZI regimens.
Results. The developed PPK model was able to adequately describe the breastmilk concentration data using a combination of sigmoid E_{max} relationships for the MPR over time. Initially the MPR was ~40 peaking at ~100 at 5-6 days then falling to ~70. Median estimated relative total infant exposure was between 5-18% for the simulated regimens. The NNH for IHPS was estimated to be as low as 60 with certain assumptions.
Discussion. This represents the most comprehensive evaluation of the transfer of AZI into breastmilk to date. Infant exposure exceeded the suggested 10% safety limit in a large proportion of simulations even with a single maternal dose. There may be advantages in reducing neonatal infections, but the association with IHPS should be monitored.

Ref: [a] Kelsey JJ et al. (1994) Am J Obstet Gynecol 170:1375-1376. [b] Sutton AL et al. (2015) Am J Obstet Gynecol 212:812 e811-816. [c] Eberly MD et al (2015) Pediatrics 135:483-488.

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Assessing the contribution of CYP3A4 to total cytochrome P450 mediated kinase inhibitor metabolism

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Introduction. Kinase inhibitors (KIs) are a rapidly expanding class of anticancer drugs that have achieved remarkable success in extending the survival of cancer patients. It is impossible to predict which patients will achieve a desired KI exposure based on current fixed dosing protocols. A practical approach to predict optimal KI dosing may be the assessment of phenotype for key pathways that determine KI exposure. In this regard, cytochrome P450 (CYP) 3A4 is reported as the 'major' enzyme involved in the clearance and metabolic activation of many KIs, however there are limited data regarding the specific contribution of CYP3A4 as a proportion of total CYP metabolism. In order to consider the capacity of a CYP3A4 phenotype to inform dosing for individual patients and KIs, it is essential to understand the precise role and importance of this enzyme for each KI.

KI	% CYP3A4 Contribution
Axitinib	99.8
Dabrafenib	57.4
Dasatinib	87.8
Erlotinib	97.0
Gefitinib	30.5
Imatinib	59.8
Lapatinib	90.4
Nilotinib	94.9
Pazopanib	90.4
Regorafenib	97.6
Sorafenib	99.7
Sunitinib	95.1
Vemurafenib	82.6

Aim. Quantify the contribution of CYP3A4 to total CYP mediated KI metabolism.

Method. *In vitro* metabolism studies were performed for a panel of 13 KIs using human liver microsomes. The rate of microsomal KI metabolism was quantified in the presence and absence of a specific CYP3A4 inhibitor (CYP3Cide) and a pan-CYP inhibitor (1-aminobenzotriazole). The contribution of CYP3A4 to total CYP metabolism was assessed on the basis of the extent of inhibition of microsomal KI metabolism in the presence of CYP3cide and 1-aminobenzotriazole.

Results. The contribution of CYP3A4 to total KI oxidative metabolism varied between 30 and 99% (see table). CYP3A4 is responsible for >80% of the total CYP metabolism of axitinib, dasatinib, erlotinib, lapatinib, nilotinib, pazopanib, regorafenib, sorafenib, sunitinib and vemurafenib.

Discussion. Exposure to these KIs is likely to be affected by CYP3A4 activity, thus providing a mechanistic rationale to consider CYP3A4 phenotype when estimating KI exposure.

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Regulation of UDP-glucuronosyltransferase 2B7 by microRNAs in liver cancer cell lines

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Introduction. Glucuronidation is defined as the covalent attachment of glucuronic acid to small lipophilic compounds and this process alters their biological activity, water solubility and thus, facilitates their excretion from the body. Glucuronidation is primarily catalysed by UDP-glucuronosyltransferases (UGTs), a drug-metabolizing enzyme superfamily. For example, UGT2B7, one important UGT that is highly expressed in the liver, has high activity towards many drugs, carcinogens and endogenous bioactive molecules. Therefore, the regulation of UGT2B7 expression and activity in the liver is critical for its substrate detoxification and clearance.

Aims. To explore the possibility of regulation of UGT2B7 by microRNAs in liver cancer cell lines.

Methods. Potential regulatory microRNAs were identified by TargetScan and their effects on UGT2B7 expression studied with luciferase reporter assays, site-directed mutagenesis, quantitative real-time polymerase chain reaction (RT-qPCR), western blotting and glucuronidation assays.

Results. Bioinformatic analysis revealed putative binding sites in the 3'-untranslated region (3'-UTR) of UGT2B7 for several microRNAs including miR-3664. Transfection of miR-3664-mimics into the liver cancer HepG2 cell line reduced UGT2B7 expression at both mRNA and protein levels as well as decreased UGT2B7 glucuronidation activity. Transfection of miR-3664-mimics also reduced the activity of luciferase reporter containing UGT2B7 3'-UTR; however, mutation of miR-3664 binding site significantly abolished this reduction. We further showed an inverse correlation between expression levels of the miR-3664 and UGT2B7 mRNA in a panel of human tissues.

Discussion. Our data indicate that miR-3664 negatively regulates UGT2B7 expression in a hepatic cancer cellular context. Given the broad substrate spectrum of UGT2B7 and its abundant expression in the hepatic and extra hepatic tissues, the miR-3664-mediated negative regulation of hepatic UGT2B7 expression may have an important role for substrate detoxification and clearance of this UGT in the liver.

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Medication errors in patients undergoing elective surgery

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Introduction. Paper medication charts are being replaced with electronic systems. Electronic prescribing and administration (ePA) systems are associated with different errors to paper systems. We sought to describe medication errors on paper charts in preparation for evaluation of electronic systems.
Aims. To describe medication errors in patients undergoing elective surgery.
Methods. The clinical records of 176 randomly selected patients admitted to CDHB hospitals for elective surgery in 2013 were reviewed. Prescribing and administration errors were recorded by study pharmacists using a validated protocol.[1] Clinical and procedural errors were correlated with patient and medicine characteristics.
Results. From a total of 3,206 prescriptions, there were 2,574 (78 %) prescribing and 726 (22 %) administration errors over 637 inpatient days. This equates to 19 errors per admission, and 518 errors per 100 patient days. The majority of errors were procedural (e.g. prescriber not identified). The medicines associated with most errors were paracetamol (151), morphine (150), enoxaparin (79) and ondansetron (72). Of the administration errors, 629 were missed doses (19 % of total errors), with paracetamol and tramadol being the most frequently missed medicines (220 and 53 doses, respectively).
Discussion. Patients undergoing elective surgery are subject to multiple medication errors with paper chart systems. The majority of errors were prescribing-related. Of administration errors, missed doses are particularly common. ePA systems have been shown to reduce procedural errors found in this study . However, most of these errors have low clinical impact and ePA systems introduce new errors (e.g. selection error), some of which are associated with significant risk.

Westbrook JI et al (2012) PLoS Med 9(1) e1001164

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Investigating selectivity of ginsenosides as positive modulators of purinergic ion channels

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Introduction: P2X7 is an ATP gated ion channel expressed primarily on immune cells and is known to play a major role in inflammatory pathways. *Panax ginseng* is a Chinese herb well known for its pro- and anti-inflammatory effects. Previous work in our lab has demonstrated that bioactive ginsenosides Rh2, Rd, Rb1 and CK, have a potent potentiating action on P2X7 channels. It is possible that the immuno-modulatory properties of ginseng *in vivo* may be due to the ability of ginsenosides to potentiate P2X7-mediated responses (1). In the present study, we further investigate the selectivity of these four ginsenosides for P2X7 by determining the effect of these compounds on closely related purinergic receptors expressed on immune cells such as macrophages.
Aims: This study aims to investigate the selectivity of four protopanaxadiol ginsenosides on purinergic receptors.
Methods: Purinergic receptor activation was measured by YOPRO and Fura-2 dye uptake on Flexstation III.
Results: Ginsenoside mediated potentiation was observed in both HEK-rat P2X7 and HEK-human P2X7 in the same rank order of CK=Rd>Rb1>Rh2, with CK causing a 5-fold increase in ATP mediated responses.Similiar results were obtained with HEK-hP2X4 cells, even though the overall magnitude of responses were lower in comparison to P2X7. In HEK-hP2X2a cells, ginsenoside CK potentiated ATP responses, by mean of 1.6-fold. The P2Y receptors mediated calcium uptake was not significantly affected by these ginsenosides.
Discussion: The potentiation of purinergic receptors can enhance the pro-inflammatory activities of immune cells expressing them and help boost the immune system.This data suggests that the selected protopanaxadiols tested may not be selective for P2X7 with these modulators affecting both P2X4 and P2X2 responses to some extent. Further experiments are required to investigate direct actions on P2X4 and P2X2 ion channels.

1. Helliwell et al, 2015, Br J. Pharm. 172: 3326-40.

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Pilot study showing "AderenceCheck" does not improve 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).
Aims. The aim was to determine whether an "AdherenceCheck", which was designed to be similar to the "MedsCheck" (with the major exception that it was performed in the homes of the older-aged rather than at the pharmacy), 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. Six months later we reassessed their management of medicines.
Results. The 23 participants at the rental retirement village had a mean age ~75 years, 43% were non-adherent or at risk of being nonadherent in the next 6 months, and only 53% had a good knowledge of their illnesses. After 6 months, 8 participants were lost to the study: 5 had left the village, 2 withdrew, and 1 had died. Of the remaining 15 subjects, 40% were nonadherent and 55% had a good knowledge of their illnesses before the "AdherenceCheck". Six months after the "AdherenceCheck", there was no change. Thus, 53% of the older-aged were nonadherent and only 55% had a good knowledge of their illnesses. After the 6 months, only 9 participants remembered receiving an "Action Plan", and 6 of these had followed-up on the "Action Plan".
Discussion. The management of medicines by the older-aged living in a low socioeconomic rental retirement villages is poor, and there is no evidence from this pilot study, that an "AdherenceCheck" with an "Action Plan" improves this.

Doggrell SA, Kairuz T. (2012) J Pharmac Pract Res 42:208-12.

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Gentamicin pharmacokinetics and monitoring in paediatric febrile neutropenic patients

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Introduction. Gentamicin is an aminoglycoside antibiotic used in the treatment of gram-negative infections in febrile neutropenic patients. Due to its narrow therapeutic range, therapeutic drug monitoring (TDM) is routinely performed to optimise this agent's efficacy and minimise toxicity.
Aims. To describe the pharmacokinetics of gentamicin in paediatric febrile neutropenia patients and assess adequacy of current initial dosing based on hospital TDM.
Methods. Data on gentamicin usage and monitoring were collected retrospective from all children with febrile neutropenia admitted to hospital from January 2012 to December 2013 who had at least two suitable gentamicin measurements for log-linear regression analysis. Gentamicin clearance, volume of distribution, area under the concentration-time curve (AUC) and maximum concentration (Cmax) were estimated based on log-linear regression analysis and compared with pre-specified hospital targets.
Results. Data were collected from 69 patients (median [IQR] age 3.7 years [2.2, 8.9]) and comprised 121 paired concentration sets characterising 80 separate admissions. The median [IQR] duration of gentamicin therapy was 4.0 [3.0, 6.0] days with a median of 2.0 [2.0, 4.0] gentamicin concentration readings per admission. The median dosage given was 7.3mg/kg [6.7, 7.5], 7.9mg/kg [7.1, 8.8] and 8.9mg/kg [7.4, 10.2] for the first, second and third paired concentration set respectively within the same admission. The median [IQR] clearance and volume of distribution of gentamicin was 8.1L/h/70kg [5.8, 12.4] and 21.8L/70kg [16.9, 29.5] respectively. In 10% of admissions pre-defined hospital targets were achieved for both AUC and Cmax; in 36% of admissions one or the other of these targets were met and in 54% of admissions neither target was achieved. Achievement of AUC and Cmax targets improved with repeat TDM during the same admission.
Discussion. In the majority of patient's, gentamicin exposure after daily IV dosing was below current hospital targets, suggesting that either an increase in the recommended starting dose of gentamicin or an adjustment of the targets is required.

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A LCMSMS method for amisulpride in human plasma and breast milk, applied to measuring transfer to a fully breast-fed neonate.

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Introduction. Amisulpride is a second generation atypical antipsychotic drug. The management of psychosis in late pregnancy or during lactation is often hampered by inadequate knowledge of risk to the baby from placental transfer or breast milk transfer of drugs. There is no specific information on adverse effects from amisulpride.

Aims. In order to gather guiding information from one mother – baby pair, we conducted a pharmacokinetic study on the 4th day of life and developed a novel liquid chromatography-tandem mass spectrometry (LCMSMS) method with application to the very small plasma volumes obtainable from a neonate and to human breast milk.

Methods. Waters Acquity UPLC I Class Binary Solvent Manager, Flow Through Needle Sample Manager, Column Manager, HSS T3 1.8um, 2.1 x 100mm column and precolumn were used. Plasma and breast milk extracts, spiked with deuterated amisulpride internal standard were separated isocratically with a NH_4HCO_3/NH_4OH-buffered water:methanol:acetonitrile mobile phase. Detection employed a XEVO-TQS tandem mass spectrometer in positive electrospray ionisation mode with multiple reaction monitoring.

Results. Linearity, sensitivity, precision, matrix effects, recovery and overall process efficiency were satisfactory for milk and plasma. No interferences were found from a broad range of psychotropic and general drugs. The breast milk $AUC_{0\text{-}12hr}$ was 10,726 µg.h/L, corresponding to C_{AVG} of 894µg.h/L. Breast milk amisulpride was 12-fold higher than simultaneous plasma concentration. The baby's plasma amisulpride concentration was 10.9% of the simultaneous maternal plasma concentration.

Discussion. An assay was developed that is suitable for therapeutic drug monitoring of amisulpride, as strongly recommended in AGNP Guidelines (Hiemke et al., 2011). Its application to breast milk and neonate plasma showed that amisulpride partitioned strongly into breast milk and that the neonate reached plasma levels that were more than desirable for a psychotropic drug.

Hiemke C et al (2011) Pharmacopsychiatry, 44**,** 195-235.

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A simple and precise assay for the determination of febuxostat in human plasma

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Introduction. Febuxostat is a selective xanthine oxidoreductase (XOR) inhibitor that reduces plasma uric acid concentrations and is registered for the treatment of gout. There is limited information on the PK and PD of febuxostat in gout patients notably those with significant renal impairment. A HPLC assay with fluorescence-detection was developed previously to determine plasma concentrations of febuxostat however the interday coefficients of variation (CV) was ≤19.2%[1].

Aims. To develop and validate a simple, accurate and precise assay for the determination of plasma concentrations of febuxostat suitable for investigation of PKPD relationships in gout patients.

Methods. Proteins in plasma samples (200 µL) were precipitated with acetonitrile (200 µL) containing the internal standard (2-naphthoic acid). The supernatants were analysed by HPLC with fluorescence-detection at excitation and emission wavelengths of 320 and 380 nm, respectively. A Luna C18 column (Phenomenex, Australia) was used to resolve febuxostat and the internal standard with a mobile phase composed of 0.032% glacial acetic acid in acetonitrile:water (55:45, v:v).

Results. Standard curves were linear in the range 0.01 to 15 µg/mL. No interference by endogenous compounds was detected. QC samples at 0.02 and 14 µg/mL had CV of 4% and 3%, respectively. The lower limit of quantification was 0.01 µg/mL (CV <7%).

Discussion. This assay has sufficient accuracy, precision and sensitivity for the determination of plasma febuxostat for PKPD studies and should be useful for therapeutic drug monitoring.

[1]Khosravan R et al (2007) Br J Clin Pharmacol 65(3): 355–363.

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The pattern of anticholinergic medication prescribing in elderly hospital inpatients

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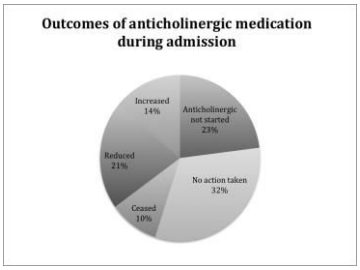

Introduction. Anticholinergic toxicity is well documented and common in the elderly. Deprescribing of medicines is an area of recent emphasis as per Poudel et al, 2015;it is unknown how prevalent this practice is within Australia.
Aims. To determine the number of elderly patients on anticholinergic medication, review associations between presentations and prescriptions and review action taken during admission by medical teams.
Methods. Retrospective descriptive study of 100 elderly inpatients from medical specialties at Redland Hospital, a 150-bed outer metropolitan hospital in Brisbane. Anticholinergic potency was described as per Duran et al, 2013.
Results. Mean age was 78 years (SD 8) and 55 subjects were female. A mean of 11.8 (SD 6.0) medicines were prescribed on admission. Seventy-three subjects (73%) were prescribed at least one anticholinergic medication. The most commonly administered anticholinergic medicines were frusemide (34% of subjects; 'unknown potency'), inhaled tiotropium (18%; 'high potency') and oxycodone (11%; 'low potency'). A total of 26 patients presented with falls or cognitive disturbances, of which seventeen (65.3%) were prescribed at least one anticholinergic. Only 2 of these cases documented polypharmacy as contributor to presentation. Evidence of a deprescribing trend during admission was found, with 31 subjects (42.5%) having a reduction in anticholinergic load on discharge, 32 (43.8%) with no changes and 14 (13.7%) increased prescribing of anticholinergic medicines.
Discussion. The majority of elderly patients admitted were on anticholinergic medications which may have contributed to presentation in some cases. Anticholinergic medications were recognised and an overall reduction in anticholinergic load was evident during admission.

Duran CE et al (2013) Eur J Clin Pharmacol 69(7):1485-1496
Poudel et al (2015) Clin Interv Aging 10: 1043-105

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Population pharmacokinetic modelling as tool to personalise gentamicin therapy

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Introduction. Bayesian forecasting is one method of tailoring drug dosages to individual patient's needs. To facilitate this, a population pharmacokinetic model is required describing expected drug exposure under a specific dosing regimen in a given patient population.
Aims. This review aims to summarise available population pharmacokinetic models developed to increase the efficiency of personalising gentamicin therapy in specific subpopulations and individual patients.
Methods. PubMed and EMBASE databases were searched for articles describing the population pharmacokinetics of gentamicin prior to May 2015. These articles were assessed and results were extracted and summarised in tables.
Results. Thirty-one studies were identified that developed a population pharmacokinetic model of gentamicin. Typical clearance (CL) and volume of distribution (V_d) of gentamicin in adults with normal renal function and paediatric patients were 4.54 L/h/70kg, 19.50 L/70kg, 3.09 L/h/70kg and 31.79 L/70kg, respectively. Variability in gentamicin exposure amongst adults and paediatrics was most commonly related to patient creatinine clearance (CL_{CR}), serum creatinine, body weight and age. In adult populations, CL_{CR} had a positive correlation with CL of gentamicin. In paediatrics, both age and body weight showed a positive correlation with CL of gentamicin. In adult populations, between-subject variability (BSV) in CL and V_d ranged from 0.3 to 83.7% and 5.8 to 84%, respectively. In paediatric populations, BSV in CL and V_d ranged from 16.1 to 52% and 10.3 to 40%, respectively. Only two studies in adults included successfully between-occasion variability (BOV) into the model.
Discussion. To predict gentamicin's pharmacokinetics in a given individual, a population model needs to be available that characterises the patient group to which the individual belongs. Large BSV for CL and V_d was observed in many studies, even after including patient characteristics influences. This makes gentamicin a suitable candidate for exposure monitoring and dose adjustment. To further evaluate the benefits of monitoring within a patient, BOV should be estimated. More population pharmacokinetics studies are required in certain populations such as children and the elderly.

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Therapeutic drug monitoring trials for gastrointestinal stromal tumors treated with sunitinib cannot be expected to detect clinical outcome improvement

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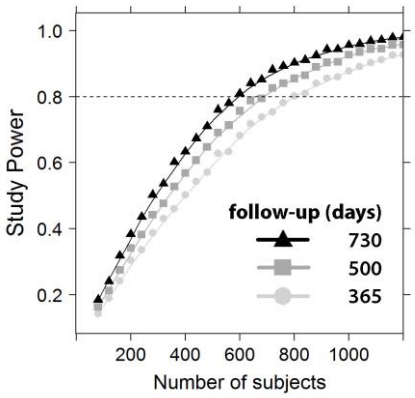
Introduction: Due to large interpatient variability, therapeutic drug monitoring (TDM) is being considered to individualise cancer treatment with tyrosine kinase inhibitors like sunitinib. However, several trials lacked statistically significant improvement in clinical outcomes with TDM—possible due to the low number of subjects included.

Aims: To investigate the feasibility of showing improved clinical outcome in prospective TDM trials of sunitinib in patients with gastrointestinal stromal tumors.

Methods: Therapeutic drug monitoring trials were simulated, using published models of the pharmacokinetics and pharmacodynamics of sunitinib. Log-rank test was used to test for statistical improvement in time of tumor progression in the simulated trials.



Results: Trials with a longer follow-up time require less subjects to be sufficiently powered for clinical outcome. However, even simulated trials with a 2-year follow-up (±90% maturity) required enrolment of about 600 subjects.

Discussion: The number of subjects needed to show an improvement in clinical outcomes with TDM about 20-fold higher than the 29 subjects enrolled in a recent sunitinib TDM feasibility trial. Pharmacometric modeling and simulation might facilitate evidence-based TDM practices when prospective TDM trials cannot be expected to show improvement in clinical outcomes due to difficulties of enrolled the required number of subjects.

Yu et al. (2015) Br J Clin Pharm 79(5):809-819
Houk et al. (2010) Cancer Chemother Pharmacol 66:357-371
Lankheet et al. (2014) Br J Cancer 110: 2441-244

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Complex Patients, Polypharmacy and Guidelines

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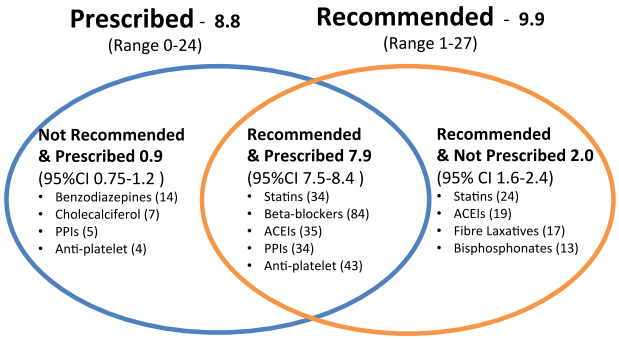
Introduction. For nearly every diagnosis in medicine there is an associated international, national or local management guideline. Guidelines recommend starting medicines but few guidelines include recommendations on stopping medicines or adapting treatment in multi-morbid, complex patients. Polypharmacy is prevalent in multi-morbid patients and is a known risk factor for medication related harms.

Aims. To compare the medicines recommended by guidelines with the medicines prescribed by general physicians to patients at hospital discharge.



Methods. A prospective cross-sectional study of 88 consecutive patients admitted acutely to two general medical teams at Christchurch Hospital. For each patient a list of current problems and current medicines at discharge from hospital was compiled. For each problem an appropriate treatment guideline was sought. The medicines recommended by each guideline were compiled in a list for each patient. The lists of current and recommended medicines were compared.

Results. The average number of current problems was 7.8 (1-19) with 5.9 (1-15) amenable to medical treatment. The average number of current and recommended medicines is shown in the figure. Following disease management guidelines would result, on average, in starting two additional medicines and stopping one medicine per patient.

Discussion. Current guidelines should be applied to complex patients with caution. Future guidelines should include when and how to stop medicines. Future guidelines should include recommendations on adapting treatment in complex patients.

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LC-MSMS method to describe the pharmacokinetics of aerosolised lincomycin and tobramycin at the site of infection.

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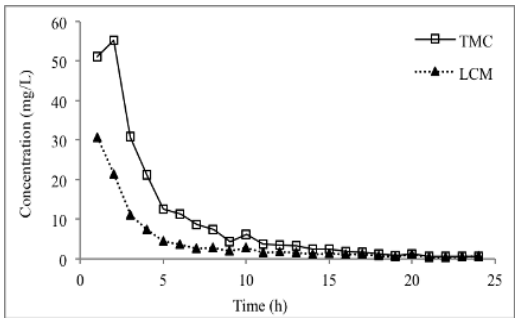

Introduction. A pharmacokinetic study was designed to investigate the target site penetration of aerosolised lincomycin and tobramycin and potential suitability for the treatment of severe lung infections.

Aims. To establish a validated LC-MSMS methods for bioanalysis of lincomycin (LCM), tobramycin (TMC) and gentamicin (GTM) in microdialysis fluid for use in an ovine PK study.

Methods. Microdialysis samples (10 µL) were diluted with the analytical internal standard, vancomycin, prior to separation using hydrophilic interaction chromatography (HILIC) and quantitation on a Shimadzu Nexera 8030+ LC-MSMS.

Results. The assays met the requirements for bioanalytical method validation over the concentration range of 0.1 to 20 mg/L for both lincomycin and tobramycin and 0.5 to 10 mg/L for gentamicin, in microdialysis fluid. Inter-assay results for lincomycin were 0.28 ± 0.01, 2.05 ± 0.04, 17.3 ± 0.1 mg/L, for tobramycin were 0.29 ± 0.01, 2.16 ± 0.01, 17.5 ± 0.1 mg/L, and for gentamicin 2.14 ± 0.03 mg/L. The figure presents the tissue concentrations resulting from a sheep receiving an aerosolised dose of 600 mg lincomycin and 400 mg tobramycin, with gentamicin infused at 8 mg/L in the microdialysis fluid to estimate the efficiency of the probe across the study period (24 h).

Discussion. We have successfully validated an LC-MSMS assay for lincomycin, tobramycin and gentamicin and subsequently applied it to an animal PK study using aspirated lincomycin and tobramycin.

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A suite of LC-MSMS assays to investigate pharmacokinetics of vancomycin in renal replacement therapy

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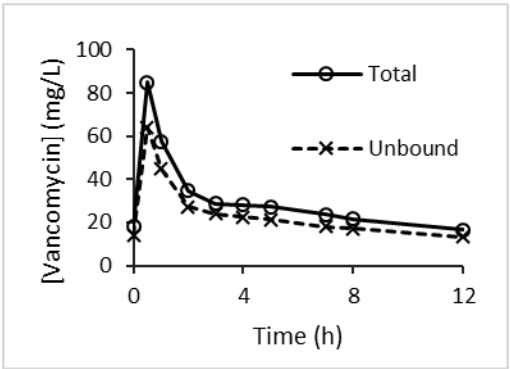

Introduction. Inadequate antibiotic therapy is a critical determinant of survival in patients admitted to an Intensive Care Unit (ICU) with overwhelming infection requiring renal replacement therapy (RRT). We have commenced a multinational pharmacokinetic (PK) clinical trial with a range of sample types to provide valuable dosing guidance for vancomycin in ICU patients on RRT.

Aims. To establish validated LC-MSMS methods for bioanalysis of vancomycin in plasma (total and unbound concentrations), dialysis effluent and urine for use in a clinical PK study.

Methods. Sample preparation for total plasma concentrations was by protein precipitation, whilst ultracentrifugation was used for isolating the unbound fraction for analysis. Urine and dialysis effluent were analysed with or without dilution, respectively. Hydrophilic Interaction Chromatography (HILIC) was used to separate vancomycin and the internal standard, with quantitation on a Shimadzu Nexera 8030+ LC-MSMS.

Results. The assays met requirements for bioanalytical method validation for measurement of vancomycin over the concentration range of 0.2 to 100 mg/L in plasma (total concentration), plasma ultrafiltrate (unbound concentration) and dialysis effluent, and over the range of 2 to 10,000 mg/L for urine. The figure presents the results from an ICU patient from the PK study receiving 1g intravenous vancomycin 12-hourly on RRT, with a vancomycin unbound fraction of 79±2%, dialysis effluent concentrations of 31 to 55 mg/L and a urine concentration of 3110 mg/L.

Discussion. The zic-HILIC guard cartridge provided robust chromatographic separation suitable for the four sample extracts. This suite of methods for measuring vancomycin has been applied to a multinational clinical PK trial.

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Quantitative bioanalytical validation of fosfomycin using innovative techniques of microsampling.

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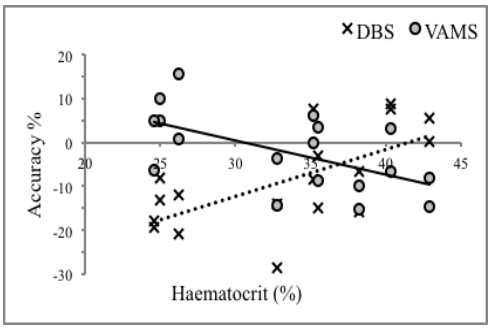

Introduction. Pharmacokinetic studies can greatly benefit from engagement with hospitals with challenging patient populations, but which may not be equipped to perform their own laboratory analysis. Innovation in technologies for sampling, such as: volumetric absorptive microsampling (VAMS), dried blood spots (DBS), and dried plasma spots (DPS) allow low volumes (<25 µL) of clinical 'microsamples' to be collected, dried, stored and shipped.

Aims. Perform a quantitative bio-analytical method validation for fosfomycin in VAMS, DBS and DPS that is suitable for use in a clinical pharmacokinetic study.

Methods. Whole blood or plasma containing fosfomycin was applied to a microsampling device or collection paper, extracted by adding 200 µL of methanol containing internal standard, and shaken for 30 minutes. The supernatant was analysed using a Shimadzu LC-MSMS (8030+) Merck SeQuant zicHILIC 2.1 x 50 mm, 5.0 µm analytical column.

Results. VAMS (at normal or high hematocrit) and DPS sampling validation data met acceptance criteria. The calibration range for fosfomycin using both VAMS and DPS was linear from 5 to 2000 mg/L. Inter-assay results for for VAMS were 14.6 ± 0.3, 188.0 ± 3.5, and 1606 ± 38; inter-assay-results for DPS were 15.7 ± 0.7, 77.8 ± 2.0, and 1632 ± 18. For DPS 75% of clinical samples from a pilot PK study were within 20% of plasma concentrations.

Discussion. Initial testing over a range of hematocrit levels (24.6 to 42.9%) suggests an inverse correlation between accuracy and haematocrit may exist with the VAMS technique (see Figure), in contrast to the DBS sampling technique. DPS compared to plasma sampling found a -16.6% bias, but results met incurred sample reanalysis criteria. VAMS and DBS require further investigations; we believe DPS is a suitable tool for PK research.

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A validated UHPLC-MS/MS method for measurement of riluzole in plasma and cardiac tissue samples

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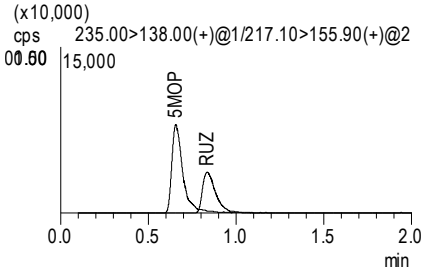

Introduction. Riluzole is a glutamate antagonist used to prolong the survival of patients with amyotrophic lateral sclerosis. Experiments in pigs have shown riluzole reduces arrhythmias and myocardial damage (Weiss et al, 2013). An assay to measure riluzole in plasma and heart tissue was required to investigate heart tissue penetration of riluzole in cardiac bypass surgery patients.

Aims. To develop and validate a chromatographic method for measuring concentrations of riluzole (RUZ) in plasma and heart tissue homogenate.

Methods. Heart tissue was homogenised in borate buffer. Dichloromethane was used to extract riluzole and 5-methoxypsoralen (5MOP, internal standard) from plasma and homogenate. The dried dichloromethane extract was reconsitituted in methanol:water 1:1. Separation was on a Shimadzu Shim-pack XR-ODS III, 2.0 x 50 mm (1.6 µm) column (see figure), with quantitation on a Shimadzu 8030+ LC-MSMS.

Results. The assay met validation criteria, being linear from 0.5 to 500 ng/mL with precision and accuracy within 6% for plasma and within 14% for heart tissue homogenate. A patient who received oral riluzole 100 mg per day for 5 days and then 200 mg immediately before surgery had plasma levels measured from 52.5 and 298 ng/mL and a cardiac tissue level estimated to be 232 ng/g.

Discussion. We have developed and validated a chromatographic method for measuring concentrations of riluzole in plasma and tissue homogenate. This assay has been successfully used in a clinical trial in cardiac bypass surgery patients. This study reports the first riluzole concentrations measured in heart tissue.

Weiss SM, Dahlstrom JE et al (2013) Clin Exp Pharmacol Physiol 40:856-863

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Metformin in pregnant women with type 2 diabetes mellitus: update on safety and efficacy

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Introduction. Metformin is considered safe in women with polycystic ovary syndrome (PCOS) who become pregnant (first trimester exposure) and in those who develop gestational diabetes mellitus (GDM) later in pregnancy (third trimester exposure). However, the safety and efficacy of metformin in pregnant women with type 2 diabetes mellitus (T2DM) is less clear. Drug information sources recommend switching these women from metformin to insulin when they present for antenatal care.

Aim. To review the studies of metformin *vs*. insulin in pregnant women with T2DM.

Methods. A systematic search was conducted in the electronic databases MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials. Keyword searches of Google Scholar and Pubmed were also performed. Inclusion criteria were; study design (RCTs and prospective cohort studies), population (women with pre-pregnancy T2DM, metformin in first trimester), comparison (insulin in first trimester), and outcomes (glycaemic control [fasting glucose, $HbA1_c$] and pregnancy and neonatal outcomes).

Results. Three RCTs and one prospective cohort study met the inclusion criteria. The RCTs were small and open-label. There were no statistical differences between metformin and insulin in glycaemic control during pregnancy or pregnancy and neonatal outcomes (Refuerzo et al. 2014; Hickman et al. 2013; Waheed et al. 2013). In Refuerzo et al., no women randomised to metformin required insulin, whereas 43% in Hickman et al. required insulin supplementation to achieve glycaemic control. Rai et al. (2009) compared metformin in one obstetric unit to insulin in another, finding similar glycaemic control between groups and no differences in pregnancy or neonatal outcomes.

Discussion. Three recent under-powered RCTs and one cohort study provide preliminary data to support the efficacy of metformin in pregnant women with T2DM. The risk of adverse neonatal outcomes with metformin is low based on first trimester data in PCOS and third trimester data in GDM. Two larger RCTs of metformin *vs*. insulin in pregnancy with T2DM are currently recruiting (NCT00678080 and NCT01353391 at www.clinicaltrials.gov).

Hickman et al. (2013) Am J Perinatol 30:483-490. Refuerzo et al. (2014) Am J Perinatol 30:483-490. Waheed et al. (2013) J Coll Physicians Surg Pak 23:866-869. Rai et al. (2009) Indian J Med Sci 63:491-497

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Safety and Pharmacokinetics of Metformin in Chronic Liver Disease

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Introduction. Metformin is the first-line treatment of type II diabetes mellitus, although its use is contraindicated in patients with severe hepatic impairment due to concerns of increasing the risk of lactic acidosis. However there is little evidence supporting this concern.

Aim. To evaluate the safety and PK of metformin in subjects with liver fibrosis.

Methods. Study A: a cross-sectional study of liver disease patients currently taking metformin (n=33). Study B: a 6-week prospective, interventional clinical trial in metformin-naïve patients with liver disease (n=13). All patients had a Fibroscan® to determine the degree of fibrosis. Biochemical parameters and metformin concentrations were monitored. Metformin concentrations in plasma were determined using HPLC. PK parameters were determined using TCIWorks (Version 1.0) and our previously published metformin PK model (Duong et al, 2013).

Results. The mean (± SD) ratio of metformin apparent clearance to creatinine clearance in all patients was 12.6 (± 4.3, n=39) which was not significantly different to values found in diabetic patients without significant liver disease, 12.9 (± 4.3, p=0.83). There was no correlation between metformin concentrations and lactate concentrations in both Study A and B, nor in Study B patients over time. There was however a significant relationship between Fibroscan® score and lactate concentrations ($p=0.04$, $r^2=0.10$), which was apparently independent of metformin.

Discussion. Metformin clearance was similar to that seen in previous literature, with concentrations remaining within the therapeutic range. This study shows that liver disease has no significant impact on metformin pharmacokinetics and safety although further studies are required.

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

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The Safe use of Metformin in patients with End Stage Kidney Disease on Haemodialysis

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Introduction. Metformin is almost entirely renally excreted and is subsequently contraindicated in patients with end stage kidney disease on dialysis. Currently, very little data exists describing the safety or pharmacokinetics of metformin in patients on haemodialysis (HD).

Aims. To investigate the safety and pharmacokinetics of metformin in T2DM patients on haemodialysis.

Methods. Patients (n=4) were dosed with 500 mg of metformin after each dialysis session (3 x 500 mg/week) for 12 weeks. Metformin dose was reduced if plasma concentrations were approaching 5 mg/L. Biochemical parameters were measured at baseline, and monitored in weeks 1-4, 8 and 12. Metformin plasma concentration was also determined 4, 24 and 48 hours post dose in weeks 1, 2 and 8. HD clearances were intensively calculated by collecting blood entering and exiting the dialyser at various time points throughout dialysis during weekly sessions (weeks 1-4, 8 and 12). Metformin concentrations were measured by HPLC.

Results. Metformin plasma concentrations did not exceed 5 mg/L and were generally constant between 4 and 48 hours post dose. Metformin concentrations in plasma decreased considerably throughout the dialysis session, while very little was cleared from RBCs. Average metformin plasma clearance was calculated to be approximately 173 ± 30 mL/min (± SD; n=3). Metformin was well tolerated in all patients with no serious adverse effects, and lactate concentrations remained below 5 mmol/L.

Discussion. Metformin is readily dialyzable from plasma. The stability of concentrations between dialysis sessions suggests no non-renal clearance. This pilot study provides the basis for further investigations with sparser sampling to firmly establish safety in this population. This work is of significant interest due to the cardiovascular risk benefits and glycaemic control metformin can provide for these patients.

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The role of dimethylarginine dimethylaminohydrolase 1 (DDAH-1) inhibition in experimental angiogenesis

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Introduction. Nitric oxide (NO) is a key mediator of the pro-angiogenic growth factor, vascular endothelial growth factor-A (VEGF-A). The hydrolytic enzyme dimethylarginine dimethylaminohydrolase 1, DDAH-1, selectively metabolises asymmetric dimethylarginine (ADMA) and N'G'-monomethyl-arginine (NMMA), endogenous inhibitors of the nitric oxide synthase (NOS) family of enzymes. The inhibition of DDAH-1 is therefore proposed as a strategy to inhibit excessive angiogenesis, e.g. in the setting of cancer, by increasing ADMA and NMMA concentrations that subsequently inhibit NO synthesis, thus reducing the function of VEGF-A.

Aims. To identify how dose-response relationships of DDAH-1 inhibition alter established phenotypic markers (tube and loop formation) of angiogenesis.

Methods. *In vitro* endothelial tube formation assays were undertaken using the two primary endothelial cell lines, HUVEC and VeraVec. Dose-response experiments with established (L-257) and recently synthesized (compound 10a, Tommasi S et al, 2015) DDAH-1 inhibitors were conducted over a 24-hr incubation period, with tube formation monitored and recorded post 5-hr incubation in matrigel.

Results. Tube formation assays in the presence of compound 10a (5μM) demonstrated a significant decrease both in tube formation (~40%) and in loop formation (~55%), relative to untreated cells. L-257 (5μM) reduced tube and loop formation by ~50% and ~62%, respectively. Increasing the concentration of both 10a and L257 to 10μM reduced the antiangiogenic response (10a: ~25% tube, ~42% loop; L-257: ~38% tube, ~52% loop).

Discussion. DDAH-1 inhibition significantly reduces NO-mediated experimental angiogenesis, with possible therapeutic applications in disease states characterized by excessive angiogenesis, e.g. cancer.

Tommasi, S et al (2015) Org Biomol Chem (submitted)

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Evaluating a natural remedy for Cholelithiasis (gallstones)

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Aim: To ratify/disprove an alternative nutritional treatment for gallstones.
Background: Gallstone disease is prevalent in modern societies. Allopathic treatment often involves long-term choleretic therapy eg with ursodiol or chenodiol 10 mg/kg/day(1) or stone fragmentation by shock wave lithotripsy or laporoscopic cholecystectomy. An alternative treatment uses dietary nutrients, apple juice (1L/day for six days), then one dose of olive oil (130 ml) with lemon juice (130 ml) preceding bowel evacuation with 'Epsom salts' $MgSO_4$(2).
Methods: (i) A retrospective survey of this treatment used in three rural naturopathic clinics in SE Qld indicated 80% of subjects diagnosed with cholelithiasis (n=30) responded with early pain relief, improved fat digestion and in many instances, presence of gallstones in their stools after $MgSO_4$ catharsis. (ii) A semi-controlled* trial was carried out at two naturopathic clinics in Brisbane after eight subjects with severely infected gallbladders gave their consent to treatment. A non-linear scanning (NLS) non-invasive device, the MetAtron (IPP Omsk) monitored severity of gall bladder (GB) disease and burden of GB-resident bacteria *E.Coli,* enterococci, etc.
Results: (Brisbane trial): response to this one-off treatment was rapid in five subjects and delayed (up to six weeks) in two others, requiring re-treatment. [Only one subject failed to respond and was referred elsewhere.] No further evidence of cholelithiasis was apparent four months later.
Conclusions: (i) The combination of malic and other Ca-chelating acids (apple, lemon juice) and a cholesterol dispersant (olive oil) was usually sufficient to reduce gallstone size and associated infection, facilitating their elimination by Mg-induced laxation. (ii) The MetAtron provided a rapid indication of response to treatment, allowing quantitation of effective dosage and assessing duration of responses.
*It was considered unethical to include an appropriate control group = $MgSO_4$ only.

Reference: 1. Schoenfield LJ et al (1981) Ann Intern Med 95:257-282
2.Lai Chiu-Nan. wwwlapislazulilight.com. Retrieved 6 September 2015

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Determination of total and free concentrations of cefalexin, cefazolin, flucloxacillin and probenecid in human plasma by liquid chromatography/tandem mass spectrometry

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Introduction. Cefalexin, cefazolin and flucloxacillin are widely used β-lactam antibiotics. Probenecid is co-prescribed for the elevation and prolongation of plasma levels of certain β-lactam antibiotics. Maximizing effectiveness and minimizing toxicity of antimicrobial agents are important and require an understanding of the pharmacokinetics and pharmacodynamics of the prescribed agents. It is important, for example, for the free concentration of β-lactam antibiotics to be above the *minimum inhibitory concentration* (MIC) of the bacteria for a sufficient period time over the dosing interval. Accurate measurement of plasma antibiotic and probenecid concentration is necessary for pharmacologic research and accurate and rapid measurement of plasma antibiotic concentration is increasingly used in clinical practice.
Aims. The objective of this work was to develop and validate a rapid LC-MS/MS for the determination of total and free concentrations of cefalexin, cefazolin, flucloxacillin and probenecid in human plasma.
Methods. Free cefalexin, cefazolin, flucloxacillin and probenecid were separated from bound drug by ultrafiltration. Samples of plasma and plasma ultrafiltrate were pretreated with acetonitrile (sample:acetonitrile = 1:4). Cefalexin, cefazolin, flucloxacillin, probenecid and four stable isotopically labeled internal standards were then resolved on a C18(2) column using gradient elution of 0.05% formic acid and methanol. The eight compounds were detected using electrospray ionisation in the positive mode.
Results and Discussion. Standard curves were linear over the concentration range 0.2 to 100 mg/L (r > 0.99) in plasma and 0.005 to 10 mg/L (r > 0.99) in plasma ultrafiltrate for the three antibiotics and probenecid. For both total and free concentrations, bias was <±10%, and intra- and inter-day coefficients of variation (imprecision) were<10%. The limit of quantification was 0.2 mg/L in plasma and 0.005 mg/L in plasma ultrafiltrate. The assay has been used successfully in pharmacokinetic studies and clinical practice.

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Diagnosis and management of early COPD in Australia: views from the coal face

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Introduction. Chronic obstructive pulmonary disease (COPD) is a major cause of disability, hospital admission and premature death in Australia. Approximately 1.45 million Australians are estimated to have COPD, although half of these people do not have a doctor's diagnosis. Under-diagnosis and under-treatment contribute to the burden of human misery and healthcare costs.

Aims. To gain insight into the diagnosis and early management practices of COPD amongst Australian general practitioners (GPs), and highlight any problem areas and management gaps for future intervention.

Methods. One thousand GPs in Australia were invited to take part in a postal survey, which collected demographics and practice details, familiarity with contemporary practice guidelines, method of diagnosis of COPD, use of lung function tests, treatment preferences, advice offered and patient follow-up.

Results. Two hundred and thirty-three GPs responded and were eligible for inclusion (23.3% response rate). While 83.7% of GPs indicated that they base a diagnosis of COPD according to guideline recommendations, 60.1% of respondents indicated that they delay the diagnosis. Overall, 87.6% of respondents routinely communicate the diagnosis to patients, although the preferred descriptive diagnostic terms differed, with one-third using terms other than 'COPD' to describe the condition to patients. Inhaled bronchodilators were the preferred treatment in initial management of COPD by approximately 90% of GPs; however, only 27.5% indicated that they routinely recommend pulmonary rehabilitation. GPs indicated that they routinely record patients' smoking status and offer smoking cessation advice, but the timing of this advice varied. Less than half of the respondents indicated that their management of COPD is informed by guidelines, and only 7.3% indicated that they were familiar with tools and resources provided by the Australian Lung Foundation.

Discussion. There is considerable scope for improvement in GPs' use of and familiarity with COPD management guidelines, tools and resources. Compilation and dissemination of guidelines and focused education on some areas in need of improvement (such as delayed diagnosis, delayed smoking cessation advice and underutilisation of pulmonary rehabilitation) are important strategies for improving patient outcomes in COPD.

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Understanding the discrepancy between perceived and actual asthma control in Australia

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Introduction. Asthma is a major chronic respiratory health issue in Australia, and is the focus of significant clinical and public health interventions. A well-documented barrier to optimal asthma management is patients' overestimation of how well their disease is controlled. This overestimation is quite prevalent in Australia, and it represents a major barrier to the design and uptake of asthma interventions. However, almost nothing is known about the factors that affect patients' overestimation of their asthma control.

Aims. To investigate the factors associated with the over-estimation of asthma control in Australia.

Methods. Australians over the age of 18 with a current diagnosis of asthma were recruited via Facebook to complete an online survey. The survey contained basic demographic questions and validated questionnaires assessing asthma control, asthma knowledge, medication adherence, medication beliefs and illness perception. Over-estimation of asthma control was determined by comparing patients' self-reported asthma symptoms to their self-rating of asthma control. Patients' over-estimation of control was analysed across the other dimensions of the survey.

Results. Of 2,971 responses, 1,950 (65.6%) were complete and eligible for inclusion. Over-estimation of control was apparent in 45.9% of participants. In multivariate analysis, older age, lower education level, use of regular preventer medication, agreement to the statement 'most medications are addictive', increased patient feelings of control over their asthma and greater experience of asthma symptoms were shown to be independent predictors of over-estimation of asthma control.

Discussion. This study demonstrates that over-estimation of asthma control is still a problem in Australia. To our knowledge, this is the first national study that has identified modifiable predictors of overestimation of asthma control. By identifying these factors, this study has paved the way for targeted interventions designed to improve the discrepancy between perceived and actual asthma control. This will empower patients, encourage them to be more proactive and help them to achieve better asthma management, including more appropriate use of medications and improved health outcomes.

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Dynorphin 1-17 and its biotransformation fragments modulate lipopolysaccharide (LPS)-induced release of interleukin-1beta and tumor necrosis factor-alpha in differentiated THP-1 cells

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Introduction. Upon release in peripheral inflamed tissues, dynorphin 1-17 (DYN-17) undergoes rapid biotransformation and yields a unique set of opioid and non-opioid fragments. Evidence has shown that some of these major fragments play a role in immunomodulation, suggesting that DYN 1-17 biotransformation fragments may modulate the release of inflammatory cytokines such as interleukin-beta (IL-1β) and tumor necrosis factor-alpha (TNF-α).
Aim. To examine the modulatory effects of DYN 1-17 and major biotransformation fragments found in the inflammatory environment on LPS-induced release of IL-1β and TNF-α in the human differentiated THP-1 cells.
Methods. LPS was used to induce the release of IL-1β and TNF-α in differentiated THP-1 cells. The cells were co-incubated with LPS (1 mg/L) and DYN 1-17 or a range of biotransformation fragments (DYN 1-6, 1-7, 1-9, 1-10, 1-11, 2-17, 6-12, 3-14 and 7-17) at 10 pmol/L, 1 nmol/L and 0.1µmol/L for 24 h. The modulation of cytokine release was also examined in the presence of ML-190 (10 µmol/L), a selective kappa-opioid (KOP) receptor antagonist. The levels of cytokine release were determined using a commercial AlphaLISA kit for IL-1β and TNF-α.
Results. DYN 1-7 significantly inhibited the release of IL-1β and TNF-α whereas DYN 1-6 increased the release of both cytokines at 0.1µmol/L, 1 nmol/L and 10 pmol/L. DYN 1-17 inhibited only IL-1β release and had no effect on TNF-α release from THP-1 cells. ML-190 antagonised the DYN 1-7 modulated release of IL-1β and TNF-α but did not alter DYN 1-17 and DYN 1-6 effects.
Discussion: DYN 1-17, DYN 1-6 and DYN 1-7 demonstrated differential modulation on the release of IL-1β and TNF-α in differentiated THP-cells. This cytokine modulation was mediated through either KOP selective or KOP receptor-independent pathways. These findings indicate that the biotransformation fragments of DYN 1-17 modulate inflammatory processes differentially as a result of enzymatic processing at the site of inflammation, highlighting pharmacological differences that might exist dependent upon disease status and severity.

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Differential expression of TRPV1 on circulating PBMC sub-populations assessed by confocal microscopy

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Introduction. The non-selective cation channel, transient receptor potential vanilloid 1 (TRPV1), is a polymodal nociceptor that is activated by vanilloids, including capsaicin, and a number of pro-algesic substances released during inflammation. We have previously reported that human peripheral blood mononuclear cells (PBMC) express TRPV1 mRNA and immunoreactivity (Saunders et al 2007). The expression of TRPV1 on PBMC suggests a role in mediating the inflammatory, immune and cancer surveillance responses of these cells. However, the differential expression patterns amongst circulating PBMC is still unclear.
Aim. The aim of this study was to use immunocytochemical staining and confocal microscopy of PBMC markers and TRPV1 to assess the differential expression of TRPV1 on these cells.
Methods. Platelet-rich PBMC preparations where prepared from healthy donors using histopaque isolation. Naïve monocytes were prepared from PBMC fractions using a paramagnetic negative isolation protocol. PBMC and monocyte fractions were immuno-stained for TRPV1, CD3 and CD14, using Alexa-Fluor secondary antibodies and counterstained with Hoechst 33342. Cells were visualised and assessed using an Olympus FV1200 laser scanning confocal microscope.
Results. Monocytes (CD14-positive) and platelets stained strongly for TRPV1, and 3D imaging showed a typical membrane pattern while lymphocytes (CD3-positive) demonstrated sparse immunoreactivity.
Discussion. These data show that monocytes and platelets strongly express TRPV1 and suggests that TRPV1 may play a role in monocyte/macrophage actions during inflammation and also affect platelet function in these conditions.

Saunders CI et al (2007) Mol Immunol 44, 1429-35

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Exploring the role of Annexin A1 in airway smooth muscle contraction and inflammation

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Introduction: Annexin A1 (ANX-A1) is an endogenous protein induced by glucocorticoids (GCs) that may mediate their anti-inflammatory actions via activation of formyl peptide receptors 1 and 2 (FPR1, FPR2). ANX-A1$^{-/-}$ mice display *in vivo* airway hyperresponsiveness (AHR) as seen in asthma and FPR-selective agonists mimicking ANX-A1 protect against cardiac inflammation (Qin *et al.*, 2015), but the influence of ANX-A1 deficiency or FPR agonists on airway contraction and inflammation are not known.

Aims: To determine if *in vitro* airway contraction is increased in ANX-A1$^{-/-}$ mice and whether synthetic FPR agonists inhibit cytokine release and from human airway smooth muscle cells (HASM) when GCs are ineffective.

Methods: Airway contraction to methacholine (MCh) was measured in lung slices (150μm) from wild-type (WT) and ANX-A1$^{-/-}$ mice (6-8 wks) treated with the inflammatory cytokine IL-13. The effects of budesonide (GC, 100nM) or Ac_{2-26}, Compound 17B and Compound 43 (FPR 1/2, FPR1, FPR2 agonists, 10μM) on IL-8 release were measured in HASM treated with TNFα/IFNγ, previously shown to induce GC-insensitivity via upregulation of the inactive isoform of the GC receptor, GRβ (Tliba *et al.*, 2008).

Results: Airways from ANX-A1$^{-/-}$ mice were not hyperresponsive to MCh (pEC_{50} WT 6.8±0.1, ANX-A1$^{-/-}$ 6.5±0.2, n=6) and contraction was not increased by IL-13 in either group. TNFα/IFNγ-induced IL-8 levels (140±21 pg/ml above basal) that was not inhibited by budesonide or FPR agonists (TNFα/IFNγ + Ac_{2-26} 198±46 pg/ml, n=5 one-way ANOVA, P>0.05). GRβ expression was unchanged following TNFα/IFNγ treatment.

Discussion: ANX-A1 deficiency is not associated with *in vitro* AHR. FPR agonists do not inhibit HASM cytokine release under GC-insensitive conditions or alter expression of GRβ. Future studies assessing the potential of FPR agonists in asthma may require a more integrated model of *in vivo* AHR and GC insensitivity.

Qin C *et al* (2015) Pharmacol Ther 148C: 47-65

Tliba O *et al* (2006) Am J Respir Cell Mol Bio 38: 463-472

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Predictive factors of probability of having *Aspergillus fumigatus* positive culture at five years of age of children with cystic fibrosis

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Introduction. *Aspergillus fumigatus* (*Af*) is the most frequent filamentous fungi isolated from respiratory culture of patients with cystic fibrosis (CF). Persistent *Af* infection during the paediatric years may have a lifelong implication on CF lung function progression (Amin et al, 2010). Most of studies investigated predictive factors of *Af* positive culture were done in patients with CF >6 years of age. However studies in children under the age of five years are still missing.

Aims. To determine factors predicting the probability of acquiring *Af* positive bronchoalveolar lavage (BAL) culture at five years of age in children with CF.

Methods. Data from the Australian Cystic Fibrosis Broncheoalveolar Lavage study was used (Wainwright et al, 2011). *Af* positive BAL cultures at five years of age and possible predictive factors from 157 children were analysed using logistic regression analysis.

Results. A multivariate analysis showed that every additional course of *Pseudomonas aeruginosa* eradication therapy received by the children prior to BAL observation increased the odds of having *Af* positive culture at age 5 by 61% (OR=1.61, 95%CI [1.23-2.12], P<0.05), and a warmer maximum annual temperature of living area at birth increased the odds of having *Af* positive culture at age 5 for by 11% for every degree Celsius (OR = 1.11 per ^{o}C, 95%CI [1.01-1.23], P<0.05).

Discussion. Interaction between antibiotics and *Af* acquisition may occur at an early stage of life in children with CF. Environmental factors, specifically temperature of living area at birth independently influence *Af* growth in the lung of young children with CF.

Amin et al (2010) Chest 137:171-6.

Wainwright et al (2011) Journal of the American Medical Association 306(2):163-71.

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Inhaled colistin is more efficacious than parenteral administration against multidrug-resistant *Pseudomonas aeruginosa* in a mouse lung infection model

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Introduction. Colistin is administered parenterally or by inhalation for the treatment of respiratory infections caused by multidrug-resistant (MDR) *P. aeruginosa*. However, limited pharmacokinetics (PK) and pharmacodynamics (PD) data are available for colistin following pulmonary delivery.
Aims. To examine the PK and PD of colistin administered intratracheally (I.T.) and intraperitoneally (I.P.) in a mouse lung infection model.
Methods. *P. aeruginosa* strains (ATCC 27853, PAO1, and PA QLD; MIC=1 µg/mL for all strains) were employed to establish lung infections in neutropenic Swiss mice. At 2 h post-infection, a single colistin dose (2.54 or 5.40 mg/kg) was administered via I.T. or I.P.. Saline-treated mice served as the growth control. Mice were humanely killed at 0, 1, 3, 6, 12, and 24 h post-treatment and viable counts (log_{10}CFU/lung) were conducted with lung homogenate (LH). Histopathological examination of the lung samples was undertaken at 4 and 24 h post treatment. Concentrations of colistin in bronchoalveolar lavage, LH and plasma were quantified using HPLC, and the volume of epithelial lining fluid (ELF) was measured using urea.
Results. I.T. administration (AUC_{ELF}=13,620 mg/L·min for 5.40 mg/kg colistin) attained much higher exposure in the ELF than with I.P. (colistin not detected). A maximum concentration in ELF of 22.7 mg/L was observed following I.T. administration. At 24 h inhaled colistin had no significant effect on lung epithelial cells for I.T. 2.54 mg/kg colistin, and only very minor damage was observed for I.T. 5.40 mg/kg. The *in vivo* efficacy after I.T. delivery was superior to by I.P. with all test strains. Even though bacterial regrowth was observed at 24 h, the efficacy of I.T. therapy was at least 1-2 log_{10}CFU/lung more killing than that by I.P. for ATCC 27853, PAO1 and PA QLD within 1 h post-treatment.
Discussion. Our study is the first to examine the PK and PD of inhaled colistin in mice. Multiple dosage regimens are being examined and will provide essential pharmacological information for optimising its use in the clinic.

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The transcriptional repressor ZBTB16 is induced by glucocorticoids in human bronchial epithelial cells

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Introduction. The transcriptional repressor ZBTB16 encoding the protein PLZF (promyelocytic leukaemia zinc finger) was first recognized for its role in the pathogenesis of a rare form of acute promyelocytic leukaemia. Its involvement in control of cell cycle and differentiation, as well as its upregulation by glucocorticoids (GCs) have been reported in different cell types. However, the potential function of ZBTB16 and response to GCs in airway epithelium remains to be established.
Aims. To investigate ZBTB16 levels in response to synthetic GCs, using different airway epithelial cell models.
Methods. BEAS-2B cells were exposed to Dexamethasone (Dex) (0.1nmol/L-1µmol/L) in the presence and absence of the GC receptor (GR) antagonist RU486 (1µmol/L). ZBTB16 mRNA and protein levels were assessed by qPCR and western blot, respectively. Similarly, the response to Methylprednisolone, Hydrocortisone, Budesonide and Fluticasone Propionate (+/-RU486) was assessed by qPCR. Additionally, the effects of different GCs on ZBTB16 mRNA levels were also investigated in primary human bronchial epithelial cells (HBECs).
Results. Dex strongly induced ZBTB16 gene and protein expression in BEAS-2B cells in concentration-dependent manner (203±64 fold vehicle, n=4, P<0.01). Similarly, other systemic and inhaled GCs also showed pronounced upregulation of ZBTB16 mRNA levels at 4 h time point (n=5-10). However, there was a lower maximum response to Hydrocortisone (78±29 fold vehicle, n=5). Addition of RU486 30 minutes before steroid treatment completely prevented the induction of both mRNA (97±1 % inhibition, n=4, P<0.0001) and protein levels (97±2 % inhibition, n=5, P<0.0001) by Dex. Additionally, strong upregulation of ZBTB16 with Dex/Budesonide was also confirmed in HBECs.
Discussion. ZBTB16 is greatly upregulated by GCs in bronchial epithelial cells. This GC-upregulation seems to be even higher compared to the cell lines explored by others (e.g. endometrial stromal cells), and it is mediated by GR. Our study provides a foundation for generation of new insights into the potential roles of ZBTB16 in mediating the effects of GCs in the airway epithelium.

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Poly I:C and Lipopolysaccharides (LPS) impair glucocorticoid sensitivity in human bronchial epithelial cells

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Introduction. Exacerbations represent the major burden of asthma that lead to hospitalization and mortality. Inhaled glucocorticoids reduce the frequency of symptoms, but not viral exacerbations. Among the respiratory viruses implicated in asthma exacerbations is respiratory syncytial virus (RSV), which impairs glucocorticoid sensitivity in the human airway epithelial cell line, BEAS-2B. However, the molecular mechanism is not understood. We hypothesize that resistance may occur through epithelium activation by pathogen-associated molecular patterns (PAMPs) with subsequent release of cytokines, including transforming growth factor-β (TGF-β), a potent inducer of glucocorticoid resistance (Salem et al, 2013 and Keenan et al, 2014).
Aims. To examine the impact of the activation of different TLRs involved in RSV sensing on Glucocorticoid responsiveness in bronchial epithelial cells.
Methods. BEAS-2B cells were transiently transfected with a glucocorticoid response element (GRE)-controlled secretory alkaline phosphatase (SEAP) reporter construct to assess the impact of the TLR3 ligand, Poly I:C, the TLR4 ligand, LPS; and the TLR2 ligand, Lipoteichoic acid (LTA) on GRE activity. Expression of GC responsive genes and plasminogen activator inhibitor-1 (PAI-1), a TGF-β-responsive gene, were assessed by RT-qPCR.
Results. Both poly I:C and LPS induced a marked suppression of the GRE activation mediated by dexamethasone (30nM) in a dose dependent manner. However, LTA has no detectable effect on GRE activation. Moreover, both poly I:C and LPS impaired the expression of GC-inducible genes including, GILZ and CDKN1C, while upregulating PAI-l mRNA expression, suggesting that these PAMPs initiated expression of endogenous TGF- β.
Discussion. Activation of TLR3 and TLR4 but not TLR2 has detrimental effects on glucocorticoid signalling in bronchial epithelium. This effect may be partly mediated by TGF-β.

Salem S et al. (2012) Br J Pharmacol 166: 2036–2048.
Keenan C.R. et al. (2014) Respir Res 15:55.

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Programming mitochondria with CUTGAPS, the ancient, intermediate-level language of innate immunity.

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Introduction. Living cells must simultaneously perform myriad biochemical computations to survive in changing environments. They achieve this with remarkable speed and energy efficiency using multitudes of temporally and spatially separated non-linear subunits wirelessly connected into complex, dynamic networks that can process and integrate exogenous and endogenous signals to produce all types of output. Outputs range from immediate on/off (digital) responses, to graded (analogue) transitions and oscillations. Such clear similarity to computing networks has inspired the field of synthetic biology, which aims to design electronic models that mimic complex biological systems and *vice versa.* Highly Organized Tolerance (HOT), a conceptual framework for studying behavior of such complex networks, defines their essential properties as self-organization, robustness and meta-stability. HOT networks are robust to "expected" fluctuations in input yet fragile when challenged by signals of frequency or amplitude outside their normal operating range. Responses overload can vary from fast, highly regulated power-up or power-down to rapid switch-off. In eukaryotic cells, mitochondria are being increasingly recognized as the organelles that orchestrate these processes. Initially, stressful stimuli trigger diffusion-limited reactions that modify existing circuitry, affecting the activities of transcription factors. Subsequent responses may ultimately entail changes in the expression of hundreds of genes, but a critical few can re-organize an entire HOT network.
Aims and Methods. Interactions that initiate mitochondrial stress responses, in particular those associated with innate immunity, were identified using information extracted from a variety of resources publicly available *via* the Internet. Metabolic fluxes were mapped and networks modelled using the methods of synthetic biology.
Results and Conclusions. Cytokine/stress-induced changes in the rates and directions of flux through rate-limiting pathways that govern mitochondrial respiration-coupled nucleotide synthesis can rapidly "program" subsequent global responses. CTP, UMP, TTP, tryptophan, glutamine, guanine and ADP occupy the most important nodes, which are wirelessly connected by phosphate and superoxide ("CUTGAPS"). We hope and expect that this framework will facilitate further investigation of network behavior under physiological and pathological conditions.

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A traditional anti-inflammatory from Tasmania: Mutton bird oil

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The mutton bird '*Puffinus tenuirostris*' feeds in the Southern Ocean but nests on the Bass Strait Islands and shores of southern New Zealand. The unprocessed preventricular (stomach) red oil from the young chicks (MBO), aka Yolla oil, is used by local Aboriginals and Maoris as a nutritional supplement and medicinal aid (1). The stomach oil contains wax esters (≥60%) in contrast to the triglyceride body fat (2).

Methods: MBO was obtained from the Aboriginal Centre, Hobart; Ocean Omegas, Lady Barron, Flinders Is and harvesters on Stewart Island NZ. Anti-inflammatory activity was assessed in rats developing experimental polyarthritis initiated by caudal injection of either heat-sterilised *Mycobact. tuberculosis* (M.tb.) dispersed in squalane ($C_{30}H_{62}$) or by bovine type-II cartilage collagen (C-II) dispersed in Freund's incomplete adjuvant (FIA). Neat MBO was administered orally for 14 days, or transdermally for four days rubbed into the shaved dorsal skin, admixed with ≤20% v/v isopropanol. Oils were analysed for fatty acid composition and astaxanthin, an antoxidant dioxo-carotenoid-diol.

Results: (i) Rubbed into the skin MBO suppressed arthritis development; proving much more potent (v/v) than other oils sourced from cold seawater animals (abalone, fish, seals, sharks, squid). (ii) Oral administration (0.5 ml/kg/day) for 14 days was also effective. (iii) MBO was not adjuvant-active ie pro-pathogenic unlike many oils, when substituted for the squalane or FIA used to sensitise the rats to M.tb. or C-II and provoke polyarthritis. (iv) Co-administered p.o. to fasted, inflamed rats with dispersed aspirin (150 mg/kg) or ibuprofen (50 mg/kg), MBO prevented NSAID gastrotoxicity two hrs later; many mineral, vegetable and marine oils (n=22) being ineffective. (v) Oil analyses indicated the presence of 18:4, 20:1 EPA, 22:1, DHA.

Conclusion: MBO contains natural anti-inflammatory, gastroprotectant and immunosuppressant agents. Some oil samples aged 20 years at ambient temperatures still retained these activities. The astaxanthin content (ca.0.2% w/w) from krill in the food chain, may contribute to these properties.

Turner A et al (2015) Progr. Drug Res. 70:155-178.
Woodward DR et al (1995) Austr J. Nutr. Diet. 52:87-91.

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Signal Transduction Pathways Activated by Insulin-like peptide 5 at Relaxin Family Peptide Receptor 4

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Introduction. Insulin-like peptide 5 (INSL5) is a two-chain, three-disulphide bonded peptide belonging to the insulin/relaxin superfamily expressed in the enteroendocrine L-cells of human and mouse colon (Grosse et al, 2014). It is the cognate ligand for relaxin family peptide receptor 4 (RXFP4) a GPCR mainly expressed in the colorectum and enteric nervous system. Currently little is known of the signal transduction pathways activated by RXFP4.

Aims. This study examined intracellular signalling pathways activated by INSL5 acting at the human RXFP4 receptor stably expressed in CHO cells.

Methods. Cell signalling was investigated using AlphaScreen® assays. Ca^{2+} flux was monitored in a Flexstation® using X-rhod-1AM. RXFP4 recruitment of $G\alpha_{i/o}$ protein isoforms were determined by rescue of ERK1/2 responses by PTX-insensitive $G\alpha_{i/o}$ C351I mutants ($mG\alpha_{i/o}$). Cell proliferation was studied by bromo-deoxyuridine (BrdU) cell proliferation ELISA. RXFP4 interactions with β-arrestins 1/2, G protein-coupled receptor kinase 2 (GRK2), KRas and Rab5a were examined using real-time BRET.

Results. Mouse INSL5 inhibited forskolin-stimulated cAMP accumulation and activated ERK1/2, p38MAPK, Akt-Ser473, Akt-Thr308 and S6 ribosomal protein (S6RP) more potently than human INSL5. No Ca^{2+} mobilisation was observed. PTX-abolished INSL5-stimulated ERK1/2 signal was rescued by $mG\alpha_{oA}$, $mG\alpha_{oB}$, $mG\alpha_{i2}$ and to a lesser extent by $mG\alpha_{i1}$ and $mG\alpha_{i3}$. RXFP4 interacted with GRK2, β-arrestins 1/2 and Rab5a but dissociated from KRas.

Discussion. INSL5 negatively regulates cAMP production and activates multiple signalling pathways important for diverse cellular functions including growth, differentiation and proliferation (ERK1/2, p38MAPK, Akt) and protein synthesis (S6RP). Following INSL5 activation, RXFP4 recruits a variety of $G\alpha_{i/o}$ and is regulated by β-arrestin 1/2 and GRK2 leading to receptor internalisation. Information on signalling pathways activated by INSL5 at RXFP4 is essential for understanding the biological roles of this novel gut hormone.

Grosse et al (2014) PNAS. 111:33-38

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Development of an MDA-MB-231 breast cancer cell line stably expressing a GCaMP6 genetically encoded calcium indicator

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Introduction. Alteration in the nature of the calcium signal in breast tumours may promote key cancer hallmarks, including enhanced cell migration and resistance to cell death. Typical methods to measure cytosolic Ca^{2+} using small molecule fluorescent dyes are often poorly suited to assessing these long-term cellular events. GCaMP6 genetically encoded calcium indicators overcome some of the barriers associated with fluorescent dyes, while maintaining comparable brightness and sensitivity. To date, no published studies have employed this calcium sensor in breast cancer cell lines.
Aims. To develop a stable cell line expressing the GCaMP6m genetically encoded calcium indicator in the basal-like breast cancer cell line MDA-MB-231.
Methods. MDA-MB-231 breast cancer cells were co-transfected with a GCaMP6m plasmid and a hygromycin resistance plasmid at a molar ratio of 10:1, using Lipofectamine 3000. Cell populations were expanded and tested for fluorescence using an automated epifluorescent microscope, ImageXpress Micro (Molecular Devices). Validation experiments were performed examining cytosolic free calcium ($[Ca^{2+}]_{CYT}$) in response to the purinergic receptor agonist ATP using the ImageXpress Micro and a Fluorescence Imaging Plate Reader (FLIPR). Expression of key markers was assessed to confirm that characterising features of MDA-MB-231 cells were retained.
Results. GCaMP6m expression was present in all imaged cells in the selected stable cell line. Increases in $[Ca^{2+}]_{CYT}$ induced by ATP stimulation increased fluorescence in a concentration dependent manner. Consistent with the expression profile for MDA-MB-231 cells, the developed cell line lacked mRNA expression of estrogen receptor alpha and the HER2 receptor, while expressing the epidermal growth factor receptor.
Discussion. The developed GCaMP6m MDA-MB-231 cell line is a promising model for examining Ca^{2+} dynamics in breast cancer cells. Application of this model in cell death and migration assays will help characterise the alterations in calcium signalling that occur during these cancer-related processes.

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Assessment of the effects of genistein and resveratrol in an MDA-MB-231 breast cancer cell line expressing a genetically-targeted calcium sensor.

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Introduction: Basal-like breast cancer is highly metastatic and has a poor prognosis due to a lack of effective treatments. Common phytochemicals such as genistein and resveratrol have been shown to inhibit proliferation of and induce apoptosis in many cancer cells including breast cancer cells. Further understanding of the effects of these compounds on cancer cell behaviour and consequent changes in gene expression and calcium (Ca^{2+}) signalling may provide an avenue for identifying agents and pathways that may enhance the efficacy of anti-neoplastic agents.
Aims: To characterise the effects of genistein and resveratrol on MDA-MB-231 basal-like breast cancer cells expressing a genetically-targeted Ca^{2+} sensor.
Methods: MDA-MB-231 cells expressing a genetically-targeted Ca^{2+} indicator were incubated with different concentrations of genistein (0 µM, 1 µM, 3 µM, 10 µM, 30 µM, 100 µM) and resveratrol (0 µM, 1 µM, 3 µM, 10 µM, 30 µM, 100 µM, 300 µM). Live cell imaging was performed over 72 hours using an automated live cell imaging analyser (JuLI Stage). RNA was isolated at 0, 24 and 48 hours using Qiagen RNeasy Plus Mini Kit.
Results: Live cell imaging revealed a dose-dependent reduction in cellular proliferation of MDA-MB-231 cells treated with genistein and resveratrol. No significant effects on cell proliferation were observed at concentrations below 30 µM for genistein and 10 µM for resveratrol. Genistein 100 µM treatment significantly altered cell morphology with treated cells showing an enlarged cytoplasm, extensive vesicle formation and stunted cell motility. Resveratrol 300 µM treatment resulted in condensed cell cytoplasm within 6 hours and apoptosis-like changes in cell morphology 30 hours after treatment. Resveratrol (30 to 100 µM) induced a spindle-like cell morphology and extensive interaction between cells via filamentous structures.
Discussion: Both genistein and resveratrol had a profound effect on MDA-MB-231 breast cancer cells. Our data also confirmed the anti-proliferative effects of genistein and resveratrol and demonstrate the utility of breast cancer cell lines expressing genetically-targeted Ca^{2+} sensors in breast cancer research.

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Morphological changes associated with cell death by Transient Receptor Potential Cation Channel V4 (TRPV4) activation in MDA-MB-468 breast cancer cells.

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Introduction. The overexpression of specific calcium permeable ion channels is a feature of particular breast cancer subtypes. Recent studies have suggested that elevated levels of TRPV4 are an important feature of some breast cancers, and that pharmacological activation of TRPV4 in MDA-MB-468 breast cancer cells produces cell death, although the type of cell death is unclear. Different mechanisms of cell death, including apoptosis, necrosis and oncosis are associated with significant changes in cellular morphology, thus providing a tool to define cell death pathways initiated by TRPV4 activation in MDA-MB-468 breast cancer cells.
Aim. To define the morphological changes associated with TRPV4 pharmacological activation and cell death in MDA-MB-468 breast cancer cells.
Methods. MDA-MB-468 cells were plated 96-well plates and 24 h after plating were treated with vehicle control (DMSO) or GSK1016790A (1 nM – 100 nM). Live cell imaging was performed for 48 h after GSK1016790A addition using a JuLi® stage automated imaging system. Experiments were performed on three independent passages.
Results. During the period of observation, distinct morphological changes were observed, which included pronounced cell membrane blebbing, failed mitosis, and swelling followed by loss of plasma membrane integrity (defined as 'popping'). Cell death associated with cell membrane blebbing and popping was induced by GSK1016790A, although popping was more pronounced at higher concentrations of GSK1016790A and often occurred more rapidly than membrane blebbing. The proportion of cells undergoing failed mitosis was not influenced by GSK1016790A treatment.
Discussion. These studies suggest that pharmacological activation of TRPV4 by GSK1016790A induces distinct cell death mechanisms in MDA-MB-468 breast cancer cells. The morphological changes observed suggest that apoptosis and oncosis occur with GSK1016790A.

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Regulation of copper transporters by platinum-based anticancer drug oxaliplatin and copper chelators in colorectal cancer cells

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Introduction. Copper transporters are important membrane-bound proteins that maintain mammalian copper homeostasis, including copper transporter 1 (CTR1), copper transporting P-type ATPase 7A (ATP7A) and 7B (ATP7B). Recent preclinical studies have provided indirect evidence implicating copper transporters in mediating the transport and cellular pharmacology of oxaliplatin, a first-line chemotherapy drug for advanced colorectal cancer (CRC). We have previously characterized the expression of copper transporters in CRC cells, however, whether these transporters can be manipulated by oxaliplatin and other chemicals such as copper chelators is poorly understood and knowing this can give us clearer understanding of the association of copper transporters with oxaliplatin cytotoxicity.
Aims. To characterize the regulatory role of oxaliplain and copper chelators in CTR1 expression; To study whether CTR1 is implicated in oxaliplatin cytotoxicity.
Methods. The temporal effect of oxaliplatin at clinically relevant concentrations and copper chelators BCS,TM and DP on the expression of copper transporters was measured by Western blotting and immunocytochemistry in CRC DLD-1 cells using transporter-specific primary antibodies. MTT assay was performed to study CTR1-mediated synergistic effect of BCS on oxaliplatin cytotoxicity.
Results. Our study demonstrated that CTR1 protein was up-regulated by oxaliplatin, BCS, TM and DP significantly. Prior incubation with BCS increased oxaliplatin cytotoxicity by ~6.7-9.4% in DLD-1 cancer cells. The distribution pattern of copper transporters CTR1, ATP7A and ATP7B was not altered apparently under these incubation conditions.
Discussion. Oxaliplatin, like the Cu chelator BCS, could up-regulate the expression of CTR1 in a time-dependent manner in CRC cells. BCS is able to enhance the cytotoxicity of oxaliplatin possibly through a transport mechanism with CTR1-mediated increased drug uptake by cancer cells. This work is supported by grants from Royal Hobart Hospital Research Foundation and Cancer Council Tasmania.

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ERK phosphorylation induced by adrenergic agonists in CHO cells expressing low levels of α_{1A}-adrenoceptors: differential effects of oxymetazoline and noradrenaline

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Introduction: ERK phosphorylation (p-ERK) induced by the activation of α_{1A}-adrenoceptors (α_{1A}-ARs) may be related to pathological conditions such as prostate cancer, benign prostatic hyperplasia and heart failure (Rapacciuolo et al., 2001; White et al., 2013). However, the signalling pathway(s) leading to p-ERK after α_{1A}-AR activation are not fully understood. Aims: This study investigates the mechanism of p-ERK activation in response to noradrenaline (NA) and oxymetazoline (Oxy), an imidazoline that is a selective agonist at α_{1A}-ARs, in CHO cells expressing low levels of α_{1A}-AR. Methods: p-ERK induced by NA or Oxy (5 min) was evaluated in an α-screen assay (Garbinson et al., 2012) in the absence or presence of α_1-AR antagonists, G-protein inhibitors and selective kinase inhibitors. Results: Both NA and Oxy caused p-ERK with Oxy being 10-fold more potent and ~45% more efficacious than NA. The p-ERK induced by NA (1 μM) but not Oxy (0.1 μM) was inhibited by phentolamine (1 μM) or prazosin (1 μM). The α_2-AR antagonist, idazoxan (1 μM) failed to block p-ERK induced by either agonist. PTX (1 ng/mL; $G_{i/o}$ inhibitor) partially blocked NA-induced p-ERK while UBO-QIC (0.1 μM; G_q-inhibitor) abolished it. However, Oxy-induced p-ERK was totally blocked by PTX while UBO-QIC was ineffective. The p-ERK induced by NA was blocked by UO126 (1 μM; MEK inhibitor) but unaffected by LY294002 (3 μM; PI3K inhibitor) or PP2 (10 μM; Src inhibitor). On the other hand, Oxy-induced p-ERK was blocked by UO126 and PP2. Discussion: NA-induced p-ERK was via α_{1A}-ARs and involved G_q-protein signaling while p-ERK induced by Oxy was insensitive to classical α_1-AR antagonists and was dependent on $G_{i/o}$-protein and Src phosphorylation. These results suggest that Oxy induced p-ERK involves another receptor/signalling pathway or binding to a site on the α_{1A}-AR distinct from that utilized by NA.

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Insights into allosteric structure-function relationships at the M_5 muscarinic acetylcholine receptor

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Introduction. The five muscarinic acetylcholine receptors (mAChRs M_1-M_5) are a family of class A G protein-coupled receptors that play a key role in regulating a wide range of physiological functions. Activation of the M_5 mAChR represents a potential path for the treatment of chronic cerebrovascular diseases, whereas inhibition of the M_5 mAChR may provide novel therapies for the treatment of addictive behavior. The design of subtype-selective mAChR selective ligands is challenging due to the conserved nature of the orthosteric pocket across mAChR subtypes. Recently, several highly selective M_5 mAChR ligands have been reported that achieve subtype-selectivity by targeting an allosteric site of the M_5 mAChR, thus enabling the closer study of the allosteric pharmacology of the receptor. Knowledge of the location, structure, and dynamics of the M_5 mAChR allosteric binding site can usher in a new era of structure-based drug design for this important protein family.
Aims. The objective of this project is to obtain a comprehensive understanding of the structural properties of the allosteric site of the M_5 mAChR and its ligands in order to address the need for novel and selective M_5 mAChR-targeting therapeutics.
Methods. Site-directed mutagenesis has been used to generate multiple M_5 mAChR mutants. Structure-function analyses, incorporating cell-based Gq protein-mediated signaling, radioligand binding, and analytical and molecular modelling, are being used in conjunction to study the effects of receptor mutation on novel positive and negative M_5 allosteric ligands and elucidate receptor regions contributing to the pharmacological properties of the modulators.
Results. We have assessed multiple mutations of residues within the M_5 mAChR allosteric pocket in the extracellular vestibule of the receptor and identified residues that are vital to the binding and/or functional effects of the modulators. The diversity of the allosteric probes involved has allowed better delineation of the putative binding site in the absence of a crystal structure.
Discussion. Although mutagenesis has revealed evidence of the location and structure-function properties of the M_5 mAChR allosteric site with respect to the first generation allosteric modulators, more information will be needed to facilitate rational structure-based drug discovery efforts. A renewed mutagenesis campaign is underway in conjunction with efforts to obtain a crystal structure of the M_5 mAChR in order to fully characterize the structural properties of the M_5 mAChR allosteric site(s).

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Calcium signalling and the acquisition of trastuzumab resistance in HER2-positive breast cancer cells

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Introduction: Human epidermal growth factor (HER2) overexpression is found in approximately 20-25% of all breast cancers and is linked to poor disease prognosis and high reoccurrence rates. Trastuzumab (Herceptin®) is a monoclonal antibody targeting HER2 and is a molecular therapy for HER2 positive patients. However, not all patients respond to trastuzumab treatment at the beginning of therapy (intrinsic resistance) and most patients develop drug-resistance (acquired resistance) within one year. Intrinsic and acquired resistance are predicted to occur via different mechanisms, although the precise pathways are not fully understood. Calcium is a key modulator of cellular pathways that are associated with trastuzumab resistance.

Aims: To assess the expression profile of more than 40 proteins involved in calcium signalling, such as purinergic receptors, calcium channels, pumps, exchangers in trastuzumab sensitive SKBR3 breast cancer cells and trastuzumab resistant SKBR3 breast cancer cells.

Methods: Proliferation assays were used to assess sensitivity to trastuzumab in sensitive and resistant SKBR3 cell lines and real time RT-PCR was used to assess mRNA levels. Pharmacological inhibition and silencing of $Ca_V3.2$ was used to assess the potential role of $Ca_V3.2$ in trastuzumab resistance. $Ca_V3.2$ overexpression was used to define effects on mRNA markers associated with drug resistance.

Results: SKBR3 breast cancer cells were continuously cultured in the presence or the absence of trastuzumab to develop resistant and age-matched control cell lines. In three of four resistant cell lines expression of the voltage-gated calcium channel $Ca_V3.2$ was significantly higher, compared to control cell lines. However, inhibition and silencing of this ion channel did not reverse resistance. Overexpression of $Ca_V3.2$ did not change the levels of mRNA markers associated with drug resistance.

Discussion: Altered expression of specific calcium channels, such as $Ca_V3.2$ may be a feature of trastuzumab resistance. However, $Ca_V3.2$ does not appear to be a driver for the acquisition of trastuzumab resistance.

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Inhibition of incretin receptors for the prevention of hyperinsulinaemia in horses

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Introduction: The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are gastrointestinal hormones that contribute to hyperinsulinaemia by amplifying glucose-dependent insulin secretion from the pancreatic β-cells. Hyperinsulinaemia and insulin resistance, two components of metabolic syndrome in humans and horses, are associated with an increased risk of type II diabetes mellitus in humans. However, horses rarely get diabetes, but hyperinsulinaemia becomes chronic. Therefore, they are an excellent model for investigating pre-diabetic hyperinsulinaemia.

Aims: This study investigated the physiological consequences of incretin receptor binding in horses and whether insulin secretion can be reduced by incretin receptor inhibition.

Methods: The viability of pancreatic tissue and isolated islet was examined by stimulation with glucose and measurement of insulin secretion (ELISA). Pancreatic islets (isolated from horses for the first time) produced a higher insulin response than tissue and were therefore used. The effective concentration (EC_{50}) of GLP-1 and GIP was determined by examining the concentration-response relationship between each compound and insulin secretion. Incretin receptor agonists (EC_{50}) were inhibited by incretin receptor antagonists (exendin (9-39) and (pro3) GIP) to investigate whether the insulin secretion could be inhibited.

Results: GLP-1 and GIP caused concentration-dependent increases in insulin secretion, with a maximum response of 336 and 299 μIU/mg of protein, respectively, and an EC_{50} of $1x10^{-9}$ M for both. Exendin (9-39) and (pro3) GIP inhibited the insulin secretory effect of GLP-1 and GIP by 29% and 30%, respectively.

Discussion. These data support the potential use of incretin inhibitors for reducing hyperinsulinaemia and preventing metabolic syndrome.

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Aryl group modification in novel anti-cancer agents based on ω-3-17,18-epoxyeicosapentaenoic acid

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Introduction. Dietary and experimental studies have shown that ω-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) inhibit the development of certain cancers. The CYP-derived 17,18-epoxide of EPA (ω-3-17,18-epoxy-EPA) and its saturated analogues impair tumour cell growth and activate apoptosis (Cui *et al.*, 2012; Dyari *et al.,* 2014). We recently developed a stable mimic of ω-3-17,18-epoxy-EPA termed CTU that rapidly killed tumour cells *in vitro* and *in vivo* in mouse xenograft models.

Aims. In this study the structural requirements for tumour cell killing by synthetic CTU analogues were evaluated.

Methods. The nature of the aromatic system in CTU was modified to produce 12 new analogues for the evaluation of steric and electronic factors in anti-tumour activity. The viability of MDA-MB-231 breast cancer cells was assessed by ATP production, apoptosis by caspase-3 activity and cell cycle kinetics by flow cytometry.

Results. Three di-substituted CTU analogues that carried electron-withdrawing groups were active. NK14, NK24 and NK18 decreased ATP production to 50±4%, 60±15% and 81±3% of control (10 µM, 48 hr) and NK14 and NK24, but not NK18, also increased caspase-3 activity to 141±6% and 137±10% of control (10 µM, 48 hr); these changes were more pronounced at higher concentrations. NK14 in particular markedly increased the proportion of cells in sub-G1 phase (31±3% versus 4.4±0.4% in control; 10 µM, 48 hr), and decreased G_0/G_1 and G_2/M populations. In contrast, CTU analogues carrying bulky aromatic or heteroaromatic substituents or that contained weakly electron-withdrawing groups were inactive.

Discussion. Like CTU, NK14 decreased the viability of MDA-MB-231 cells by activating apoptosis, impairing energy metabolism and disrupting cell cycle progression. CTU and several other ω-3-17,18-epoxy-EPA mimics show promise as potential anti-tumor agents.

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

561

Drug Discovery Twist – Are Aminooxazolopyridine Compounds Carcinogenic?

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Introduction. Carcinogens are agents that cause DNA damage (genotoxicity) in cells. The presence of DNA damage is indicated by the phosphorylation of a specific histone protein known as ɣH2AX. If DNA damage is not repaired properly or the cells continue to proliferate, this DNA damage can lead to DNA mutations and cellular transformation. Aminooxazolopyridine compounds (AOPs) are attractive drug candidates are structurally similar to the known carcinogen 2-Amino-1-methyl-6-phenylimidizado (4,5-*b*)pyridine, (PhIP). We hypothesised that AOPs are also genotoxic.

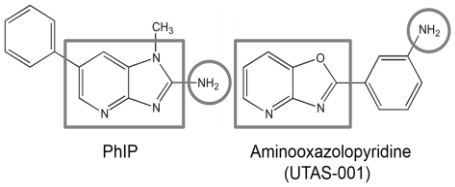

Figure 1: Similarity in chemical structures highlighted by the squares and circles

Aims. To evaluate the cytotoxicity and genotoxicity of the novel AOP UTAS-001.

Methods. Cytotoxicity and genotoxicity of UTAS-001 in HepG2 and HCT-116 cells were determined using colony formation, ɣH2AX immunostaining and soft agar invasion assays.

Results. UTAS-001 was non-cytotoxic in HepG2 and HCT116 cells but induced significant ɣH2AX foci in a dose dependent manner down to low nano-molar concentrations. Significant increases in numbers and colony volume in soft agar were observed in HepG2 cells exposed to UTAS-001.

Discussion. Our results indicate that UTAS-001 is exhibiting the hallmarks of a genotoxic and mutagenic compound. Despite a lack of overt cytotoxicity and normal DNA repair kinetics, the induced DNA damage incorporated errors into the DNA, which likely account for the increased transformation potential observed.

Zharkov DO (2008) CMLS 65:1544-1565
Ferrins L et al (2003) Eur. J of Med Chem 66:450-465

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Assessment of the effects of NH125 on HCC1569 human breast cancer cells.

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Introduction. The monoclonal antibody trastuzumab (Herceptin®) has revolutionised the treatment of women with HER2 positive breast cancers. However, the effectiveness of trastuzumab is often compromised through acquired or intrinsic resistance. HCC1569 is a human breast cancer cell line with intrinsic resistance to trastuzumab despite the maintained overexpression of HER2. Recent studies suggest that the reported atypical alpha-kinase inhibitor NH125 can increase the sensitivity of HER2-expressing breast cancer cells to trastuzumab in a model of acquired trastuzumab resistance.
Aims. To assess the effects of NH125 on the proliferation of HCC1569 breast cancer cells and their sensitivity to trastuzumab treatment.
Methods. Relative HCC1569 cell number was assessed using a [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) based assay (CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay, Promega) in the absence and presence of trastuzumab (0 – 10 μg/mL).
Results. HCC1569 breast cancer cell proliferation was sensitive to NH125 (IC50 ~ 1.97 μM). However, NH125 did not promote the sensitivity of HCC1569 breast cancer cells to trastuzumab.
Discussion. HCC1569 cell proliferation is sensitive to the reported atypical alpha-kinase inhibitor NH125. However, in contrast to an acquired trastuzumab resistant model, NH125 does not reverse trastuzumab resistance in this model of intrinsic resistance. These studies suggest different mechanisms may be responsible for acquired and intrinsic resistance to trastuzumab.

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Transient Receptor Potential Cation Channel V4 (TRPV4) expression in breast cancer subtypes and potential expression regulators in breast cancer cells.

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Introduction. Breast cancer is a heterogeneous disease that can be defined by histopathological subtype, hormone receptor expression or by molecular subtype. These subtypes have significantly different prognoses. Elevated TRPV4 is a feature of some breast cancers, however the association between TRPV4 and breast cancer subtype and regulators of its expression in breast cancer is not fully understood.
Aims. To define breast cancer subtypes associated with elevated TRPV4 and identify potential regulators of TRPV4 in breast cancer cells.
Methods. Bioinformatics and publically available RNA-Seq and miRNA-Seq data from breast cancer patient samples (The Cancer Genome Atlas) and breast cancer cell lines was used to define associations between TRPV4 and breast cancer molecular markers, transcription factors and microRNAs. TRPV4 DNA regulatory elements were assessed to define possible transcription factors important in altered TRPV4 expression in breast cancer cells and potential microRNA-mRNA interactions.
Results. Tumour samples were clustered according to topological overlap, which revealed 19 modules of co-expressed genes. The module that had the strongest correlation with TRPV4 was associated with molecular markers of the poor prognosis triple negative and basal molecular breast cancer subtypes. Putative DNA regulatory elements controlling TRPV4 transcription were identified using chromatin state maps and were used to identify a number of potential transcription factor regulators. In-silico analysis also identified potential microRNA-mRNA interactions that may effect post-transcriptional regulation of TRPV4 expression in breast cancer cells.
Discussion. These studies suggest that elevated TRPV4 may be a feature of breast cancer subtypes associated with poor prognosis and that a variety of mechanisms may be responsible for TRPV4 expression in breast cancer.

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"Glucose lowering effect of *Teucrium polium*; mechanistic insight"

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Introduction: *Teucrium polium* is a Mediterranean herb that has folk reputation for its medicinal uses, especially antidiabetic potential. A previous study conducted in our laboratory indicates that the aqueous extract of the aerial parts of this plant acutely lowers blood glucose levels in rats in a manner that is similar in efficacy to insulin. Although many of the chemical constituents of the plant have been previously identified, the bio-actives are not yet known and their individual or collective effects are yet to be determined.

Aims: We are currently investigating whether the extract 1) directly acts like insulin; promoting glucose uptake by insulin sensitive cells or, by ii) indirectly lowers blood glucose levels by inducing the release of insulin from pancreatic beta cells.

Methods: Aerial parts of the plant were extracted in alcohol and freeze dried. The effect of various doses of *Teucrium polium* (Tp) on insulin release was investigated using cultured rat BRIN BD11 (transformed islet beta cells). The level of glucose consumption was also studied by cultured C2C12 (transformed muscle cells) using fluorometer in the presence of Teucrium polium.

Results. The plant extract (250µg/ml) acutely promotes insulin release ($p<0.01$) from cultured rat BRIN BD11 (transformed islet beta cells). It stimulates insulin secretion from pancreatic beta cells in a dose-dependent manner. This plant extract (500 µg/ml) appears to increase glucose consumption significantly in differentiated C2C12 mice muscle cell culture.

Discussion: This data suggests that *Teucrium polium* acts as an insulin secretagogue to modulate glucose consumption. Preliminary results also recommends that *Teucrium polium* works as an insulin mimetic agent. This observation now requires further validation in other insulin sensitive cell lines and animal models.

Stefkov G et al (2011) Pharmaceutical Biology 49(9): 885–892
Salvucci M et al (2013) Plos one 8: e52611

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Pharm-Ecology: The mutualistic interactions of two disciplines brings research opportunities

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Pharmacology and ecology are both integrative disciplines, drawing on core biological and chemical knowledge. Both fields study chemical-biological interactions and their effects on individual organisms. Recent collaborative studies have demonstrated the value of using pharmacological approaches (PK and PD) to answer ecological questions. Examples come from studies of plant-herbivore interactions in which plants use defensive chemicals to protect themselves from herbivores, which in turn have evolved mechanisms to avoid toxicity. Many plant chemicals are used as pharmaceuticals, and the human metabolism and transport systems that eliminate drugs are a legacy from ancestral herbivores. Pharm-Ecology studies have provided fresh insights into foraging behaviour and translation of those impacts on populations and communities (Forbey et al, 2013). These studies have investigated the absorption, blood levels and elimination of different plant defence chemicals; differences between the chemical defences of individual plants; and the detoxification mechanisms of generalist herbivores and specialists which have co-evolved with their host plants. Mechanistic studies of the effects of plant defence chemicals on cells and self-medicating behaviour of herbivores (Moore et al, 2013) have the prospect of leading to the discovery of new pharmaceuticals that are bioactive (eg anti-helminthics, anti-microbials) and can overcome multidrug resistance. Pharmacology may also be enriched by the investigation of PK and PD of plant chemicals in co-evolved herbivores versus non-coevolved animals along with comparative biochemical studies of detoxification enzymes of these animals. These studies extend pharmacological knowledge beyond humans and a small number of inbred laboratory species.

Forbey JS et al (2013) J Chem Ecol 39: 465-480
Moore BD et al (2013) Science 340: 1041

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Endogenous allosteric modulators of G protein-coupled receptors – unappreciated mediators in health and disease?

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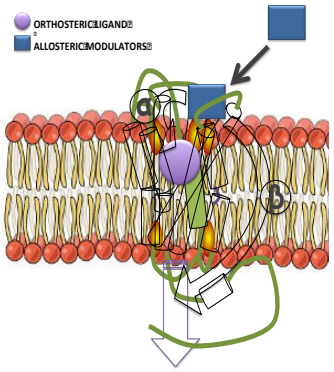
Introduction. Synthetic allosteric modulators of G protein-coupled receptors (GPCRs) now have validated pharmacological and therapeutical potential. However, the fact that most (if not all) GPCRs possess allosteric sites begs the question as to whether some of the sites of action of such molecules represent unappreciated 'orphan' allosteric sites for hitherto unidentified natural modulators (van der Westhuizen et al., 2015). One example is major basic protein (MBP), previously implicated in asthma symptomatology via antagonism of neuronal autoinhibitory M_2 muscarinic receptors (mAChRs) in the airways (van der Westhuizen et al., 2015).



Aims. To identify and characterise novel putative endogenous allosteric modulators at the M_2 mAChR.

Methods. Using CHO cells stably expressing the M_2 mAChR, we performed [^{3}H]NMS radioligand binding and cell-based functional studies to assess the allosteric effects of highly basic endogenous peptides including MBP, dynorphin A, myelin basic protein, apelin-13 and -36.

Results. All endogenously expressed peptides inhibited [^{3}H]NMS specific binding with potencies ~10μM.. Additionally, all peptides significantly altered [^{3}H]NMS dissociation from the orthosteric site, a hallmark of allostery. Moreover, several of these endogenous peptides also modulated the functional properties of the endogenous orthosteric agonist, ACh.

Discussion. These results highlight that M_2 mAChR functionality can be modulated by endogenous peptides, suggesting that the study of putative endogenous allosteric modulators of GPCRs may represent a new frontier for exploring (patho)physiological context-specific GPCR biology and drug discovery.

van der Westhuizen ET et al. (2015) J Pharm Exp Ther 353(2):246-60.

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Role of cyclic AMP in BRL37344 and isoprenaline mediated glucose uptake by β_2-adrenoceptors

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Introduction: Cyclic AMP (cAMP) is a key second messenger involved in β_2-AR mediated glucose uptake in skeletal muscle but in vivo causes adverse effects including altered vasoreactivity and hypertrophy. It has been suggested that BRL37344, a dual β_2/β_3-AR agonist originally developed for the treatment of obesity, increases skeletal muscle glucose uptake via β_2-ARs independently of cAMP, but the mechanism has not been rigorously examined (Nevzorova et al 2002). This study examines the signalling mechanisms activated by isoprenaline (Iso) and BRL to promote glucose uptake in CHOβ_2GLUT4myc cells and L6 skeletal muscle cells.

Methods: We measured glucose uptake with ^{3}H 2-deoxy-glucose, the production of cAMP was determined using a LANCE cAMP assay and Förster resonance energy transfer (FRET; pmEpac2, cytoEpac2), and Bioluminescence resonance energy transfer (BRET; β-arrestin, Kras) was performed to measure receptor internalization.

Results: In L6 cells, BRL increased glucose uptake with a similar potency and efficacy to Iso (pEC_{50} 7.41±0.2 and 7.45±0.3; Emax 168.1±4.6 and 186.8±7.9 respectively, n=6), but was a partial agonist when measuring global cAMP levels (Iso pEC_{50} 8.44±0.1 Emax 15.5±0.5pmol cAMP/well; BRL pEC_{50} 6.57±0.1 Emax 6.9±0.3pmol cAMP/well; n=4-6). However glucose uptake to both Iso and BRL was partialy inhibited by PKI, a competitive inhibitor of cAMP-dependent protein kinase activity (control glucose uptake 100%, +PKI 87±3%, Iso 151±11% + PKI 113±5%, BRL 135±5% + PKI 116±4%; n=5). FRET studies in CHOβ_2GLUT4myc cells showed that BRL and Iso activated the plasma membrane cAMP sensor pmEpac2 to the same degree but that BRL caused significantly less activation of the cytoplasmic cAMP sensor cytoEpac2. BRET studies showed that Iso, but not BRL, caused interaction between the β_2-AR and β-arrestins, movement of the receptor away from Kras, and desensitisation.

Discussion: Our results show that BRL has equivalent effects to Iso on glucose uptake, preferentially increases cAMP at the plasma membrane and does not produce desensitization or internalization of the receptor.

Nevzorova, J., et al. (2002) Br J Pharmacol, 137:9-18.

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New synthetic targets for the inhibition of dimethylarginine dimethylaminohydrolase (DDAH): A pre-synthetic computational study

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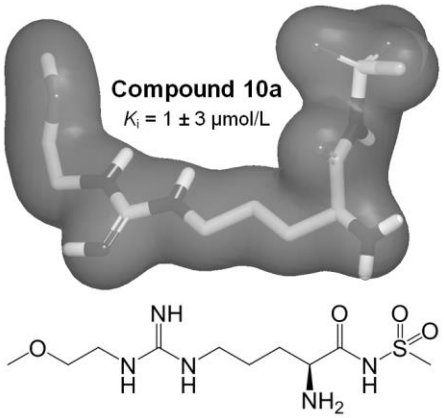
Introduction. DDAH metabolises asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS). Excessive NO production, associated with several disease states such as septic shock, neurodegeneration and cancer, can be prevented via the pharmacological mediation of DDAH. Arginine derivatives such as the experimental compound L-257 are effective DDAH inhibitors (Leiper et al 2011). Recent reports indicate that proton pump inhibitors (PPIs) may also inhibit DDAH (Ghebremariam et al 2013).

Aims. To identify new chemical entities (NCEs) with improved pharmacokinetic parameters that inhibit DDAH with high selectivity.

Methods. *In silico* overlay of NCEs against a database of compounds compiled of known DDAH inhibitors and related structures (OpenEye).

Results. A number of engineered NCEs display favourable Tanimoto scores relative to the prototypic DDAH inhibitor compound 10a synthesised by our groups (Figure; Tommasi et al 2015). The PPIs rank poorly relative to compound 10a, yet appear similar in chemical structure to several non-arginine DDAH inhibitors that exhibit poor inhibitory constants (K_i) *in vitro*.

Discussion. This study provides a rational approach in the design of new DDAH inhibitors.



Ghebremariam, YT et al (2013) Circulation 128:845-853
Leiper, J et al (2011) Nat Rev Drug Discovery 10:277-291
Tommasi, S et al (2015) Org Biomol Chem (submitted)

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Ethnicity information included in US Prescribing Information on initial registration: 2006 to 2015

Romina A Nand[1], Carol MY Lee[1], Sophie L Stocker[1], Michael J Dolton[1], Carwyn Davies[1], Christine S Clifton[1], Jonathan L Palmer[2] & Annette S Gross[1], GlaxoSmithKline R&D, Ethnopharmacology, Sydney[1], NSW, Australia, Stockley Park[2], Middlesex, UK.

Introduction. Ethnicity can contribute to variability in drug response and inter-ethnic differences in drug doses can result. The initial Prescribing Information (PI) text approved by the US Food & Drug Administration (FDA) has been surveyed to evaluate the information related to ethnicity obtained during global drug development programmes. This survey builds upon the results initially reported in 2011 (Lee et al, ASCEPT 2011 Abstract #42).

Aim. To identify ethnicity/race information reported in the PI for small molecule drugs (SMD) at the time of initial approval by the US FDA.

Methods. PIs for SMDs approved by the US FDA from 01 Jan 2006 to 30 June 2015 were obtained from Drugs@ FDA. All information included on race/ethnicity and pharmacokinetics (PK), efficacy and safety was recorded. A 4-category rating scale (no ethnicity information, no ethnic difference, ethnic difference, not studied/inadequate studies) was applied to record the race/ethnicity information reported.

Results. During this time 196 SMDs were approved. In most cases, there was either no mention of race/ethnicity information in relation to PK 86(44%), efficacy 159(81%) or safety 175(89%) or it was stated that race/ethnicity was not studied/studies were inadequate (PK 12(6%), efficacy 2(1%), safety 4(2%)). No influence of ethnicity on PK, efficacy or safety was reported for 75(38%), 28(14%) and 15(8%) SMDs, respectively. Inter-ethnic differences in PK, efficacy or safety were reported for 23(12%), 7(4%) and 2(1%) SMDs, respectively. Ethnic differences in PK were reported for East Asians (15), Asians (3), Blacks (6), Hispanics (3) and South Asians (1) relative to Caucasians/Overall population. Of the 7 SMDs with an inter-ethnic difference in efficacy, 6 related to Blacks (aliskiren, azilsartan, boceprevir, crofelemer, nebivolol, telaprevir) and 1 to East Asians (eltrombopag) relative to Caucasians. Inter-ethnic differences in safety were reported for simepravir (East Asians) and afatinib (Asians). An ethnicity-based dose regimen was recommended for only 1 SMD, eltrombopag (East Asians).

Discussion. For most SMDs, drug development programmes provided limited information on potential inter-ethnic differences in drug response. Most of the ethnicity information was available for PK rather than efficacy or safety.

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Allosteric modulators of metabotropic glutamate receptor subtype 5 show biased agonism and modulation.

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Introduction. Altered glutamatergic neurotransmission has been implicated in schizophrenia and depression, with the metabotropic glutamate receptor subtype 5 ($mGlu_5$) emerging as a promising therapeutic target in this regard. Allosteric $mGlu_5$ modulators offer the advantage of spatial and temporal fine-tuning of endogenous agonist activity, increased selectivity and reduced adverse effects. However, current $mGlu_5$ modulators have been classified based almost exclusively on their effects in modulating $mGlu_5$–mediated intracellular calcium responses ,but this likely represents an underappreciation of the full scope of drug action given the known pleiotropic coupling of this receptor. It is thus possible that allosteric compounds may differentially modulate signalling pathways arising from this receptor, a phenomenon referred to as 'biased' agonism and modulation.

Methods. Intracellular calcium mobilisation (iCa^{2+}) and inositol phosphate (IP_1) accumulation were assessed in stably transfected $mGlu_5$ HEK293A cells and mouse embryonic cortical neurons. Allosteric interactions with glutamate were operationally quantified to delineate affinity, cooperativity (β) and relative agonist efficacy.

Results. In recombinant cells, glutamate was more efficacious in iCa^{2+} vs IP_1, whereas the opposite was true for allosteric agonists. VU0360172 was a biased modulator, potentiating glutamate-iCa^{2+} ($\log\beta=0.83\pm0.12$) and inhibiting glutamate-mediated IP_1 ($\log\beta=-0.12\pm0.04$*; $p<0.05$, one-way ANOVA). Both CDPPB and VU0424465 were allosteric agonists for IP_1 and iCa^{2+}, however, CDPPB displayed a trend toward greater efficacy in the absence of extracellular calcium. These profiles were largely retained in primary cortical neurons, with the allosteric ligands displaying agonism of IP_1 accumulation, and VU29 and VU0424465 showing greater potency ($pEC_{50}=6.09\pm0.17$ and 7.70 ± 0.21 respectively) relative to the reference orthosteric agonist DHPG ($pEC_{50}=5.27\pm0.04$). All modulators were biased toward IP_1 vs iCa^{2+} relative to DHPG.

Discussion. Our results highlight that for $mGlu_5$, biased agonism/modulation is operative between pathways traditionally considered to be sequentially linked. Ultimately, such findings can lay the groundwork for rational drug design of pathway-targeted compounds in the treatment of refractory CNS disorders.

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Compounds Australia – Bringing Biologists and Chemists Together

Moana Simpson and Sally-Ann Poulsen, Compounds Australia, Eskitis Institute for Drug Discovery, Griffith University, Nathan, Queensland 4111, Australia

Compounds Australia is a national collaboration engine enabling drug discovery research projects across universities, research institutes and biotech industry within Australia. Compounds Australia actively sources small molecules, including synthetic compounds, pure natural products and natural product extracts, from the Australian chemistry research community and consolidates them into a central compound management facility. Compounds Australia then facilitates academic and not-for-profit members to access this unique small molecule collection for screening.

The Compounds Australia operating model is simple and allows its members to lodge and access compounds, without any claim on intellectual property. This presentation will highlight how to interact with Compounds Australia and how this can help your research to reach further.

[1] Simpson, M. and Poulsen, S.-A. An Overview of Australia's Compound Management Facility: The Queensland Compound Library. *ACS Chem. Biol.*, 2014, 9 (1), pp 28–33.

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Design and synthesis of arginine analogues incorporating carboxylate bioisosteres as DDAH-1 inhibitors.

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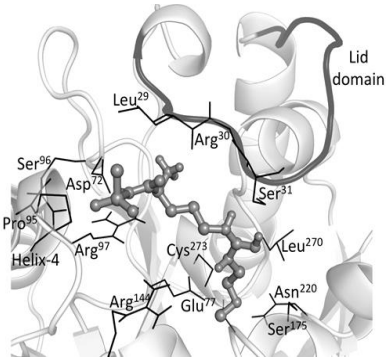
Introduction. Excessive synthesis of nitric oxide (NO), a pleiotropic messenger in mammals, plays a key role in several pathological conditions. The enzyme dimethylarginine dimethylaminohydrolase-1 (DDAH-1) metabolizes asymmetric dimethylarginine and monomethyl arginine, endogenous inhibitors of NO synthase. DDAH-1 inhibition is a promising strategy to limit excessive NO synthesis without affecting its homeostatic role. (Leiper and Nandi 2011)



Aims. To synthesize and characterize the kinetic parameters, IC_{50} and K_i, of novel human DDAH-1 inhibitors,.

Methods. Recombinant DDAH-1 was expressed in HEK293T cells. *In vitro* pharmacokinetic parameters of DDAH-1 inhibitors were derived by detecting L-citrulline (CIT) formation via UPLC-MS. Investigations of the putative mechanism of DDAH-1 inhibition by the most potent inhibitor were undertaken by molecular dynamics simulations.

Results. Three carboxylate bioisosteres (compounds 10a, 14a and 14b) demonstrated strong DDAH-1 inhibitory activity, acting as reversible competitive inhibitors. Compound 10a showed a 13-fold greater inhibitory activity relative to the known DDAH-1 inhibitor L-257. Molecular dynamics simulations using compound 10a suggest the presence of several H-bonds and stabilizing polar interactions with key amino acids within the DDAH-1 active site (Fig-1), thus providing a mechanistic basis for the kinetic data.

Discussion. Three novel arginine analogues incorporating carboxylate bioisosteres demonstrated significant human DDAH-1 inhibitory activity, with potential for further development.

Leiper J and Nandi M (2011) Nature Reviews Drug Discovery. 10:277-291.

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Consequences of Transient Receptor Potential Cation Channel V4 (TRPV4) activation in MDA-MB-468 breast cancer cells

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Introduction. The calcium permeable ion channel TRPV4 is over-expressed in basal breast tumours and the basal-like breast cancer cell line MDA-MB-468. Pharmacological activation of TRPV4 with GSK1016790A causes cell death and altered calcium homeostasis in MDA-MB-468 breast cancer cells, however the pathways activated during this cell death are still unclear.

Aims. To assess the pathways associated with GSK1016790A induced cell death in MDA-MB-468 breast cancer cells.

Methods. Cell viability assays were carried out using MTS assay (Promega). Morphological changes were assessed using the JuLI stage live cell imaging system (NanoEnTek). Western blot analysis of cell death related proteins were carried out following incubation with GSK1016790A and various pharmacological inhibitors of specific cell death pathways (24 h). ATP levels were measured using a Celltiter-Glo 2.0 luminescent assay (Promega) after GSK1016790A treatment (0-24 h).

Results. Cell viability of MDA-MB-468 cells decreased significantly with GSK1016790A at concentrations ranging from 3 nM to 10 µM. Western blot analysis of apoptotic related proteins caspase-3 and poly (ADP-ribose) polymerase-1 (PARP-1) showed no effect of GSK1016790A on caspase 3, but cleavage of PARP-1 with a 85kDa fragment observed with GSK1016790A at 10 nM. The cleavage of PARP-1 was not attenuated by the pan-caspase inhibitor Z-VAD-FMK, implicating a role for a caspase independent cell death pathway. ATP levels were reduced by GSK1016790A in a time and concentration dependent manner.

Discussion. The TRPV4 activator (GSK1016790A) induced cell death in MDA-MB-468 breast cancer cells may involve multiple mechanisms, one of which is associated with PARP-1 cleavage.

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Small molecule inhibitors on LOXL2 for fibrosis

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Introduction. Lysyl oxidases play a key role in fibrosis as they catalyse the formation of crosslinking aldehydes in collagen and elastin. Lysyl oxidases have five members, namely LOX, LOXL1, LOXL2, LOXL3 and LOXL4. LOXL2 activity and expression has been shown to be associated with fibrosis and cancer in particular a functional antibody has been shown to ameliorate fibrosis in various animal models (Nat Med 16:1009-1017, 2010). Interestingly, a functional LOX antibody also dampened fibrosis in animal models (Cancer Res 73:1721-1732, 2013), a possible indication of the lysyl oxidases can be complimentary in diseases.
Aims. To develop selective and efficacious small molecule inhibitors of LOXL2 for the treatment of fibrosis.
Methods. The CCl4-induced liver fibrosis model has been used to assess the efficacy of the small molecule LOXL2 inhibitors to reduce fibrosis.
Results. The inhibitors that have been developed in-house are mechanism-based inhibitors, and are highly selective for LOX or LOXL2. In a CCl4-induced fibrosis model, the compounds significantly reduced the area of fibrosis and α-smooth muscle actin.
Discussion. Small molecule selective inhibitors of LOXL2 can be developed and these compounds show high efficacy in inhibiting fibrosis in animal models. Pharmaxis LOX and LOXL2 inhibitors show great promise for the treatment of a number of fibrosis.

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A fat chance for improving brain cancer treatment?

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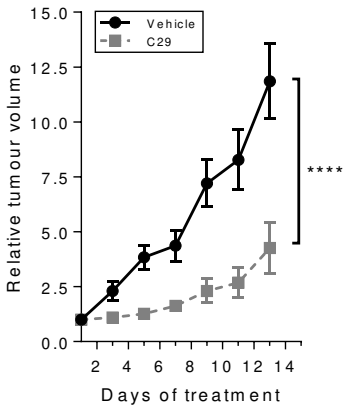
Introduction. The anti-cancer effects of omega-3 fatty acids have stimulated the development of synthetic fatty acid analogues for cancer research. Fatty acids are readily taken up by the brain and are important for normal brain development and function. In contrast, the majority of chemotherapeutics are inefficiently taken up by the brain and can fail to reach therapeutic concentrations. This is a major challenge for developing effective brain cancer treatments. In this study we investigated synthetic epoxy omega-3 fatty acid analogues, C29 and C15, as novel anti-cancer agents.
Aims. Investigate the potential for agents C29 and C15 in the treatment of brain cancers.
Methods. Ectopic and orthotopic tumour mouse models of brain cancer with the GL261 glioma were used to determine activity of these agents. LC-MSMS was used to determine the tissue distribution of the agents in mice.
Results. Treatment with C29 significantly delayed subcutaneous tumour growth ($P<0.0001$) and prolonged survival (log-rank, $P=0.05$) versus the vehicle treated group. Survival advantage was not observed in the orthotopic intracranial tumour mouse model. C29 exposure in the brain (AUC) was 2.5-fold lower than ectopic tumour exposure. Agent C15 did not inhibit tumour growth in either of the models, although the AUC of C15 in the brain was 5-fold higher than that in ectopic tumour tissue.
Discussion. C29 demonstrated promising activity against subcutaneous GL261 tumours in mice. Further understanding of the enhanced brain uptake of C15 may aid the development of analogues with improved activity against intracranial tumours.



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Rilmenidine increases mTOR-independent autophagy enhancing disease progression in a mouse model of Motoneuron Disease

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Introduction. Autophagy eliminates misfolded proteins and damaged organelles linked to neurodegeneration. Rilmenidine, a clinically approved clonidine analogue with preferential affinity for imadazoline receptors, induces autophagy allowing clearance of mutant (m) huntingtin (1). Misfolded superoxide dismutase 1 (SOD1) accumulates in motor neurons (MNs) in MN Disease. Enhancement of autophagy with rilmenidine is a potential therapeutic approach to enhance degradation of mSOD1 as imadazoline receptors are expressed by MNs in spinal cord.

Aims. To profile autophagy induced by rilmenidine and its impact upon disease progression in SOD1G93A mice.

Methods. SOD1G93A mice received rilmenidine (10 mg/kg, ip, 4x/week) from 60 days of age. Autophagic markers were assessed by immuno-blotting and cytochemistry performed in spinal cords of wild-type and SOD1G93A mice at presymptomatic (30 & 60 days), disease onset (90 days) and advanced (120 days) stages. Mice were examined for weight loss, motor function and survival. Spinal cords and brains were analysed for MN survival, glial cell activation, and mSOD1 level and aggregation. Molecular, cytochemical and functional techniques as described (2,3).

Results. Microtubule associated light chain 3 (LC-3) and sequestosome 1 (SQTM1, p62) levels were elevated in cords of SOD1G93A mice from disease onset ($P<0.05$). Rilmenidine upregulated LC3II and reduced voltage-dependent anion channel 1 levels in spinal cord ($P<0.05$), indicative of autophagy activation. Soluble mSOD1 levels were diminished ($P<0.01$) in spinal cords of rilmenidine treated mice consistent with autophagy. Disease onset was unaltered by rilmendine, survival was reduced ($P<0.05$) and disease progression accelerated in male SOD1G93A mice.

Discussion. Rilmenidine likely drives mTOR-independent autophagy (1) and enhances disease progression in mSOD1 mice, suggesting that autophagy activation contributes to pathology in this MN Disease model.

(1) Rose C et al (2010) Hum Mol Genet 19: 2144-2153
(2) Turner BJ et al (2014) Neurobiol Aging 35: 906–915
(3) Higgins GC et al (2011) Cell Mol Life Sci 68: 3725-3740

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The Inhibitory Effects of Anti-Oxidant Polyphenols on Beta Amyloid Fibrillisation and Neurotoxicity in PC12 Cells

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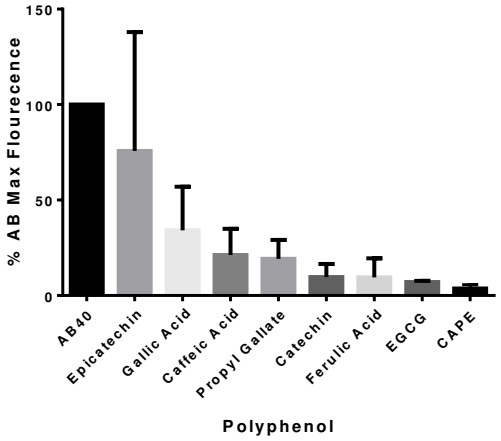
Introduction. Beta amyloid (Aβ) fibrillisation in Alzheimer's disease has been previously linked to neurotoxicity. Fibrillisation and neurotoxicity can be inhibited *in vitro* by a variety of anti-oxidant polyphenols, especially gallates like epigallocatechin gallate (EGCG). Recently, coffee consumption has been associated with decreased Alzheimer's risk. Caffeic acids which are found in coffee have been shown to inhibit transthyretin (TTR) fibrillisation, which is a similar amyloid protein to Aβ. Thus caffeic acids may inhibit Aβ fibrillisation.

Aims. To determine if caffeic acids cause inhibition of Aβ fibrillisation and reduce amyloid toxicity

Methods. Aβ fibrillisation was assessed with Thioflavin T (ThT) fluorescence assay in the presence of gallic and caffeic acid derivatives (100μM). Neurotoxicity was tested using an MTT assay on PC-12 cells, in the presence of 0.5 μM Aβ and 3, 10, 30, and 100 μM of polyphenol.



Results. Amyloid fibrillisation was reduced in the presence of all compounds tested, especially with EGCG (93%±0.6) and caffeic acid phenyl ester (CAPE; 96.7%±1.7). No compound reduced the toxicity of preformed fibrils on PC-12 cells. Paradoxically, CAPE, increased the toxicity of Aβ by 10-20%.

Discussion. Polyphenols significantly reduced fibrillisation of Aβ, and the caffeic acids were as potent as the gallic acids. Addition of these compounds to prefibrillised Aβ did not affect Aβ toxicity, suggesting that the polyphenols cannot reverse the fibrillisation process. In fact, CAPE is the greatest inhibitor of Aβ fibrillisation, but caused an increased toxicity in PC12 cells. The reason for this effect needs further investigation.

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Stroke-related cachexia and muscle wasting: role of the neuroendocrine hormone ghrelin

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Introduction. The outcome of stroke patients is not only defined by the extent of brain injury, but also by post-stroke complications such cachexia and sarcopenia. However, the mechanisms remain largely unknown. Ghrelin is a neuroendocrine hormone, which regulates energy balance largely through its actions on the hypothalamus and pituitary. Indeed, production of ghrelin (acylated form) by the stomach increases in response to negative energy balance, resulting in food intake, adiposity, and muscle mass gain. A recent study has reported that ghrelin levels are lower in stroke patients. Thus, suppression of ghrelin's actions may contribute to cachexia and sarcopenia.
Aims. Examine whether stroke is associated with suppression of the neuroendocrine ghrelin system.
Methods. Stroke was induced in mice by middle cerebral artery occlusion. At 6, 24, 72, and 7 d post-stroke, we measured: 1) serum levels of ghrelin (ELISA); 2) *ghrelin* and *GOAT* (enzyme responsible for ghrelin synthesis) mRNA in the stomach (RT-PCR); and 3) *GHSR1α* (ghrelin's receptor), *Npy,* and *Agrp* mRNA in the hypothalamus, and *GHSR1α* mRNA in the pituitary (RT-PCR).
Results. Despite profound and sustained weight loss (15±2%), and muscle wasting (20±3%), levels of ghrelin were reduced by >50% over the 7 d period (e.g. 24 h: stroke, 596±102 vs. sham-op, 1307±127, pg/ml, n=6), which occurred in parallel with a ~50% reduction in *ghrelin* and *GOAT* mRNA in the stomach (n=8). In the hypothalamus and pituitary, expression of GHSR1a was reduced by at 6 and 24 h (n=6), but was unchanged at 72 h and 7 d (n=6). Under normal conditions ghrelin increases *Npy* and *Agrp* mRNA in hypothalamic neurons to promote food intake. However, our preliminary data thus far indicate that *Npy* and *Agrp* mRNA expression are decreased after stroke, and levels do not increase after ghrelin administration (n=4).
Discussion. Our data thus far indicate that the ghrelin system is suppressed both peripherally and centrally in mice after stroke, which we propose may contribute to stroke-related cachexia and muscle wasting. As such, we propose that restoring the actions of ghrelin after stroke may be an effective therapeutic approach.

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Establishment of a rat model of mechanical low back pain

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Introduction. Chronic low back pain (LBP), a common health problem in humans, is difficult to alleviate with clinically available analgesics. Discovery of novel pain-killers is hampered by poor knowledge of the pathobiology of this condition. Previously reported rat models of LBP are confounded by concurrent neuropathic pain, and so our research was designed to address this issue.
Aims. To establish an optimised rat model of mechanical low back pain that is not confounded by a concurrent neuropathic pain component.
Methods. Ethics approval was from The University of Queensland Animal Ethics Committee. Adult male Sprague-Dawley rats (200-225g) were anaesthetised with a 1:1 mixture of zoletil (50mg/kg ip) and xylazine (8mg/kg ip). Rats underwent a surgical procedure to induce 10 small punctures (each 2mm deep) in two adjacent lumbar intervertebral discs (IVDs) at L4/L5 and L5/L6. Sham rats underwent the same surgery but without IVD puncture. Pressure algometry thresholds (PATs) in the deep axial tissues at L4/5 were measured using a pressure algometer pre-surgery and at weekly intervals until study completion on day 49 post-surgery. Paw withdrawal thresholds (PWTs) were measured in the bilateral hindpaws using calibrated von Frey filaments prior to surgery and at weekly intervals until study completion on day 49 post-surgery.
Results. Mechanical hyperalgesia developed in a temporal manner in the deep axial tissues at L4/L5 in LBP-rats but not sham-rats. Importantly, PWTs remained unaltered for the 49-day study period.
Discussion. A novel rat model of LBP that is not confounded by a concurrent neuropathic component has been established, and it will be used in our future work to gain insight on the pathobiology of chronic mechanical LBP.
Andersson GB (1998) Acta Orthop Scand Suppl 69(1):28-31

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Mapping morphine-induced analgesic tolerance

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Introduction. Chronic pain is one of the most challenging-to-treat conditions today and morphine is considered the gold standard for pain relief. However, the manifestation of analgesic tolerance after repetitive administration of morphine hinders its analgesic benefits. The exact mechanism of tolerance to morphine is not clear and therefore mapping its progression is a vital process to help us understand how it manifests and how more effective pain management strategies can be developed for its clinical use.

Aims. To map the tolerance profile of morphine-induced analgesia in animals taking daily morphine and identify the role of progressive increased dosing in its manifestation.

Methods. Sprague Dawley rats (n= 26) were administered chronically with daily doses of subcutaneous morphine sulphate (MS; various regimens 2.5-15mg/kg, ≥15 days depending on the treatment group). Pain responses were measured by tail-flick and hot-plate analgesic assays every day 5 mins before and 15, 30, 60 and 120 minutes after administration. Analgesic tolerance was defined as a significant reduction in analgesia after MS administration. An increased dose of MS was given to all animals 2-3 days after tolerance was observed.

Results. Animals receiving 2.5mg/kg MS daily produced analgesic tolerance within 3 days, whereas animals receiving 10mg/kg MS daily produced tolerance in 6 days. Animals receiving escalating doses of 2.5-5-10-15mg/kg over a period of 21 days presented significantly less overall analgesia (as AUC) compared to animals with 5-15mg/kg dose regimen over a period of 15 days ($p<0.005$).

Discussion. Our data reveal that the starting dose of morphine and the increments of dosing required for reinstating analgesia after the development of tolerance, play a key role to the extent of tolerance developed, the speed of its progression and the overall analgesic profile produced. Our results challenge the current therapeutic approach "*start low, go slow*" which is followed in the clinic for chronic opioid use (see Australian Therapeutic Guidelines, Analgesic v6, 2012). We show here that this approach results in faster tolerance developed and less overall analgesia after chronic morphine dosing. Further studies are needed to optimize the balance between opioid dosing, side-effects and manifestation of tolerance in order to develop the most effective therapeutic strategy.

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Identification of novel splice variants of GABA transporters in the central nervous system

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Introduction. GABA transporters (GATs) play an important role in the reuptake of inhibitory neurotransmitter, GABA. Changes in the distribution of GATs and GABA uptake in the brain is potentially a contributor to development of seizures such as that typically observed in epilepsy (Pow DV et al, 2005). There are 3 main GATs, known as GAT-1, GAT-2, and GAT-3. Unlike the glutamate family of transporters where numerous splice variants have been identified, there is limited published data examining the potential for novel splice variants of the GATs.

Aims. To clone and sequence potential novel splice variants of GABA transporters.

Methods. Rat GAT cDNA were amplified with specifically designed primers targeting SLC6a1 (GAT-1) from 991bp, SLC6a13 (GAT-2), and SLC6a11 (GAT-3). Amplified products were then examined electrophoretically for splice variants that ran at different sizes.

Results. PCR amplified products for GAT-1 showed a band at approximately 822 bp and a second band that is approximately 400bp lower. GAT-2 showed two close but distinct bands, one at approximately 1800 bp and a second band that was 10-50 bp smaller. GAT-3 showed single band at approximately 1800 bp.

Discussion. Results showed no indication of splice variants for GAT-3. Commonly, splice variants have exon skips or insertions that can change the size and the function of the transporter. Cloning, sequencing and then functional analysis of the smaller GAT-1 band and the two GAT-2 bands are ongoing.

Pow DV, Sullivan RKP, Williams SM et al (2005) Cell Tissue Res 320(3):379–92.

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Morphine displays a distinct behaviour of antidepressant activity in the Learned Helplessness model compared to that of imipramine

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Introduction. Long-term exposure to stressful uncontrollable life events can precipitate depressive symptoms and contribute to the development of major depressive disorder (MDD). Current medications used in depression (such as imipramine) are drugs that increase the brain levels of serotonin and/or noradrenaline, exhibiting numerous side-effects. New targets and drugs with novel mechanisms of antidepressant action are urgently needed. A number of studies have looked at the potential antidepressant action of morphine in MDD animal models (such as Learned Helplessness; LH). However, its complex locomotion effects (drowsiness and euphoria) makes the study of its antidepressant activity a real challenge to study using standard monitoring equipment. Here, we used a fully-automated animal-behaviour monitoring system (Multi-Conditioning System) in a fully enclosed and insulated environment, in an effort to distinguish the antidepressant effects of morphine from its locomotion effects.
Aims. To compare the antidepressant activities of morphine and imipramine in the LH model.
Methods. Sprague-Dawley male rats (n=26) were subjected to LH conditioning for the induction of depressive symptoms by non-escapable minor electrical shocks. These were then divided in two treatment groups (n=10 for s.c. morphine sulphate 5mg/kg/day and n=10 for s.c. imipramine 50mg/kg/day; from 24h after induction) and one non-treated group (n=6). Appropriate internal controls were also used (n=14). All animals were subjected to shock-tests with available escape for 3 days, at 48 hours after induction. A number of monitored animal activities were used for behavioural assessment (e.g. escape failures, transfers, avoidance). Complete locomotion analysis was conducted prior to and after LH testing, using an Open Field arena. Monitoring & analysis was conducted using automated tracking software and infra-red cameras
Results & Discussion. Morphine showed a similar antidepressant activity to imipramine as characterised by the reduced escape failures in the LH paradigm. However, morphine exhibited an overall distinct antidepressant profile compared to imipramine that involved the manifestation of increased learning potential during the test-period and enhanced perception of stimuli as shown by the significantly higher avoidance scores compared to imipramine.

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Medications and memory: The two big "M"s. A clinic population profiling exercise

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Introduction. As the ageing population rapidly grows in proportion, so does their medical and medication use burden. Age-related decline in some aspects of memory is to be expected, however the literature suggests that certain medications, especially those targeting the central nervous system (CNS) may also be associated with impairments in memory.
Aims. The aim of this study is to determine baseline CNS drug exposure and its association with memory in a broad spectrum population group with self-reported memory issues.
Methods. Help-seeking individuals aged 44-89 years were recruited from the Healthy Brain Ageing Clinic, a specialist research clinic for older adults at the Brain and Mind Centre, Sydney, Australia. Patients self-reported medication use was collected in a clinic session with a neurologist/psychiatrist. At baseline, all participants completed a standardised battery of neuropsychological tests, including the Wechsler Memory Scale (WMS) and Rey Auditory Verbal Learning Test (RAVLT), two common validated assessments used to assess memory. Inclusion criteria for analysis encompassed subjects taking at least one medication and the availability of either WMS or RAVLT memory scores for the subject. Descriptive statistics were performed.
Results. A total of 712 individuals (mean age 67, SD=9.19, 53.8% female) were included in our analysis. The average cumulative illness index rating scale total score was 5.08 (SD=3.77). On preliminary analysis, 29.7% of participants were taking an antidepressant, 8.1% benzodiazepines and 6.1% antipsychotics. Average percentage retention score (calculated using trials 7 and 5) for the RAVLT was 62.89% (SD=32.18). Average percentage retention score for the WMS (calculated using logical memory 1 and logical memory 2 scores) was 73.28% (SD=25.704).
Discussion. This preliminary analysis suggests that middle aged to older CNS medication users self-report memory problems, though objective measurement of memory scores for this population indicate good cognitive function. Medication use and perception of memory decrements need to be examined closely in future research.

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Non-medical prescription and self-medication practices preceding to remedy in district Multan,Pakistan

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Introduction: In developing countries; non-medical prescription (NMP) and self-medication(SM) are common practices prior to visit a qualified medical doctor.
Aim: Evaluation of prevalence and causes of SM and NMP among patients of Multan, Pakistan
Methods: A questionnaire was used for randomized collection of data from 515 outdoor patients of 3 Basic health units11.6%, 3 Rural health centers 14.5%, 2 Tehsil hospitals15.53%, 1 District Hospital15.5%, 1 Teaching Hospital15.5% , 1 Charity Hospital 7.76 % and 10 private clinics19.4%. Data was analyzed by using SPSS and Graph Pad Prism.
Results: Overall, 59.02 % of participants(age range 20-50 years) practiced SM and NMP due to affordability 34.9 %, access to health facility 15.14 %, availability (24/7) of doctor 33.2 %, carelessness 2.7 %, faith 5.4 % and multiple reasons8.4%.These patients had fever 15.5%, digestive disorders 18.4%, sleep disorders 0.29%, malaria 0.5%, urinary tract infections 8.3%, respiratory tract infections 13%, skin problem 2.3%, joint pain 6.2%, and dental problems 4.8%. Among these 64.37% were illiterate and 35.63% were literate. Ratio of male was 76.6% and female 23.34%.Patients had hypertension 3.9%, diabetes 1.6%. While female have pregnancy 0.65% and breast feeding 0.98%.
Discussions: A large number of People in Multan practiced SM and NMP

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New Zealand community pharmacists' views on pharmacovigilance for natural health products

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Introduction. Natural health products (NHPs) are a popular healthcare choice. Typically, users believe NHPs are 'safe', but some can cause adverse drug reactions (ADRs). At present, identification of suspected ADRs associated with NHPs relies on spontaneous reporting schemes, but few reports are submitted. Additional methods of monitoring safety of NHPs are required; the Intensive Medicines Monitoring Programme (IMMP) model, used in NZ for prescription medicines, has been modified to enable monitoring safety of NHPs and is soon to be piloted.
Aims. To explore community pharmacists' awareness of and views on (a) the feasibility of a pharmacy-based pharmacovigilance model for NHPs and (b) the closure of the IMMP and the implications of this.
Methods. Qualitative telephone interviews (n=13) with a random sample of New Zealand community pharmacists. Interviews were transcribed verbatim; a general inductive approach was used to analyse the data.
Results. Participants had mixed views on the likelihood of customers reporting suspected NHPs ADRs to pharmacists or identifying NHP use as a possible cause of ADRs. Participants were unaware of current pharmacovigilance systems for monitoring NHP safety. Some thought NZ's spontaneous reporting scheme might accept NHP-associated reports, but none had reported suspected NHP-associated ADRs. Participants expressed positive views about a new pharmacy-based pharmacovigilance model for NHPs and expressed an interest in participating. Potential barriers to participation were time, customer access to internet and staff training; potential enablers included increased NHP knowledge, provision of a unique service and financial incentives. Pharmacists acknowledged that they do not routinely keep records of NHP sales. All participants had previously sent dispensing reports to the IMMP for monitored medicines, but not all were aware of the suspension of IMMP's activities. Participants believed the suspension would negatively impact drug safety, but would have little impact on pharmacy.
Discussion. Participants did not doubt pharmacists' role in NHP pharmacovigilance. Generally, participants agreed a proposed 'pharmacy-based' pharmacovigilance model for monitoring NHP safety would be feasible, but several practical barriers would need to be addressed. Not all participants were aware of the closure of the IMMP; nonetheless, participants believed that the closure would have a negative impact on drug safety.

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Antiretroviral adherence and treatment outcomes among adult Ethiopian patients

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Introduction. Developing appropriate strategies to sustain optimal medication adherence among the increasing number of HIV-positive patients taking antiretroviral therapy (ART) in sub-Saharan Africa is a major challenge.
Aims. The objective of this study was to determine patient, regimen, disease, patient-provider, and healthcare-related factors associated with adherence with ART over a one-year period, and assess the impact of adherence on treatment outcomes.
Methods. We performed a prospective, observational study among 246 patients who were initiated on ART in Ethiopia, from December 2012 through March 2013. All patients who attended 12 months' follow-up and had completed self-reported adherence data were included in this analysis.
Results. Of 172 patients who completed follow-up, 130 (75.6%) had ≥ 95 % self-reported (combined dose and time) adherence. In multivariate analysis, lower body mass (BMI) (OR 1.2; 95% CI 1.0, 1.4) and lower HIV symptoms and adverse reaction distress scores (OR 1.1; 95 % CI 1.0, 0.9) and the use of medication reminder devices (OR, 9.1; 95% CI 2.0, 41.6) were associated with higher adherence. CD4 count increase was significantly higher in adherent patients compared to non-adherent patients at 12 months (159 cells/µL [IQR, 72-324 cells/µL] vs. 132 cells/µL [IQR, 43-190 cells/µL]; p = 0.026).
Discussion. Our findings indicate that interventions aimed at improving adherence, and thereby treatment outcomes in patients initiated on ART, should promote the use of reminder devices, as well as monitor HIV symptoms and adverse reaction distress and nutritional status.

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Histologically confirmed myositis and statin exposure, a case-control study

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Introduction. Statins are widely prescribed for cardiovascular risk reduction. Muscular adverse effects including myalgia and rhabdomyolysis are well recognised. The relationship between statin use and specific histologically characterised non-acute myopathic illness is less certain.
Aims. To compare patients with histologically confirmed myositis with a control population, in relation to statin exposure.
Methods. Cases of myositis were identified from the South Australian Myositis Database and were histologically confirmed, aged > 40 years. Controls, matched 3:1 to cases, were identified from the North West Adelaide Health Study. Rates of statin exposure in case and control population were measured.
Results. There were 221 cases (89M/132F), of whom 68 were exposed to statins (20M/48F). Types of myositis were dermatomyositis (11%), polymyositis (38%), inclusion body myositis (29%), necrotising myositis (10%) and non-specific chronic inflammatory (7%). There were 662 controls, of which 143 were exposed to statins. Calculated OR of exposure to statins in patients with histologically confirmed myositis was 1.6 (95% CI 1.15-2.27). The majority of cases were not exposed to concomitant interacting medications (CYP3A4 inhibitors).
Discussion. Patients with histologically confirmed myositis were at increased odds of statin exposure compared with a control population. This warrants further investigation in view of increasing population exposure to statins.

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Evolving Australian utilisation patterns of immunosuppressants after transplantation, including comparison to Northern European countries

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Introduction. The increasing utilisation of and expenditure for immunosuppressant medications is a worldwide challenge as more people successfully live with transplanted organs.
Aims. To characterise utilisation and expenditure patterns for mycophenolate, tacrolimus, cyclosporin, sirolimus and everolimus in Australian transplant recipients between 2010-2014, including use of the newer formulations and compare these patterns to Norwegian, Danish, Swedish and the Netherlands use.
Methods. Australian utilisation and expenditure were captured through Pharmaceutical Benefits Scheme and Highly Specialised Drug databases. Norwegian, Danish, Swedish and Netherlands utilisation were retrieved from their respective healthcare databases. Utilisation was compared as defined daily dose per 1000 population per day (DDD/1000 population/day). Data on kidney transplant recipients, the predominant group prescribed these medicines were obtained from international transplant registries.
Results. From 2010-2014 Australian utilisation of mycophenolate, tacrolimus and everolimus increased 1.5-fold, 1.7-fold and 1.7-fold respectively. Conversely, cyclosporine utilisation decreased by 5% while sirolimus remained unchanged. Australian utilisation was significantly lower than Northern European countries (2013;Chi-squared $p<0.0001$ in all cases); however overall utilisation in Australia was increasing at a faster rate. Australian expenditure increased by AUD$10 million over the 5-years to AUD$95 million. The new formulation EC-MPS doubled over 5 years to 26% of mycophenolate used, and XR-Tac increased from 0 to 10% of tacrolimus used in this 5 year period.
Discussion. In line with changing evidence, use of calcineurin inhibitors in Australia has moved from cyclosporin to tacrolimus, similar to changes in Northern Europe. With an increased number of people successfully living with transplants, the observed growth predicted from the comparison Northern European data, and the switching to newer, more expensive formulations, this group of medicines can be expected to continue consuming an increasing share of Australian pharmaceutical expenditure into the future. Policies to increase transplantation rates need to include consideration of increased utilisation of these maintenance immunosuppressants.

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Lifestyle factors among statin users and non-users: Do statin users adhere to a healthy lifestyle?

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Introduction. Lifestyle modifications are the cornerstone of primary and secondary prevention of cardiovascular disease (CVD). For eligible patients, lifestyle and dietary advice typically precedes or accompanies the prescription of statins. However, evidence for adherence to this advice is sparse.
Aim. The aim of this study was to compare saturated fat intake, exercise level, alcohol consumption and smoking between statin users and non-users in Australia.
Methods. This was a cross-sectional analysis of 4,614 people aged ≥37 years participating in the national *Australian Diabetes, Obesity and Lifestyle (AusDiab) study* in 2011-2012. Statin use was self-reported using a self-administered questionnaire. Saturated fat and alcohol intake were measured via a food frequency questionnaire. Smoking status was based on questions on current and past tobacco smoking. Exercise was collected via self-report. Multinomial logistic regression was used to compute adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between statin use and each of the four lifestyle outcomes. All four models were adjusted for age, sex, number of general practitioner visits, body mass index, hypertension, diabetes and prior history of cardiovascular diseases.
Results. In total 1108 (24%) participants used a statin. There was no evidence that users and non-users of statins had different levels of smoking, alcohol consumption or exercise. However, statin users had lower saturated fat intake as a proportion of total energy intake than non-users of statins (OR=0.72 for highest quartile; 95% CI=0.55-0.94).
Discussion. There was no evidence that people who use statins have a different pattern of smoking, alcohol consumption or exercise than people who do not use statins, even after controlling for other cardiovascular risk factors. However, statin users consumed less saturated fat.

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Optimising or minimising client health outcomes: intended responses by Cambodian pharmacies to clients with prolonged cough and TB-related symptoms

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Introduction. Proactive investigation and timely referral of clients presenting at primary healthcare facilities with prolonged cough increases early detection of tuberculosis cases and reduces transmission in settings with high tuberculosis burden. A cohort of private sector Cambodian pharmacies voluntarily participated in a Public/Private Mix Tuberculosis Referral Program to initiate timely referral of clients with cough and symptoms suggestive of tuberculosis. Staff at Referral Program pharmacies were trained to identify, counsel and refer symptomatic clients to public sector clinics for diagnosis and care. Clinical actions initiated by these pharmacies may optimise or minimise positive outcomes for clients.
Aims. The objective of this study was to compare intended clinical actions between those Referral Program pharmacies who would refer and those who would not refer clients with prolonged cough and other symptoms suggestive of tuberculosis.
Methods. A random sample of 180 Referral Program pharmacies in Phnom Penh was selected to participate in an interview survey. Trained interviewers administered a hypothetical case scenario to pharmacy staff trained in tuberculosis referral. Participants provided 'yes'/'no' responses to five clinical actions presented in the scenario. Actions were not mutually exclusive. All interviews were conducted in the Khmer language. Data were tabulated and compared using chi-square tests.
Results. Overall 156 (92%) participants would have referred the client to public sector clinics for care. These participants were less likely to sell a cough medicine (42% vs. 100%, $p<0.001$) and less likely to sell an antibiotic (19% vs. 79%, $p<0.001$) than those who would not have referred. These would be positive results for the Referral Program. Nevertheless, 41 (24%) participants reported they would sell non-prescribed antibiotics during referral.
Discussion. These actions may delay diagnosis and increase out-of-pocket expenses for symptomatic clients. Ongoing training is needed to improve pharmacies' awareness of the implications of their clinical actions when referring clients with prolonged cough and symptoms suggestive of tuberculosis.

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Demographic, clinical and lifestyle factors associated with high intensity statin therapy in Australia

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Introduction. The association between demographic, clinical and lifestyle factors and statin intensity has not been explored in Australia.
Aims. To determine the demographic, clinical and lifestyle factors associated with high intensity statin therapy in an Australian cohort.
Methods. Cross-sectional analysis of 1108 statin users participating in the 2011-2012 wave of the Australian Diabetes, Obesity and Lifestyle (AusDiab) study were conducted. High and moderate intensity statin therapy were defined as the use of a statin that reduces low density lipoprotein cholesterol level by ≥50% and 30 to <50%, respectively. People on low intensity therapy (n=35) were excluded. Demographic, clinical and lifestyle factors included age, sex, smoking status, body mass index, physical activity, eGFR, total cholesterol to HDL ratio, prior cardiovascular events, diabetes, cardiovascular disease (CVD) risk score, CVD medicines (yes/no) and alcohol consumption. Logistic regression was used to compute adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association between these factors and high (vs. moderate) intensity statin therapy.
Results. The prevalence of high and moderate intensity statin therapy was 33% (n=341) and 67% (n=696), respectively. Overall, 45% of people with prior CVD events used high intensity statins. There were adjusted associations between insufficient physical activity (OR=1.548, 95% CI=1.086-2.205), a prior CVD event (OR=2.083, 95% CI=1.386-3.13) and risky drinking (OR=1.509, 95% CI=1.034-2.202) and high intensity statin therapy. Age ≥65 years (OR=0.61, 95% CI=0.426-0.873) was inversely associated with high intensity statin therapy.
Discussion. Despite a strong association between prior CVD events and high intensity statin therapy, less than half of people with a prior CVD event use high intensity statins. High intensity statin users are more likely to engage in risky drinking and exercise less than moderate intensity statin users.

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Why is polypharmacy increasing in aged care facilities? The views of Australian healthcare professionals

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Introduction. The prevalence of polypharmacy in residential aged care facilities (RACFs) is high and increasing. Although not necessarily inappropriate, polypharmacy has been associated with drug interactions, adverse drug events, geriatric syndromes and hospital admissions.
Aims. To identify and prioritise factors contributing to the increasing prevalence of polypharmacy in RACFs.
Methods. Seventeen healthcare professionals from metropolitan and regional Victoria and South Australia identified and prioritised factors using a modified nominal group technique.
Results. The top five factors ranked from most important to fifth most important were 'changes in resident mix', 'increasing numbers of prescribers and the reluctance of one prescriber to discontinue a medicine commenced by another prescriber', 'better adherence to clinical practice guidelines', 'increasing reliance on locums' and 'greater recognition and pharmacological management of pain'.
Discussion. Reasons for the increase in polypharmacy are multifactorial. Understanding the factors contributing to polypharmacy may help to guide future research and development of interventions to minimise and manage polypharmacy in RACFs.

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The association between polypharmacy and medication regimen complexity with quality of life in residents of aged care facilities

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Introduction. Polypharmacy and complex medication regimens are highly prevalent in residential aged care facilities (RACFs). There has been little research on medication-related factors associated with quality of life (QoL) in RACFs.
Aims. To investigate the association between polypharmacy and medication regimen complexity with QoL in RACF residents.
Methods. A cross-sectional study of 383 residents from six Australian RACFs was conducted. The primary exposures were polypharmacy (≥9 regular medications) and the Medication Regimen Complexity Index (MRCI). The outcome measure was staff informant rated quality of life assessed using the Quality of Life Alzheimer's disease (QoL-AD) scale. Covariates included age, sex, Charlson's Comorbidity Index (CCI), activities of daily living (ADL) and dementia severity. Logistic quantile regression was used to quantify the association between polypharmacy and QoL-AD (model 1) and MRCI and QoL-AD (model 2).
Results. The median age of the 383 residents was 88 years and 297 (78%) residents were female. In total, 243 (63%) residents were exposed to polypharmacy and the median MRCI (range) was 43.5 (4-113). After adjusting for the covariates, there was no evidence that polypharmacy was associated with QoL-AD scores (coefficient: -0.02; 95% CI: -0.165, 0.124, p=0.78). Similarly, there was no evidence for an adjusted association between MRCI and QoL-AD scores (coefficient: -0.0009, 95% CI: -0.005, 0.003, p=0.63).
Discussion. These findings suggest that polypharmacy and regimen complexity are not associated with the staff informant rated QoL. Further research is needed to investigate how specific medication classes may impact change in quality of life over time.

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Glucose monitoring in new users of second generation antipsychotics in older people: a population-based study

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Introduction. Consensus statements published world-wide have highlighted concerns of increased metabolic risks associated with second generation antipsychotics (SGAs) (Cohn and Sernyak, 2006).

Aims. The aim of the study was to evaluate glucose monitoring rates for new users of SGAs in older people.

Methods. The study was a population-based retrospective cohort of SGA new users (365 days without pre-exposure to antipsychotics) from 1 January, 2006 to 31 December 2012. Pharmaceutical collections data was used to identify older people dispensed SGAs. Individual patient data were linked to the National Minimum Dataset and Laboratory Claims database. Chi-square (χ^2) statistics were used to compare proportions of individuals monitored within specified time frames and after guideline introduction on monitoring blood glucose in SGA users in New Zealand.

Followed 25,603 new users (days)	One-off dispensings	SGAs first dispensings	Subtotal monitored	Percent (%)
Baseline (≤30)	3173	88	3261	20.05
31-60	1535	68	1603	9.86
61-90	1373	54	1427	8.78
91-180	2923	137	3060	18.82
181-270	1886	100	1986	12.21
271-365	1389	61	1450	8.92
> 1 year	3275	199	3474	21.36
Total people monitored	15554	707	16261	100

Results. Of 25,603 new users dispensed SGAs, 63.5 % received plasma glucose monitoring at least once during the study period. Of these, only 20.1 % were monitored at baseline, 38.7 % were monitored for plasma glucose within the first 90 days and proportion of patients monitored at baseline were independent (χ^2 = 6.1; P = 0.4) of pre- and post-recommendation. Plasma glucose monitoring within the first 180 days increased to more than half (57.5 %) of the SGA new users.

Discussion. Glucose monitoring was underutilised in new SGA users. Prescribers must be cautioned about the metabolic risks posed by SGAs and recommend glucose monitoring.

Cohn, TA and Sernyak, MJ (2006). Can J Psychiatry 51:492-501.

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A systematic review of interventions to deprescribe benzodiazepines and other hypnotics among older people.

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Introduction. Long-term use of benzodiazepines in older adults is associated with harms including falls, physical and cognitive impairment. However, there is limited evidence on which interventions are safe and beneficial in withdrawal of benzodiazepines in older patients.

Aims. To critically evaluate the effectiveness and outcomes of interventions used to reduce benzodiazepine and other hypnotic use among older people.

Methods. A search was conducted in PubMed, Embase, Informit, International Pharmaceutical Abstracts, Scopus, PsychINFO, Cochrane Central Register of Controlled Trials (CENTRAL), and CINAHL for studies conducted in older adults (≥65 years) published between January 1995 to July 2015 using relevant key words. Two authors independently reviewed all articles for eligibility and extracted the data.

Results. Seven studies of benzodiazepine withdrawal were identified. No studies were found which investigated withdrawal of other hypnotics. The benzodiazepine discontinuation rates ranged from 27% to 35% in studies which trialled patient education and tapering (n=2), whereas the combination of psychological support and tapering yielded an 80% discontinuation rate at six months in one study. Three studies trialled pharmacological substitution and one study trialled academic detailing, with successful discontinuation rates reported between 62-80%. Out of the seven studies, five measured clinical outcomes following benzodiazepine discontinuation. Most studies observed no difference in withdrawal symptoms or sleep quality, while one study reported an improvement in sleep quality in those who discontinued benzodiazepine versus those who continued.

Discussion. Current evidence shows that benzodiazepine withdrawal is feasible in the older population, but may vary according to type of intervention employed. However, as the benefits and sustainability of these interventions are unclear, further studies should be conducted to explore this.

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Warfarin bleeding risk schemes: a systematic review

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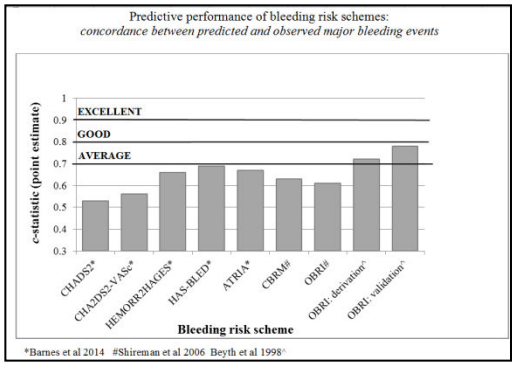

Introduction. Warfarin, an oral anticoagulant, is efficacious for thromboembolic prophylaxis when used in the appropriate therapeutic range. Deviation from the optimal dosage level can cause major bleeding. Suitable candidates may be identified through the use of bleeding risk prediction tools.

Aims. To conduct a systematic review of warfarin bleeding risk schemes.

Methods. An EMBASE, MEDLINE and Cinahl search was performed, using MeSH headings *haemorrhage, warfarin* and *risk.* Keywords were overlapped between databases and included *warfarin, bleeding, haemorrhage* and *risk prediction*. All primary research studies deriving or validating a bleeding risk scheme for long-term warfarin patients were critically evaluated. Data items relevant to developing a bleeding risk model were extracted.

Results. Five studies involving 38,418 patients explored nine risk prediction tools. All schemes were moderate at classifying high-risk patients and only considered biological factors. The different risk factors and methodology between the schemes did not enable an accurate comparison of predictive performance. Conversely, the overlapping factors, age, gender, duration of therapy and prior haemorrhage were compared.

Discussion. Age and gender showed an unclear association because of the demographic characteristics in the included studies. Duration of therapy did not appear to influence bleeding risk, however, highlighted the importance of periodic testing for long-term warfarin users. It remained unclear if gastrointestinal haemorrhage was a risk factor because major bleeding episodes are also common in other body sites. Current tools may inaccurately predict bleeding risk among warfarin users, especially if they are at high risk. Biological risk factors should be examined further and psychosocial factors should be incorporated. For patients initiated on warfarin it is essential to use a bleeding risk scheme as part of a holistic decision making process.

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Survival outcomes with lenalidomide for relapsed or refractory multiple myeloma at five large Queensland public hospitals.

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Introduction. In Australia, lenalidomide (LEN) is an immunomodulatory agent subsidised for use as a second line treatment in relapsed or refractory multiple myeloma (rrMM). The cost of LEN on the PBS was $64.3 million in 2014. The subsidy was based on two randomised controlled trials. They showed an increased survival in patients on LEN plus dexamethasone (DEX) compared to DEX (34.4 vs. 30.7 months, p=0.02). Patients enrolled in trials are often different to patients seen in usual clinical practice. We do not know the effectiveness of LEN in the 'real world' setting.

Aims. We aimed to determine survival outcomes for LEN in rrMM patients using data from five large Queensland public hospitals.

Methods. We extracted data from an electronic oncology prescribing system (Charm®) and pathology results for rrMM patients planned to start LEN between 01/09/2009 and 01/09/2013. We used descriptive statistical analyses, including Kaplan-Meier curves, to describe treatment and calculate overall survival (OS).

Results. There were 137 planned LEN-containing protocols, 136 patients who received at least one LEN dose and 2,234 LEN doses were ordered. The median age was 69 years and 55% were male. Two LEN-containing protocols were considered and the patients were split into two groups: 90% had LEN + DEX; 10% had LEN + DEX with cyclophosphamide based chemotherapy. The median LEN dose was 15mg (day 1-21) of a 28 day cycle. Median time on treatment was 279 days. Median overall survival from first dose of LEN was 45.4 months.

Discussion. The median survival was considerably longer in our study compared to the survival reported in the trials, despite our patients being older and using lower LEN doses. These data provide reassurance that outcomes for rrMM patients treated at Queensland public hospitals are at least as good as those observed in trials.

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Factors associated with use of falls risk increasing drugs in older people with cancer

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Introduction. Older people with cancer are at increased risk of falling. Falls risk increasing drugs (FRIDs), comprising psychotropics and medications that cause orthostatic hypotension, are a potentially modifiable risk factor for falls.
Aims. To determine the prevalence and factors associated with use of FRIDs in older people with cancer.
Methods: Patients aged ≥70 years presented to a hospital outpatient clinic between January 2009 and July 2010 were included in the study. Information on current medication use, falls in previous six months and frailty criteria were collected. Multinomial logistic regression was used to compute odds ratios (OR) and 95% confidence intervals (CIs) for factors associated with levels of FRIDs use (1, 2, ≥3).
Results: Overall, 76.1% (n=293) of 383 patients used FRIDs. This comprised psychotropics (31.2%, n=120) and medications causing orthostatic hypotension (69.9%, n=269). In total, 10.4% (n=40) of patients were frail and 24.0% (n=92) of patients reported falling in the previous six months. Being frail and history of falls were associated with use of psychotropics but not orthostatic hypotension drugs. Patients with a history of falls had increased odds of using psychotropics (≥3 psychotropics, OR 13.50, 95%CI 2.64-68.94). Likewise, frail patients had increased odds of using psychotropics (≥3 psychotropics, OR 27.78, 95%CI 6.06-127.42).
Discussion. Risk factors for falling were associated with the use of psychotropics. This suggests that clinicians either do not recognize or under-estimate the contribution of medications to falls in this high risk patient group. Further efforts are needed to rationalize medication regimens at the time of patients' first presentation to outpatient oncology services.

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Laxative use and constipation in community-dwelling adults in Australia

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Introduction. Constipation is a common condition in the community which is frequently self-treated with laxatives. The annual expenditure on laxatives in Australia is estimated to be $145 million but little is known about how laxatives are being used, factors associated with their use and their association with constipation.
Aims. To explore laxative use and constipation among community-dwelling adults.
Methods. A prospective survey of the adult population using a national representative sample was conducted online. The prevalence of constipation and laxative use was determined, as was the use of laxatives for treatment compared to prevention of constipation. Logistic regression was used to identify factors associated with laxative use.
Results. The survey of community-dwelling adults (n=2,024) revealed a high prevalence of both constipation and laxative use. Almost 60% of participants reported constipation in the last 12 months and almost 40% of participants used laxatives to either treat or prevent constipation during this period. Factors associated with laxative use included female gender, working, smoking, recent surgery and healthcare professional consultation. Other non-pharmacological management of constipation was also investigated. Only 22% of those reporting constipation sought the advice of a health care professional.
Discussion. Constipation is a significant health issue for many Australian adults. The results of this study will provide a better understanding of the issues involved in self-treatment and prevention of constipation, leading to better management of constipation in the community.

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Prevalence of cytochrome P450 (CYP) inhibitor-substrate interactions in patients on clopidogrel and omeprazole/esomeprazole and frequency of poor CYP2C19 metabolisers

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Introduction. There is conflicting evidence that the cardiovascular protective action of clopidogrel is reduced when co-prescribed with omeprazole and esomeptrazole (CYP 2C19 inhibitors). Both CYP2C19 and CYP3A4 are important in activating clopidogrel (Sangkuhl, 2010). Activation may be compromised by the co-prescription of CYP2C19 and/or CYP3A4 inhibitors, and also poor metabolism due to genetic variants of CYP2C19.

Aims. To determine the prevalence of patients on clopidogrel and omeprazole or esomeprazole, who are co-prescribed other CYP2C19 and CYP3A4 inhibitors and their genetic status.

Methods. Predicted gene-drug and inhibitor-substrate interactions were determined using self-reported medication data and genotyping using Affymetrix Kaiser Axiom arrays and imputed data from the 1000 Genomes and HapMap Phase II European reference panels for 2,642 patients aged over 55 in the Hunter cohort community study. A table of clinically relevant CYP inhibitors was used to determine the co-prescribed drugs of interest (Flockhart, 2007).

Results. Of 92 patients on clopidogrel, 9 (28%) were co-prescribed omeprazole and 8 (25%) esomeprazole. Genotype data was available for 12 of these 17 patients. Four (33%) were predicted to be poor CYP2C19 metabolisers of clopidogrel and 2/17 (12%) of patients were also on a strong CYP2C19 inhibitor (fluvoxamine).

Discussion. About 1/3 of this small patient sample were predicted 'poor metabolisers' and 12% were on a strong inhibitor. Studies assessing the efficacy of clopidogrel in the presence of proton pump inhibitors may need to consider genetic status and co-prescribed medications. Alternative medications such as prasugrel and rabeprazole may be better in patients who are poor metabolisers and/or on other relevant strong inhibitor drugs.

Flockhart DA (2007) http://www.medicine.iupui.edu/clinpharm/ddis/clinical-table/ Accessed 8/09/15

Sangkuhl K et al (2010) Pharmacogenet Genomics 20:463-5

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From personalized medicine to personalized justice: The promises of translational pharmacogenomics in the justice system. Pharmacogentic assessment of three homicide cases.

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Aims: To investigate three persons who committed homicide while taking prescribed antidepressants with suicidal intent using a medication history, reasons the drugs was were prescribed, blood levels, pharmacogenetic testing, combined with a recognition of adverse drug reactions that triggered a series of medication changes. All antidepressants are associated with suicide aggression, and homicide and tear are thousands of reports of both.

Results: Examination of the metabolic consequences of their diminished metabolism, genotypes for cytochrome P450, combined with the examination of drugs, their doses and co-prescribed inhibitors enabled the investigators to theorise elevated blood levels exceeding therapeutic which were was confirmed in two cases while in the third, blood was taken too late.

Discussion: This expertise is useful to courts and coroners some of whom remain reluctant to admit new science. It explains the multiple pharmacological and pharmacogentic causes of the adverse drug event, homicidal aggression. It recommends early examination of blood. Pharmacogentic science may exonerate perpetrators with an involuntary intoxication defence, and bring justice to victims of homicide and suicide and prevent such events occurring in the future.

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The effects of over supplementation of vitamins and minerals on oocyte and embryo quality, and subsequent pregnancy rates in sub-fertile patients undergoing IVF treatment in South Australia.

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Introduction. Multivitamins and other dietary supplements are commonly used by sub-fertile women before IVF treatment. However, what effect(s) excessive consumption of vitamins and minerals have on oocyte and embryo quality and pregnancy rates has not been explored. Ideally, a single multivitamin containing folate (Elevit™) or a single of folate supplement is recommended during the peri-conception period. It is unknown what effect consumption of additional multivitamins, high folate supplements and other vitamins and minerals have on female gametes and their treatment outcomes.
Aims. To determine if maternal consumption of Elevit™, folate, and additional vitamins and minerals alter oocyte quality, embryo development and pregnancy rates.
Methods. Data was obtained from IVF patient medical records between 2010-2015. Couples fitting strict selection criteria based on age, treatment type and other confounding factors were sorted into groups according to the number of medicines/supplements consumed. Females who used Elevit™, folate and excessive vitamin and minerals were compared and statistically analysed using Analysis of Variance (ANOVA).
Results. Taking one or many dietary supplements was beneficial compared to not taking any. Women who consumed high folate supplements had less mature oocytes ($P<0.05$) and delayed embryo development ($P<0.05$), however pregnancy rate (of transferred embryos) was unaffected. Pregnancy rate was reduced in women who consumed excessive vitamins and minerals ($P>0.05$).
Discussion. Results from this retrospective observational study suggests that high doses of folate may be detrimental to oocyte maturation and embryo development. Excessive dietary supplement intake may affect pregnancy outcome. More robust research is required to confirm these results.

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Caffeine adulteration in herbal weight loss preparations

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Introduction. The use of complementary and alternative medicines is becoming increasingly popular as it is thought that they are inherently safer compared to conventional pharmaceuticals. However, in recent years there has been evidence of the addition of pharmaceutical drugs, illegal substances and botanical materials which are not listed as ingredients, posing potential negative health effects. Although adulteration of complementary and alternative medicines is well documented in overseas literature, there is limited information regarding the products available in Australia. Caffeine is one of the most common pharmaceuticals in weight loss supplements due to its stimulant properties. Consumption of greater than 400mg/day of caffeine is potentially harmful, and if weight loss products do not list their caffeine content, this limit may be easily exceeded inadvertently.
Aims. To determine if there is accurate labelling of caffeine concentrations within a representative sample of weight loss products available for sale in Australia.
Methods. Herbal weight loss products were obtained from online vendors, chemists and health food stores around Adelaide. These products were screened using liquid chromatography coupled with mass spectrometry to identify components within the supplements.
Results. High levels of caffeine were detected in 5 out of 6 supplements which were not disclosed on the products labels. In some supplements other agents were present that could potentiate the stimulant effect of caffeine.
Discussion. High levels of caffeine consumption can cause potential health problems. The failure to specify the exact concentration of caffeine within herbal weight loss preparations can potentially lead to caffeine intoxication and subsequent medical problems.

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***In vitro* cytotoxicity induced by Pituri, the Aboriginal Australian smokeless tobacco**

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Introduction. The Aboriginal population of Central Australia use a range of *Nicotiana* species to make a smokeless tobacco product known as pituri. It is prepared by mixing dried leaves of wild tobacco plants with ash, usually from burned Acacia twigs, and macerated into a 'quid' that is chewed or stored in their mouth for prolonged absorption of nicotine. Although the main biological and physiological outcomes of tobacco consumption is attributed to its major alkaloid nicotine, however the smokeless tobacco extract contain large spectrum of other biologically active compounds that might also contribute to its adverse effects on health.

Aims. The objective of this study was to compare induced toxicity of pure nicotine and pituri extract at the same concentrations of nicotine.

Methods. An aqueous extract of pituri and Coresta reference smokeless tobacco (CRP2) were quantified for nicotine alkaloids using HPLC-UV. A range of concentrations of the pituri and CRP2 extracts and corresponding concentrations of nicotine standard were tested using a one-step MTT assay (MTS) to assay human lung epithelium cell (A549) survival and proliferation. Appropriate negative and positive cellular control and replication were used. Results. Survival of cells treated with aqueous extracts of smokeless tobacco products pituri and CRP2 was remarkably lower than the cells treated with nicotine standard.

Discussion. *Nicotiana* leaves used for preparing smokeless tobacco products such as pituri contain nicotine along with other alkaloids such as nornicotine, anatabine, and anabasine, which upon drying can give rise to carcinogenic chemicals called tobacco-specific N-nitrosamines. Therefore, higher inhibition and cytotoxic effects of pituri and CRP2 is not primarily due to their nicotine content and could be due to the presence of these nitrosamines and other compounds such as benzo[a]pyrene.

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Toxicological analysis of traditional Vietnamese herbal medicines for pharmaceuticals and residual Agent Orange compounds

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Introduction. Traditional herbal medicines have been used throughout Asia for many centuries, and remain popular today. However, recent studies show that herbal medicines are subject to adulteration (such as addition of synthetic pharmaceuticals to increase efficacy), and contamination (such as environmental exposure to herbicides). Adulteration and contamination is widespread in Traditional Chinese Medicines, yet it is unknown whether Traditional Vietnamese herbal medicines are affected.

Aims. To determine whether a sample of nine Traditional Vietnamese herbal medicines contain adulterants or contaminants, using toxicological analysis.

Methods. Nine Traditional Vietnamese herbal medicine samples were sourced from Ho Chi Minh City, Vietnam. Samples underwent basic and ethanol extraction. Extracts were analysed using gas-chromatography/electron-capture-detector, liquid-chromatography/quadrupole-time-of-flight, and gas-chromatography/nitrogen-phosphorous-detector/mass spectrometry. Results were compared against several compound libraries using Mass Hunter Software to determine a match. Specific standards were used to target residual compounds of Agent Orange (2,4 dichlorophenoxy acetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)).

Results. No peaks attributable to exogenous pharmaceuticals or herbicides were found in the nine herbal medicine samples.

Discussion. Maintaining high quality traditional medicine preparations is crucial to healthcare in Vietnam, since many people cannot access conventional medicines (either for geographical or financial reasons). While the current study was limited to a small sample size, each herbal medicine preparation contained a complex mixture of traditional plant ingredients. The strength of this study is the comprehensive qualitative screen using a variety of standardized analyses. While it is possible that the level of some pesticides/herbicides may be lowered by post-harvest processing (e.g. drying), none were detected in these traditional medicines, despite abundant spraying of defoliant Agent Orange on agricultural areas of Southern Vietnam during the Vietnam War (1962 – 1971).

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Dofetilide-induced hypoxia during rat embryonic development

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Introduction. Dofetilide is a class III anti-arrhythmic drug which blocks the cardiac repolarising current I_{Kr} (I_{Kr} = rapid component of the delayed rectifying potassium current). Previous studies have shown that dofetilide is teratogenic in rodents during the sensitive period of development – gestational day 9 (GD 10) to GD 14 – causing craniofacial defects at GD 11 and limb defects at GD 13 [1]. A previous study showed dofetilide treatment of pregnant rats at GD 13 was associated with bradycardia and hypoxia in the developing limbs (GD13) resulting in limb malformations (G20) [2].
Aims. This study was undertaken to determine if hypoxia is associated with dofetilide treatment in the rat embryo at GD 11.
Methods. Pregnant rats were treated with a teratogenic dose of dofetilide (single oral, 4 mg/kg) or water on GD 11. Dofetilide treatment was followed 2, 4, 8, 12, or 24 hours with iv dosing of an hypoxic marker, pimonidazole (60mg/kg). Embryos were collected and the intensity of pimonidazole adducts were analysed in the 1st brachial arch region (developing upper lip) of mid-saggital embryo sections.
Results. Dofetilide induced hypoxia in the region of the developing upper lip of the rat embryo at 2, 4 and 8 hours post-treatment, showing signs of recovery at 12 hours and return to near control levels at 24 hours.
Discussion. These results support the hypothesis that the teratogenic effect of dofetilide on craniofacial development is associated with pharmacologically induced embryonic hypoxia in the rat. Both in this study and in a previous study, the use of the hypoxia probe pimonidazole confirmed that *in vivo*, the embryos become hypoxic during the period of bradycardia [2]. Dofetilide has also been shown to induce bradycardia (~60% reduction in EHR) when added in culture to developing rat embryos on GD11 and 13 [3].

1. Webster WS et al., (1996). Teratology 53:168-173.
2. Ritchie HE et al., (2013) Birth Defects Res Part B, 98: 144-53.
3. Nilsson et al., (2013) Birth Defects Res Part B 98(5):416-27

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The *in vivo* and *in vitro* cardiovascular effects of Russell's viper venom

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Introduction: Snake venom is made up of a cocktail of proteins that are toxic to humans. Clinical and animal studies have demonstrated the hypotensive effects of some snake venoms, which can be life threatening.
Aim: To investigate and characterize the hypotensive effects of Sri Lankan Russell's Viper (*Daboia russelli*) venom (RVV).
Methods: Venom and eight size-exclusion chromatography (SEC) fractions were examined *in vivo* in anaesthetized (100 µg/kg ketamine/xylazine cocktail 10:1 ratio, i.p.) rats or *in vitro* in rat isolated small mesenteric arteries.
Results: RVV (100 µg/kg, i.v., n=3) caused cardiovascular collapse in anaesthetized rats. None of the individual SEC fractions caused hypotension *in vivo* (100 µg/kg, i.v., n=3) but when the fractions were recombined, hypotension was observed. RVV (1-1000 ng/ml, n=3) caused concentration-dependent relaxation *in vitro* (pEC_{50}~16ng/ml; R_{max}= 96.7±0.9%). This relaxation was markedly attenuated by high K^+ (R_{max}=12.8±3.6%), and by repetition of the concentration-response curve (R_{max}~19%, n=2). However, the relaxation was not significantly affected by nitric oxide synthase inhibitor, L-NAME or removal of the endothelium.
Discussion: *In vitro*, RVV causes endothelial-independent relaxation possibly involving potassium channels. *In vivo*, several venom components are synergistically causing this effect. Prior exposure to RVV prevented recurrence of the hypotensive effects. Further investigation of these toxins is required to elucidate the pathophysiology of hypotension following envenoming.

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Depressed effect of aging on contractile response of porcine distal ureter to 5-hydroxytryptamine via 5-HT2A receptor subtype

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Introduction. Aging has been reported to increase the risk of ureteral calculus development. This condition is frequently accompanied with ureteral colic which is understood to be caused by constriction of the ureteric tube, initiated by smooth muscle contractions (Canda et al, 2007).

Aims. To investigate the effects of age on 5-HT-mediated contractile responses of the porcine distal ureter and to pharmacologically characterise the subtype/s of 5-HT receptor mediating these responses in young and old animals.

Methods. Contractile responses of isolated smooth muscle to 5-HT were examined in distal ureteral tissues from old (56 weeks) and young (20 weeks) pigs, in the absence and presence of 5-HT2A receptor antagonist ketanserin (10nM, 30nM, 100nM), 5-HT2 and 5-HT1 antagonist methiothepin (10nM), 5-HT2C antagonist RS 102221 (30nM), 5-HT3 antagonist ondansetron (30nM), and 5-HT4 antagonist GR 113808 (100nM). Tissues developed spontaneous contractile activity and responses were expressed as AUC as a percentage of the maximal contraction.

Results. pEC50 of 5-HT in ureteral tissues from young and old pigs were similar. Ureteral strips from the younger animals produced a greater maximum contraction in comparison to the older animals ($p>0.0001$, $n=8$). Treatment of ureteral strips with ketanserin produced a rightward shift of 5-HT concentration-response curves in animals of both age groups. Using EC40 values for 5-HT in the presence of ketanserin, the affinity estimates (pKb) were 8.67 and 8.19 in younger and older tissues respectively. The Schild plots had slopes of 0.79 ± 0.07 and 1.25 ± 0.18 in younger and older animals which are both not significantly different from unity. 5-HT antagonists GR-113808, methiothepin, ondansetron and RS 102221 failed to cause any significant shift of 5-HT concentration-response curves.

Discussion. Affinity estimates for ketanserin were comparable to those reported in the literature (8.9) at 5-HT2A subtype (Bonhaus et al, 1997). Our findings indicate that the 5-HT-induced contractile response of the pig distal ureter is mediated by the 5-HT2A receptor subtype in both young and old animals.

Bonhaus et al (1997) Neuropharmacology 36:621-629
Canda AE et al (2007) Urol Int 56:23-33

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Effect of inflammatory cytokines on urothelial cell ATP release in vitro

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Introduction. Enhanced release of ATP has been demonstrated in several models of inflammatory bladder pathology. Symptoms associated with bladder inflammation, urgency, frequency and pain are thought to be mediated by purinergic signaling.

AIM. This study used a cell culture model to examine the effects of inflammatory mediators on release and breakdown of ATP.

Methods. UROtsa cells were pre-incubated with inflammatory cytokines (INF-ɣ,IL-1β,TNF-α,IL-6) and the effect on control and hypotonic stimulated ATP release determined over 10minutes. Phosphate liberation from nucleotide breakdown (100μM,ATP,ADP,AMP) was measured over 30minutes.

Results. INF-ɣ caused a significant decrease in control ATP release but had no effect on ATP release stimulated by hypotonic media. INF-ɣ was also seen to stimulate the breakdown of ATP by UROtsa cells ($P<0.05$). IL-1β had no effect on ATP release however it increased breakdown of ATP and ADP ($P<0.05$). TNF-α caused a decrease in ATP release ($P=0.003$) but had no effect on nucleotide breakdown. IL-6 stimulated ATP release in control conditions ($P=0.001$) but had no effect on hypotonic mediated induced ATP release.

Discussion. The interactions between inflammatory mediators and purinergic signaling in the urothelium are complex. Some pro-inflammatory cytokines (INF-ɣ, TNF-α,) appear to inhibit ATP release while others stimulate urothelial cell ATP release (IL-6) suggesting that the response of the urothelium to infection and inflammation will be dependent on the cytokine profile stimulated.

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Altered relaxant responses to isoprenaline and 5'-N-ethylcarboxamidoadenosine observed in whole bladders from diabetic mice.

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Introduction. Bladder dysfunction is a common problem reported in over 45% of patients with Type II diabetes mellitus (Bang et al, 2014). They present with overactive bladder and symptoms include urinary urgency, frequency and incontinence. The β_2-adrenoceptors in mice as opposed to the β_3 adrenoceptors in man have been reported to induce relaxation of detrusor (Wuest et al, 2009). All four subtypes of adenosine receptors have been identified in the bladder of experimental animals (Yu et al, 2009), however, little is known regarding adenosine receptors in murine bladders.
Aims. To investigate contractile and relaxant responses to agonists in the bladder from control and diabetic mice.
Methods. Bladders were dissected from 5 month old C57 and db/db heterozygote (control), and db/db homozygote (diabetic) mice and placed in organ baths filled with McEwans solution and gassed with carbogen. Concentration-response curves were generated for carbachol and potassium chloride (KCl) and relaxant responses to isoprenaline and 5'- N-ethylcarboxamidoadenosine (NECA) were obtained from carbachol precontracted tissues.
Results. Diabetic mice had higher blood glucose and body weights than control mice with values of 16.7 ± 1.8 mmol/l and 52.9g (n=6) versus 11.5 ± 0.8 mmol/l and 26.5 ± 1.7g (n=8) respectively, ($P<0.05$). No effect was observed on contractile responses to carbachol or KCl in the isolated bladder from diabetic mice when compared to tissues from control mice ($P>0.05$). Bladders from diabetic mice exhibited attenuated relaxant responses to higher concentrations of isoprenaline and increased relaxant responses to the adenosine receptor agonist NECA ($P<0.05$).
Discussion. Diabetes did not affect contractile responses of the bladder. Reduced relaxant responses to isoprenaline with diabetes, suggests diminished sympathetically medicated relaxation of the bladder. The adenosine receptor agonist NECA induced relaxation of the mouse bladder, an effect that was enhanced in bladders from diabetic mice.

Bang et al (2014) Urology 84:670-674.
Yu, W et al (2006) Am J Physiol Cell Physiol 291:C254-C265
Wuest M et al (2009) J. Pharmacol. Exp. Ther. 328:213-222

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Expression and localisation of CALHM1 and Pannexin1 in porcine bladder and their involvement in modulating ATP release

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Introduction. Pannexin 1 (Panx1) is well known as ATP release channels in many tissues, including rat urinary bladder (Negoro et al., 2014). Calcium homeostasis modulator 1 (CALHM1), a pore-forming Ca^{2+} permeable ion channel, has been recently surfaced as an ATP release channel in primate taste bud cells (Taruno *et al.,* 2013).
AIM. This study was aimed to localize CALHM1 and Panx1 expression and to determine their involvement in mediating ATP release in the porcine bladder, the best human bladder model.
Methods. Immunohistochemistry (IHC) was conducted to localize CALHM1 and Panx1 expression in intact porcine bladder tissues. Isolated urothelial, suburothelial and detrusor muscle cells were cultured to measure CALHM1 and Panx1mediated ATP release in response to hypotonic (~50%) induced stretch and Ca^{2+} depletion ($[Ca^{2+}]_0$, ~17nM).
Results. CALHM1 and Panx1 showed similar cellular distributions in the porcine bladder. Intensive staining was found on the cell membrane of urothelial cells, highlighting the lining of the bladder tissue. Positive CALHM1 and Panx1 signals were also seen on the surface of detrusor cell membranes, as well as on some spindle shaped cells of suburothelial layer that likely correspond to suburothelial myofibroblasts. Stretch induced a significant rise in ATP release from all three cell populations ($P<0.01$), which was attenuated by ^{10}Panx1 (100 µM, $P<0.05$), an inhibitory peptide of Panx1, ruthenium red (20 µM), and CALHM1 antibody ($P<0.01$). $[Ca^{2+}]_0$ also stimulated ATP release from urothelial cells, which was inhibited by CALHM1 antibody and ruthenium red, but not by ^{10}Panx1.
Discussion. Here we provided compelling evidence that CALHM1 and Panx1 are densely expressed in three different layers of porcine bladder and they may function as ATP release channels in response to bladder distension during the storage phase of micturition reflex. Moreover, modulation of extracellular Ca^{2+} may also regulate ATP release in porcine bladder through voltage gated CALHM1ion channel.

Negoro H et al (2014) Plos One 9(8), e106269
Taruno A et al (2013) Nature 495: 223-229

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Will the circle be unbroken? An electronic model of chronic hepatitis C virus infection and its treatment.

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Introduction. Hepatitis C virus (HCV), a small RNA virus, is a major cause of hepatitis worldwide. Most HCV infections become chronic and often cause fatal liver disease. New drugs have recently become available to treat chronic hepatitis C. Mathematical models of viral clearance kinetics have been devised to describe and predict treatment outcomes, but current models are needlessly complex and difficult to implement and interpret. Synthetic biology is a new field that aims to produce electronic models that mimic complex biological systems and *vice versa*. The vertebrate liver is the main regulator and supplier of nucleotides, which are essential for myriad vital processes including nucleic acid synthesis. Hepatocytes can synthesize nucleosides *de novo,* but salvage of nucleotides produced by RNA turnover is a more significant and energy-efficient alternative, so RNA synthesis is autocatalytic. The capacity for nucleotide supply normally exceeds demand and ultimately depends on mitochondrial function. Homeostasis is achieved by positive and negative feedback, which confers robustness and adaptability as well as the potential for oscillatory behavior.

Aims. To generate an electronic model that is capable of describing and predicting nucleotide flux in uninfected and virus-infected liver and to use it to re-assess current concepts of HCV kinetics.

Methods. Established models of genetic oscillators were used as the basis for development of a simple nucleotide-powered circuit that can be described and analyzed by means of ordinary differential equations. At the core of the model is a mitochondrial power oscillator.

Results and Discussion. The energy demand imposed by HCV infection is predicted to up-regulate the normally repressed mitochondrial power supply. Its higher output rate may be sustainable and provide some advantage in the short term, but will eventually cause mitochondrial “burnout”, triggering signals that initiate transit to a metastable low-energy state which may ultimately lead to cell death. Successful anti-HCV treatment will re-establish normal nucleotide homeostasis and divert nucleotide power back into the negative feedback loop. This concept and model are testable experimentally by computational simulation and by “real” hard-wired electronic devices. They offer a simpler and more versatile approach to modelling viral kinetics than those currently in use.

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A Sarawak jungle ginger - *Costus speciosus* inhibits nerve mediated smooth muscle contraction of the isolated rat prostate gland

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Introduction. *Costus speciosus* is widely used as a food source and dietary supplement. Traditionally it has been used by Sarawak natives to treat several illnesses including urological disorders such as Benign Prostatic Hyperplasia (BPH) which is a progressive condition characterized by increased prostatic contractility and prostate enlargement. Medicines which are able to relax prostatic smooth muscle are the most effective agents at relieving the lower urinary tract symptoms (LUTS) that accompany such disorders.

Aims. To assess the efficacy of *C. speciosus*, in treating urological disorders by investigating its effects on prostate contractility.

Methods. *C. speciosus* rhizome and roots were harvested from Sarawak. Extracts of the dried and ground plant materials were obtained using water at different temperatures. The extracts obtained were freeze dried and the activity of these extracts was evaluated pharmacologically by assessing their effects on contractions of isolated preparations of rat prostate gland in a modified Krebs-Henseleit solution bubbled with carbogen and maintained at 37 ^{o}C. Contractions were evoked electrically (0.1-20 Hz, 0.5 ms pulse duration, 60 V) or by application of exogenously administered agonists. Experiments were conducted in the presence or absence of pharmacological tools to identify mechanisms of action.

Results. *C. speciosus* rhizome and root decoction (boiling, ~100 ^{o}C) extract (3.0 mg/mL) inhibited electrical field stimulation induced contractions of rat prostatic smooth muscle by 44% and 48% at frequencies of 1.0 Hz and 2.0 Hz, respectively ($P = 0.031$, n=4); whereas cold water (room temperature, ~25^{o}C) extract inhibited contractions by 73% and 76% at 1.0 Hz and 2.0 Hz, respectively ($P < 0.0001$, n=4) (0.5 ms pulse duration, 60 V, 10 pulses or 10 s trains). Contractions mediated by exogenous administration of noradrenaline, acetylcholine or ATP were not inhibited by rhizome extract. Electrical field stimulation induced contractions were still attenuated by the rhizome extract in the presence of prazosin (300 nM), suramin (30 μM), yohimbine (1 μM), idazoxan (1 μM) or propranolol (1 μM).

Discussion. This study shows that extracts of *C. speciosus* rhizome and root inhibit contractility of rat prostatic smooth muscle, which may be beneficial in the treatment of urinary symptoms associated with BPH. The *C. speciosus* rhizome extract does not directly affect prostate smooth muscle contraction but inhibits neurotransmitter release. The increased bioactivity of the cold water extract from both rhizome and root is most likely due to the thermal decomposition of active components at the higher temperatures used in the decoction protocol.

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