## 570

**Current tapering recommendations for discontinuing psychotropic medications: a systematic review.**

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Introduction. Abrupt cessation of psychotropic medications has the potential to cause serious adverse effects. These effects form a distinct cluster of somatic and psychological symptoms. Current pharmacy texts prescribed by the Pharmacy Board (eg. AMH, APF, eTG) contain tapering recommendations.
Aims. To review current tapering recommendations and compare these to the recommendations contained in pharmacy texts prescribed by the Pharmacy Board.
Methods. Twelve major medical databases were searched using standardized search strategy back to 1 January 2000. Resultant titles, abstracts then full text articles were screened for relevance, with the inclusion criteria of a quantitave recommendation for psychotropic discontinuation.
Results. A total of 43,266 articles were identified, resulting in 40,902 articles after duplicates. Title screening produced 216 abstracts, then 45 full-text articles resulting in 7 articles included. Quantitative tapering recommendations were found for benzodiazepines, SSRIs, venlafaxine, and duloxetine. No data were found for antipsychotics, tricyclic antidepressants, MAOI and other newer antidepressants. Standard texts provide little quantitative information about withdrawal protocols.
Discussion. Older medications were unlikely to be covered in the literature from 2000. The terminology was a complication in the literature search with a range of terms such as 'deprescribing', 'withdrawing', 'discontinuing', 'tapering' and 'ceasing' and variants needing to be included and increasing the noise to signal ratio. However, there are still some newer drugs which require discontinuation protocols and this could be the subject of further research and publication.

## 571

**Creating a smoke free University of Canberra.**

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Introduction. The University of Canberra campus aims to be smoke free by 2016.
Aims. To identify factors which influence staff and students when attempting to quit tobacco smoking and those that are successful in order to design a pharmacist-led smoking cessation clinic based on practical application of the evidence.
Methods. Semi-structured interviews were conducted, audio-recorded and transcribed verbatim from a convenience sample of current and ex-smoker staff and students. Content analysis of the transcripts was conducted using grounded theory to identify major themes.
Results. Between November 2011 and March 2012, 10 participants (6 staff, 4 students; 2 current smokers, 2 recent ex-smokers, 6 ex-smokers) were recruited. Barriers identified included peer pressure, stress, culture, addiction, habits, work breaks, invincibility of youth and weight gain. Facilitators included smoking areas, health and negative advertising. Individual motivation and new habits were cited as requirements for a successful quit attempt.
Discussion. This study identified that staff and students who attempt to quit smoking experience barriers which vary according to the individual. Each potential quitter requires support to address their own barriers in order for a smoking cessation clinic to be effective. Culturally appropriate quit plans should be tailored for each individual. Where appropriate, nicotine replacement therapy (NRT) should be supplied on campus and allied health referrals for weight management, stress reduction and exercise advice can be provided. The smoking cessation clinic needs a flexible design to tailor quit solutions to individual needs.

## 572

**Development of Health Professional relationships in an Interprofessional Learning workshop.**

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Introduction: Interprofessional relationships are increasingly seen as essential in improving patient outcomes. This can be challenging in primary care given the limited opportunity for GPs and pharmacists to interact. IPL based on the Collaborative Working Relationships Framework (CWRF) may provide a solution.
Aims: To evaluate the impact of Interprofessional Learning (IPL) on health professional (HP) relationships between GPs and pharmacists, based on the CWRF.
Methods: Four pharmacists and 3 GPs practicing within the Central Sydney General Practice Network, participated in an interactive IPL workshop. HPs were randomly selected from 7 GPs and 26 pharmacists for in depth-analysis. Guided by a semi-structured format, participants jointly discussed and reflected on a problem-based-learning scenario. The workshop was video and audio taped. Content was transcribed and observed behaviours and interactions were analysed in NVivo8. Development of HP relationships and collaborative group processes where analysed along the CWRF domains of trust, respect, role definition, communication, team-leadership and professional competence.
Results: Communication was critical in positively transforming all group processes. Increased verbal and non-verbal exchanges were observed in latter stages of the workshop. Although respect was evident from the start as evidenced through HP listening and feedback provided between participants, trust only became apparent in latter stages when HPs began to express vulnerabilities and sought professional advice. Professional competence was demonstrated as trust and communication progressed through enquiry and response. One non-vocal participant (PH4) who consequently failed to demonstrate competence was overlooked by other participants. Most participants (except PH4) evolved as team members contributing to discussion whilst one GP (GP2) took on a facilitator role, guiding the group towards collaborative solutions.
Discussion: The findings indicate that bringing HPs together in IPL can positively transform relationships between GPs and pharmacists along the CWRF domains. Further work should explore the sustainability toward long-term collaborative commitment within primary care.

## 573

**"I just have to get off my arse" Barriers to medication adherence in young adults with chronic disease.**

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Introduction. Non-adherence to medication regimes contributes to the unnecessary worsening of chronic disease. Young adults are different to adolescents in terms of relative freedom and independence, particularly when they move away from home. Their priorities differ from older adults who often have more experience managing chronic disease. Pharmacy is in an ideal position to monitor adherence and empower patients to improve their adherence.
Aims. To explore the barriers and enablers that influence medication adherence in young adults with chronic disease and the role of pharmacists and other health professionals.
Methods. Semi-structured interviews were conducted with nine young adults living away from home, who had either asthma, type 1 diabetes or rheumatoid arthritis. Data was analysed using an inductive, thematic approach..
Results. Three main barriers to medication adherence emerged: the loss of routine that participants experienced when they moved out of home, a self-reported lack of knowledge about the disease and medications, and potential social issues that impact medication adherence.
Discussion. All participants acknowledged that poor adherence needed to be addressed, but only some had attempted to improve this. Lack of motivation can come from insufficient knowledge, especially if diagnosed from a young age, being unaware of implications of non-adherence, and assumptions of knowledge by health professionals. Social acceptance influenced young adults decisions and priorities, which, combined with their newfound freedom was a significant barrier to medication adherence. Pharmacists and other health professionals need to be more cognisant of the needs of young adults with chronic diseases.

## 574

**Community pharmacy-consumer/carer relationships are key in mental health care**

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Introduction. Community pharmacy is well placed to support people with mental health issues living in the community. While medication is an integral part of mental health care, insight is needed into consumer/carer views on the role of community pharmacy.
Aims. To explore mental health consumer/carer perspectives of needs, expectations and experience of community pharmacy in supporting mental health care.
Methods. Seventy-four mental health consumers/carers from Queensland, Northern Rivers (NSW) and Western Australia participated in nine focus groups and eight semi-structured interviews between November 2011, and February 2012. Qualitative data was analysed using thematic analysis informed by general inductive approach.
Results. Consumers/carers identified that information about medication side effects, occurrence rates and how to manage them was a key unmet need. Some reported that because they were unaware of what to expect, side effects led to negative outcomes. Pharmacists were viewed as an appropriate source of information and formation of a trusting relationship helped to create a sense of safety, facilitating this information exchange and a collaborative approach to mental health care.
Discussion. Good relationships between consumers/carers and community pharmacy staff are crucial in the provision of mental health care. These findings highlight the importance of information provision in quality and safe use of medicines, the need to support consumers/carers in developing self-management strategies and for collaborative relationships in a safe environment within the pharmacy.

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## 575

**Utilization of actigraphy to assess undiagnosed sleep disturbances among healthy adults in home-based settings**

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Introduction: Normal sleep duration and sleep/wake patterns can vary markedly by individual, culture and lifestyle. Many people believe they have normal and quality sleep, however they may display undiagnosed sleep disturbances. Early detection can improve the condition and may prevent future long-term use of sleep medications. Sleep assessment and monitoring are therefore vital, thus an appropriate validated measuring tool is essential to assess sleep quality and quantity in a home-based setting.
Aim: This study aims to identify undiagnosed sleep disturbances in healthy adults using actigraphy.
Methods: Individuals with no history of sleep-related problems were invited and informed about the study prior to providing informed consent. Each participant was provided with an actigraph (similar to a wrist watch) to be worn for 24-hours/day for seven consecutive days to record the sleep/wake patterns. At the end of the duration, the researcher downloaded the actigraph data using SleepConsultant™ software to generate an easy to interpret sleep report and participants completed a set of questionnaires.
Results: Acceptability of the actigraphy was supported unanimously by all participants. Only 43.8% subjects achieved the normal range (7-9 hours) of Total Sleep Time (TST), and surprisingly 75% of them had an average of Sleep Efficiency (SE%) below the normal rate (normal rate SE% value >85%).
Discussion: Utilization of actigraphy to assess sleep/wake patterns in healthy adults is possible to gain early detection of sleep disturbances before the condition worsens. Community pharmacies could use actigraphy to implement future interventions/services to improve sleep-related problems in primary care.

## 576

**Investigating pharmacists interventions on discharge from hospital**

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Introduction. Medication-related hospital admissions remain a significant problem in Australia. Failure to convey accurate and complete information and the presence of medication discrepancies across the interfaces of care is a major avoidable risk to patient safety. Discharge liaison pharmacists (DLP) have a key role in ensuring continuity of care.
Aim: The aim of this study was to define the activities of DLP, investigate the nature and clinical significance of DLP interventions made at discharge and their communication in discharge summaries and review an activity log maintained by the DLP to explore the trend of interventions.
Methods: A prospective observational study over 20 days using a data collection sheet to capture DLP interventions was undertaken. Clinical significance of interventions was ranked by a multi-disciplinary panel using a similar approach to previous studies. The percentage of interventions made in the discharge summary and time taken to incorporate such changes was documented.
Results: Seventy patients were reviewed and a total of 103 interventions were recorded; 20% due to an omission/discrepancy. Of the 62 discharge summaries reviewed, 32 were incomplete/inaccurate requiring DLP intervention. Seventy five percent (24/32) of the doctors amended the summary as a consequence of the DLP's intervention. The time taken to document ranged from immediately to one day after the suggestion was made. In 8 cases the DLP's intervention(s) were never amended on the summary. Retrospective analysis revealed that interventions comprised 11% of the DLP activities. On average, the DLP reviews 81 patients a month (range 57-108) and intervenes 61 times (range 24- 114).
Discussion: This study has identified that a substantial percentage of discharge summaries are incomplete/inaccurate requiring DLP intervention. The role of the DLP to reduce medication discrepancies, identify and correct drug related problems and facilitate continuity in medication management to potentially improve patient health outcomes has been articulated.

## 577

**Developing a methodology to target and individualise interventions to improve medication adherence in community pharmacies**

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Introduction. Improving medication adherence can reduce the risk of adverse health outcomes and health care expenditure. More innovative approaches to improve medication adherence are required. Interventions to improve medication adherence have largely been implemented on a non-targeted population using a non-individualised intervention. More innovative approaches to improve adherence are required.
Aim. To develop a methodology to target and individualise interventions to improve medication adherence.
Methods. A literature review was conducted on interventions implemented to improve medication adherence and on validated medication adherence scales. From the results of these literature reviews a methodology to identify non-adherence and the reasons for non-adherence in patients attending a community pharmacy was developed.
Results. Interventions that had an overall effect on adherence were often complex and not tailored to individual patient reasons for non-adherence. The two adherence scales, MAQ and BMQ have been validated extensively in a number of chronic diseases. The proposed methodology will screen patients coming to a community pharmacy to identify patients who are adherent and non-adherent using the MAQ. The BMQ will be used to elicit patient medication beliefs. A researcher in community pharmacy will utilise the information on non-adherence and reasons for non-adherence to develop and implement targeted and individualised interventions to improve patient adherence. To minimise bias a randomised control non-adherent group will be included.
Discussion. It is hoped that identifying reasons for non-adherence and tailoring interventions to the individual will improve non-adherence.

## 578

**What are validated adherence scales really measuring?: A systematic review**

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Introduction. Medication non-adherence is a significant health problem. There are numerous methods for measuring adherence, but no single method performs well on all criteria.
Aims. The purpose of this systematic review is to (i) identify medication adherence measurement scales, (ii) assess how these scales measure adherence and (iii) explore how these adherence scales have been validated.
Method. Cinahl and PubMed databases were used to search English-language articles involving the development or validation of the adherence scale. The search terms used to conduct the review were *medication adherence, medication non-adherence, medication compliance* and the names of each adherence scale. Various data, such as number of items, barriers identified and validation control measures were extracted from the studies and compared.
Results. Sixty articles were included in the systematic review, which consisted of 43 adherence scales. Five adherence scales, which did not meet the inclusion criteria, were excluded from the review. Adherence scales include items that either directly assess the patient's medication-taking behaviour and/or attempt to identify barriers to good medication-taking behaviour. Validation studies, however, all focus on how well the adherence scale measure medication-taking behaviour. No studies attempted to validate the type of non-adherence or the barriers to good medication-taking behaviour identified by the adherence scale.
Discussion. Adherence scales are easy to administer and provide a great opportunity to identify barriers to good medication-taking behaviour. There is a strong body of research validating and using existing adherence scales as a proxy for medication-taking behaviour. There is surprisingly little research on how well these scales identify types of non-adherence or barriers to good medication-taking behaviour. This presents an important and exciting avenue for further research.

## 579

**Community pharmacists' awareness of secondary prevention of cardiovascular disease: a preliminary study**

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Introduction. Pharmacists' interventions have been reported to help patients with established cardiovascular disease (CVD) reach treatment goals (Amariles et al, 2012). Awareness of and knowledge about secondary prevention of CVD is crucial to enable community pharmacists to support patients after a cardiovascular event (Ponniah et al, 2007).
Aims. To investigate community pharmacists' awareness of guidelines and elements of secondary prevention of CVD and of clients' cardiovascular conditions.
Methods. In-depth, semi-structured face-to-face or telephone interviews were conducted with a convenience sample of eight metropolitan and four rural NSW community pharmacists. Interviews were audio-recorded and transcribed *ad verbatim*. Data were analysed using thematic content analysis.
Results. The term prevention of CVD was frequently defined as actions to delay the occurrence of the disease (primary prevention) and to avoid recurrent events (secondary prevention). Some pharmacists, however, could not clearly differentiate between the two. Risk factors such as hypertension, hypercholesterolemia and diabetes were stated as the most common types of CVDs. Although several pharmacists could specify guidelines that deal with secondary prevention of CVD, few were able to identify all components of medication and lifestyle recommendations specified in guidelines. Several respondents indicated that fostering patient adherence to medication was their main focus when dealing with the patients with CVD. Only half of the participants actively identified clients' cardiovascular conditions.
Discussion. Community pharmacists' awareness of secondary prevention of cardiovascular disease was limited. Indeed, pharmacists play a major role in supporting medication adherence. However, both appropriate drug therapy and intensive lifestyle interventions are critical in secondary prevention of CVD. Without adequate understanding, pharmacists cannot provide comprehensive support to patients with CVD. Additional education is required to extend pharmacists' knowledge in the secondary prevention of CVD.

Amariles P et al (2012) J Manag Care Pharm 18:311-23.
Ponniah A et al (2007) J Clin Pharm Ther 32:343-52.

## 580

**Career perspectives of final year Australian pharmacy students**

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Introduction: Changes in disease burden, the ageing population and increasing demand for health services is challenging the Australian health workforce and reshaping the practice of pharmacy. Recently, concern has been raised over growing numbers of pharmacy schools and graduates in Australia; increasing competition for traditional employment opportunities. However, there is limited understanding of students' current perspectives of pharmacy career options.
Aims: We aimed to investigate students' reasons for pursuing a pharmacy career, satisfaction with this choice, perceptions of different career pathways and interest in the pharmaceutical industry.
Methods: A cross-sectional, anonymous, voluntary survey (30 questions) of final-year pharmacy students from the Universities of Sydney, Queensland and South Australia.
Results: Of the respondents ($n$=261), 25.3% were very/extremely satisfied while 39.1% were not/slightly satisfied with deciding to study pharmacy. 90% ranked "an interest in health and medicine" as an important/very important reason to study pharmacy. 67.4% maintained an intention to practice pharmacy in future, mostly in community (49%) or hospital (19.5%), while 3.1% intended to pursue the pharmaceutical industry. Nevertheless, 69.7% indicated interest in considering the pharmaceutical industry. The most frequent descriptive themes of community pharmacy were "changing", "business" and "patient contact"; contrasting with hospital pharmacy, described as "clinical/knowledge", "competitive" and "education/learning". The pharmaceutical industry was mostly associated with "business/cooperation" and "research".
Discussion: While significant proportions of students maintain intentions for traditional roles in community or hospital practice, there remains substantial interest in the pharmaceutical industry. This may relate to competition for traditional roles and to dissatisfaction with the perceived work in community practice. These results have implications for the pharmacy industry for attracting and retaining a satisfied workforce and for pharmacy educators in curriculum development.

## 581

**The use of herbal medicines in lactation among breastfeeding women in Western Australia: A population-based survey**

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Introduction. Main concerns for lactating women on medications is the transfer of drugs into breast milk and their effect on quantity and quality of breast milk, which may impact on exclusivity, duration and success of breastfeeding. Despite increasing popularity of herbal medicines, there are currently very limited data available on the use and safety of herbal medicines during breastfeeding.
Aims. Provide current information on prevalence and pattern of herbal medicines used during breastfeeding in Western Australia.
Methods. Study was conducted using self-administered survey of questionnaire validated by two pilot studies. Participants were 18 years or older, currently breastfeeding or have breastfed in the past 12 months. Participants were recruited from parenting groups, child care centres, Child Health and Immunisation Clinics in the Perth metropolitan area.
Results. 60.6% (95%CI: 53.9% - 66.9%) of women have used at least one herb for medicinal purposes during breastfeeding; 58.6% of users have indicated that the reasons for use are breastfeeding-related; 36.8% of users have reported use of at least one herb to increase breast milk supply. Most commonly used herbs were fenugreek, ginger, dong quai, chamomile, garlic, cranberry, blessed thistle, fennel seed, aloe vera and withania. Majority of participants either strongly agree or agree that currently there is a lack of resources available. Only 28.3% of users have made their doctor aware of their decision to use herbal medicine(s) during breastfeeding; 71.2% have refused or avoided drug treatments due to concerns regarding safety of breastfed infants.
Discussion. Study has demonstrated the imperative need of further research and documentation about safety of herbal medicines in breastfeeding. Evidence-based information should be available to breastfeeding women who wish to consider use of all medicines, including complementary medicines, to avoid unnecessary cessation of breastfeeding, while allowing mothers to receive appropriate pharmacotherapy without compromising breastfeeding performance and infant's health.

## 582

**Accessibility of compounded progesterone products**

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Introduction. There are few proprietary progesterone ($P_4$) products on the market in Australia, so women who use $P_4$ have to obtain their products from compounding pharmacies.
Aims. To identify components of accessibility to compounded $P_4$ products for Australian women.
Methods. A cross-sectional quantitative study was used to survey women about their experience of $P_4$. Participating pharmacies, across all Australian states, included a questionnaire with $P_4$ products they dispensed. Principal components analysis and multi-way ANOVA were used to identify components of accessibility.
Results. Principal components analysis revealed that 18 items could be grouped into 5 independent components to accessibility: easy access; have enough information for their needs; concerned about other treatments and value natural treatments; value information gathered from a variety of sources; and rurality and disadvantage. Respondents had less easy access if other treatments not working well enough had had a large influence on them starting $P_4$ treatment. Some groups of women were less likely to be concerned about other treatments and naturalness than others: younger women ($\leq$50 years); if their doctor's influence to start $P_4$ treatment had been large; if initiating $P_4$ treatment had not been influenced by other treatments not working well enough; and women whose prescribing doctor had been seen prior to commencing $P_4$. Women were more likely to have enough information for their needs if their doctor had not influenced starting treatment than if their doctor had had been a large influence. A university education enabled women to overcome the impediments of rurality and socioeconomic disadvantage.
Discussion. Women who have accessed $P_4$ value information they gather from a wide variety of sources including their doctor, relatives and friends, presentations and health professionals. They are more likely to have overcome impediments to access if other treatments have not worked; they are concerned about other treatments and value natural treatments, or have had a university education.

## 583

**Medicines prices to patients in Australia**

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Introduction. In 2009, 75% of Australian prescription medicines were subsidised by the Pharmaceutical Benefits Scheme (PBS) whilst under co-payment prescriptions accounted for 18%. Under co-payment prescription medicines are able to be discounted by community pharmacies, as the patient pays the total cost. Some banner group pharmacies use aggressive pricing business strategies to attract consumers in contrast to other banner groups and independent pharmacies. In April 2012, the prices of 237 PBS listed products decreased as a result of the PBS reforms and price disclosure policies. Lower PBS prices theoretically decrease the ability of pharmacies to discount medicines as they decrease mark-up margins.
Aims. To compare the consumer prices of under general co-payment prescription medicines between banner group pharmacies with pricing strategies and pharmacies that don't, and to assess the impact of PBS policies on the discounts that are offered.
Methods. The consumer prices of 31 under co-payment medicines were collected from banner group websites and individual pharmacies prior to and after April 2012. PBS maximum prices were obtained from the PBS website. Price and percentage differences between PBS and pharmacy groups were calculated.
Results. Before April 2012, banner group pharmacies provided discounts to patients of around 40% per prescription, whilst other pharmacies provided discounts of around 15%. Total price savings were on average $8 per prescription at banner group pharmacies and $3.50 at other pharmacies. Percentage discounts did not change greatly after April 2012, even with price decreases that occurred on the PBS.
Discussion. Banner group pharmacies with pricing strategies are able to provide greater discounts to patients compared to other pharmacies. Community pharmacies still have the ability to provide substantial discounts after the April 2012 price reductions. Further research needs to be done to assess the impact on patients' welfare and the quality of the pharmaceutical services provided.

## 584

**Medicine information accompanying OTC medicines: Do labels and leaflets adequately support safe and appropriate use?**

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Introduction. Over-the-counter (OTC) medicines must be supplied with appropriate, user-friendly information to maximise medication safety in consumer self-management. Product labels and written medicine information leaflets (WMI) are viable, highly accessible OTC medicine information sources for consumers. Comprehensibility, content and design of OTC labels and WMI contribute to their usefulness, and require further examination to better inform OTC medicine information optimisation strategies.
Aims. To investigate the comprehensibility, content and design of labels and WMI, specifically for OTC medicines.
Methods. Medline, Embase, International Pharmaceutical Abstracts and PubMed database searches were performed to identify studies exploring OTC labels and/or WMI comprehensibility, content and/or design. Additional author and reference list searching of identified papers maximised the breadth of literature identified for review.
Results. Characteristics of OTC labels or WMI were explored in the identified studies either via researcher evaluation alone or through measurement of impact on consumer outcomes. Studies involving consumers revealed variable comprehensibility of OTC labels, ranging from adequate consumer understanding to misunderstanding with cause for concern. Limited studies have examined the content of OTC labels and WMI. Design influenced outcome measures, where implementation of good information design principles, such as appropriate font size and spacing, generally improved consumer preference and/or performance of OTC labels and WMI.
Discussion. The appropriateness of information included in OTC labels and WMI requires further investigation. Opportunities exist to improve OTC medicine information design and comprehensibility. Importantly, comprehensibility, content and design of OTC labels and WMI should be considered simultaneously in their development, where well-designed studies are necessary to assess their comprehensibility with consumers.

## 585

**Usage of heart failure medications in frail and robust older inpatients**

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Introduction. Chronic heart failure (CHF) is common in the older population; however there is little evidence on the usage, safety and efficacy of medications used to treat CHF in both frail and robust older individuals.
Aims. Describe medication usage in the treatment of CHF in frail and robust older hospital inpatients.
Methods. A cross-sectional observational study of patients aged ≥65 years with CHF admitted to Royal North Shore Hospital (July-September 2012) was conducted. Data were collected from medical notes and patient interviews on demographics, medications, CHF aetiology, CHF severity and frailty (using the Reported Edmonton Frail Scale).
Results. 100 patients were recruited, with a mean age of 84±8 years (mean±SD); 52% were male and 68% were frail. Robust individuals had a higher prevalence of systolic CHF than frail individuals (60.7%, 44.0%), however, this difference was not significant. Ischaemic heart disease was the most common aetiology in both robust (40.6%) and frail (42.6%) individuals. The prevalence of more severe CHF symptoms was significantly lower in robust patients compared with frail (18.8% robust, 55.9% frail, $P<0.001$). The use of angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor antagonists (A2RAs) was significantly higher in robust patients compared with frail (69.0% robust, 52.9% frail, $P<0.001$). While there was no significant difference in use of other medications for treatment of CHF between the groups, compared to frail patients, robust patients had a trend towards more prevalent use of beta blockers (59.4%, 45.6%), spironolactone (43.8%, 39.7%), digoxin (40.6%, 26.5%) and frusemide (87.5%, 79.4%).
Discussion. Compared to frail patients, robust patients had a significantly lower severity of CHF symptoms and higher use of ACEIs/A2RAs. Further studies are required to determine the safety and efficacy of CHF medications in older patients, particularly the frail, who are not well represented in clinical trials.

## 586

**Retrospective review of initial management of febrile neutropenia at Flinders Medical Centre: Time to first dose of antibiotic therapy and risk stratification.**

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Introduction. Febrile neutropenia (FN) is a potentially life threatening adverse event among patients on chemotherapy for cancer. Such presentations require urgent administration of empirical antibiotics to reduce the risk of sepsis related complications. The Oncology and Haematology units at Flinders Medical Centre (FMC) introduced a protocol for initial management of FN in June 2010, similar to Australian guidelines (Tam CS 2011). This protocol states that patients with suspected FN should receive empirical antibiotics within one hour of their presentation.
Aims. To determine the timing of first dose of empirical antibiotics at presentation of patients with suspected FN to the Emergency department (ED) at FMC.
Methods. A retrospective audit of case notes of cancer patients with suspected FN admitted to the ED over a 12 month period from July 2010 to June 2011 is being conducted to determine whether the time of administration of first dose of antibiotics fulfilled FMC protocol.
Results. Initial review identified 44 patient encounters between July 2010 and September 2010. Only 17 had confirmed neutropenia with absolute neutrophil counts $<1.0 \times 10^9$ cells/L. However, 8 patients have been transferred from another hospital after receiving appropriate empirical antibiotic therapy. Their time to antibiotic dosing could not be retrieved. Of the remaining 9 patient encounters, only 2 (22.2%) received empirical antibiotics as per the FMC protocol of within 1 hour of presentation. The rest: 4 received in 1- 2 hours, 2 received in 2-3 hours and 1 patient after 8 hours of presentation.
Discussion. Preliminary data indicates that there has been a poor adherence to the timing of antibiotics when patients present with FN in the first three months after the introduction of FN protocol. We plan to compare the timing of antibiotic administration in subsequent periods and analyse the outcomes of each encounter.

Tam CS (2011) Int Med J 41:90-101

## 587

**A longitudinal study of constipation and laxative use in a community-dwelling elderly population**

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Introduction. Laxatives are widely available in the community and frequently used for self-medication. Whilst constipation is a common condition affecting approximately one quarter of the elderly population domiciled in the community, little is known about changes in constipation or laxative use over time.
Aims. To determine changes in the prevalence of constipation and laxative use in the community-dwelling elderly over a 10-year period.
Methods. Data from the Australian Longitudinal Study of Ageing (ALSA), a longitudinal multi-dimensional population based study of human ageing, were used for this study. ALSA participants with complete constipation and medication-related data in both wave 1 (1992/3) and wave 7 (2003/4) were included in the analysis (n=239). Constipation was self-defined and laxative use was determined from patient interview and PBS prescription data.
Results. The prevalence of self-reported constipation in the cohort increased from 14% to 21% over the 10-year period. A corresponding increase was also observed for laxative use (6% to14%). Females were more likely to report both constipation and laxative use than males. However this gender difference decreased over time for both constipation (prevalence ratio of females to males decreased from 2.42 to 1.51) and laxative use (prevalence ratio of females to males decreased from 4.28 to 1.35). In both waves, laxative use was associated with self-reported constipation in only 24 to 30% of cases, indicating that laxatives are being used more for prevention rather than treatment.
Discussion. An increase in constipation and laxative use was observed with age, with the largest increase seen in males. The majority of laxatives appeared to be used for preventative purposes, rather than for treating existing constipation. Given the diversity of laxative options, opportunities exist to optimise constipation management and laxative use in the community-dwelling elderly population.

## 588

**The impact of a pharmacist-led educational program on the psychotropic medication knowledge of aged care nurses**

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Introduction. High rates of psychotropic use are reported in aged care homes. Nursing staff administer psychotropic medications but they also appear to strongly influence prescribing. Yet, how much do nursing staff know about these medications? Can an educational program improve their knowledge?
Aims. To assess the baseline psychotropic knowledge of aged care nurses and to determine the effect of an educational intervention on their knowledge.
Methods. The 10-question 'Old Age Mental Health Psychotropic' (OAMHP) self-administered quiz was developed and validated in order to evaluate nurses' knowledge regarding psychotropic medications. Registered nurses (RNs) and enrolled nurses (ENs) at 13 homes were invited to attend two pharmacist-led educational sessions conducted 3 months apart. Before the first session, the nurses completed the OAMHP quiz, which was repeated at the end of the second session. Paired t-tests were conducted to assess the difference in pre- and post-intervention scores.
Results. A total of 97 nurses (53 RNs, 46 ENs) completed the baseline quiz, with 47 (26 RNs, 21 ENs) completing the follow-up quiz. The mean baseline scores for RNs and ENs were 64% (SD 20) and 50% (SD 17), respectively. Questions related to adverse effects of psychotropic medication or recommended therapy duration were answered poorly. For instance, less than half of the nurses knew that benzodiazepines should be used for 2-4 weeks and few were aware that hyperglycaemia is a potential adverse effect of olanzapine. The quiz scores significantly improved after attending the education sessions: for RNs the pre-training score increased by an absolute mean of 18% ($p = 0.0005$); for ENs the score increased by 28% ($p < 0.0001$).
Discussion. The baseline knowledge of nursing staff regarding psychotropic medication was moderate, particularly around adverse effects and recommended duration of use. An educational intervention led to significant improvements in psychotropic knowledge.

## 589

**Investigation of benzodiazepine utilisation in older people admitted to hospital**

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Introduction: It is well established that inpatient initiation of hypnotics and hospitalisation itself can result in continued use of these drugs in the post-discharge setting. In Tasmania, the use of benzodiazepines is high but it remains unclear as to where patients are first initiated on these agents, with blame often shifting between general practice, nursing homes and hospitals. More data regarding the utilisation of benzodiazepines in hospitals may assist in developing strategies to decrease their use.
Aim: To investigate the prevalence of use of benzodiazepines at the major Tasmanian teaching hospital and to investigate the characteristics of patients taking benzodiazepines on admission.
Methods: A retrospective audit of medical records was conducted for patients aged 70 years and over who were discharged from medical and surgical units of the Royal Hobart Hospital, from July-September 2011.
Parametric tests were conducted to test for associations between variables, including age, gender and place of residence. Multivariate logistical analysis was used to show the statistical significance of place of residence and pre-admission benzodiazepine use and account for possible confounding factors that may have influenced this.
Results: A total of 558 patients were included in the audit. Almost 25% of patients were using benzodiazepines on admission and one in four previous non-users were initiated on one in hospital. For patients newly initiated, only 12/99 (12%) received a discharge prescription. The only significant independent predictor of benzodiazepine use prior to admission was residing in a nursing home (odds ratio 1.86, 95% CI 1.15-3.00).
Discussion: Benzodiazepines were prescribed in the hospital setting at similar levels to older Australian data. Only a small number of patients initiated received a discharge prescription, suggesting discouragement of post-discharge use. The fact that residing in a nursing home was the only significant predictor of use prior to hospital admission warrants further investigation.

## 590

**An audit of antibiotic prescribing for urinary tract infections in palliative care hospices in Scotland**

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Introduction. Urinary tract infections (UTIs) are one of the most common reasons to prescribe antibiotics for palliative care patients and the main cause of bacteraemia in the older person.
Aim. To determine whether antibiotic prescribing for UTIs in palliative care hospices adheres to local guidelines and to explore possible reasons for prescribing behaviours adopted.
Methods. A cross-sectional retrospective audit of the medical records for 587 patients across three hospices in West Central Scotland was conducted. Ninety-eight patients with suspected or diagnosed UTIs were identified and treatment was evaluated with regard to current guidelines. Qualitative data were collected using semi-structured interviews and focus groups with 12 prescribers, and were evaluated using grounded theory.
Results. Low adherence to guidelines in regard to antibiotic selection (46%, 95% CI 44.9–47.1) and duration (29%, 95% CI 27.6–30.0) was detected. Several prescribers perceived that guidelines were not fully applicable to palliative care patients in a hospice setting. Feedback from nurses in relation to residents' cognitive status also influenced prescribing. Patient-specific factors that influenced prescribing included life expectancy, ease of administration, and the patients' inability or unwillingness to be administered antibiotics. Two algorithms for antibiotic prescribing for UTIs were proposed after consideration of the study findings.
Discussion. Low adherence to guidelines and perceived barriers to evidence-based prescribing suggested the need for alternative treatment algorithms for UTIs in palliative care patients. Future research should focus on evaluating palliative-care specific guidelines for treating UTIs.

## 591

**A systematic review of healthcare interventions for asthma management during pregnancy**

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Introduction. In Australia, asthma is the most common chronic disease affecting pregnant women, complicating more than 12% of pregnancies (Sawicki E et al, 2011). Pregnant women with asthma are considered a high-risk group for whom additional care, including self-education, monitoring and optimising asthma management, may be required (Wen SW et al, 2001). There are many published review articles about asthma management in pregnant women; however, none has discussed the effectiveness of non-pharmacological interventions for optimising asthma management in pregnant women.
Aims. To identify healthcare interventions for optimising asthma management during pregnancy and to examine their effects on maternal asthma control and neonatal outcomes.
Methods. The following electronic databases were searched until 31 July 2012: The Cochrane Central Register of Controlled Trials (CENTRAL, Cochrane Library), MEDLINE, EMBASE, PsycINFO, CINAHL Plus, and International Pharmaceutical Abstracts (IPA). Two reviewers independently assessed to ensure studies met the eligibility criteria. The effects of the intervention were assessed qualitatively.
Results. A total of seven studies were identified of which four were excluded according to the exclusion criteria. Three studies were included in the final review, which described an education programme, progressive muscle relaxation (PMR) and Fraction of exhaled Nitric Oxide (FeNO) guided management of asthma in pregnant women. The PMR and FeNO interventions showed significant improvement in maternal asthma control (i.e. lung function, and quality of life) and neonatal outcomes (i.e. birth weight).
Discussion. Limitations such as small sample size, no comparison group and high number of dropouts were evident. The effects of educational interventions and PMR in pregnant women with asthma at different gestational ages are unknown. Further evidence from well designed studies for optimising asthma management during pregnancy is warranted.

Sawicki E et al (2011) Aust N Z J Obstet Gynaecol 51:333-8
Wen SW et al (2001) Ann Epidemiol 11:7-12

## 593

**Computational Modeling of Powder Dispersions in Turbuhaler®**

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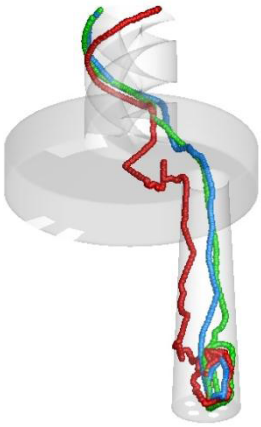
Introduction. Dry powder inhalers (DPIs) have been used to deliver dry powder formulations of active drugs for a local or systemic effect via the pulmonary route (1). We recently investigated airflow and particle impaction using Computational Fluid Dynamics (CFD) and Discreet Element Method (DEM) on the Aerolizer® inhaler (2). However, with just one inhaler, it is difficult to generalise the results with other inhalers in the market.



Aims. This study aims to investigate the dispersion process in a popular commercial DPI Turbuhaler® using simulation tools.

Methods. The effects of key variables associated with airflow rates and particle sizes were investigated based on the CFD and Discreet Phase Modelling (DPM) approach.

Results. Smaller particles moved faster in the inhaler and experienced more impactions. However their impact energy was low. Larger particles, while having large impact energy, were more inclined to be traveling slower inside the inhaler.

Discussion. Particle size affects both velocity and impact energy for powder dispersion. This study highlighted an important role of numerical modeling to provide further insight into the effect of airflow inside DPIs.

1. Hans Bisgaard COC, Gerald C. Smaldone (2002) Drug Delivery to the Lungs. New York: Marcel Dekker Inc.
2. Tong ZB et al (2010) Chem Eng J 164:432-41.

## 594

**Elucidating drug release mechanisms of biomacromolecule-containing lipid particles during lipolysis**

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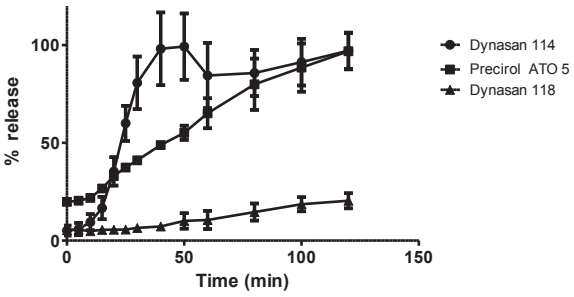
Introduction. Lipid particles are being investigated as carriers for oral delivery of biomacromolecules. Therefore an *in vitro* model is needed to elucidate the drug release mechanims from these particles during lipase degradation.




Aims. To establish an *in vitro* model for measuring release from biomacromolecule-loaded lipid particles and investigate drug release mechanisms during *in vitro* lipolysis.

Methods. Lysozyme was encapsulated in solid lipid microparticles using a melt dispersion technique. Dynasan 114 (C-14 triglycerides), Dynasan 118 (C-18 triglycerides), and Precirol ATO 5 (C-18 blend of mono-, di- (primary component) and triglycerides) were used as lipid excipients. The particles were subjected to a novel lipolysis model using a microbial lipase in biorelevant media with pH kept at 6.5 by NaOH addition. $CaCl_2$ was added continuously.

Results. Different drug release profiles were observed from the different lipid particles. The Dynasan 114 particles showed a fast and complete release of lysozyme (in 40min) whereas Dynasan 118 gave rise to a much slower release (20.4±3.9% release after 120min). The release of lysozyme from the Precirol ATO 5 particles was complete after 120min. The triglyceride formulations were superior in encapsulating the lysozyme inside the particles (t=0min) compared to the lipid blend. The NaOH addition correlated well with the drug release profiles. Experiments without lipase addition showed minimal release in 120min. All results are shown with standard deviations.

Discussion. The NaOH-addition correlates with the amount of fatty acids released and thereby the lipase activity. As minimal release was seen without lipase and the release of lysozyme was accurately predicted from the NaOH-addition it indicates that the release from the lipid particles was governed by the lipase activity on its excipient. The data therefore supports an enzyme-mediated degradation-based release mechanism. The established lipolysis model is a promising method for investigating the release of biomacromolecules from lipase-degradable particles.

## 595

**A comparison study on the effect of dry and moist heat sterilisation on lecithin microemulsions**

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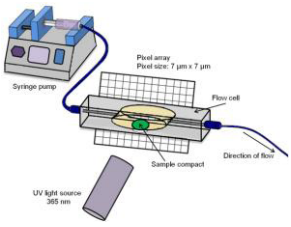
Introduction. Moist heat sterilisation is one of the methods of sterilisation commonly used for parenteral preparations. However, heat sterilization may affect the stability, chemical and physical properties of microemulsions.
Aims. This study aims to find out the effects of heat sterilizations on the stability of microemulsions..
Methods. Microemulsions containing 70% w/w water, 9 % w/w Isopropyl Myristate and 21% w/w surfactant/co-surfactant (Polysorbate 80/lecithin) at a Km of 2:1 were subjected to a moist heat and dry heat sterilisation cycles and were then assessed for physical stability (visual appearance) as well as pH, particle size and zeta potential measurements. The data obtained from two different heat sterilisation were then compared with those of the microemulsions without heat sterilisation treatment.
Results. Phase separation of the microemulsions occurred upon completion of both methods of heat sterilisation. Subjecting the separated microemulsions to sonication led to the reformation of the microemulsions. There were no noticeable differences in particle size and zeta potential measurements, but pH readings showed minor increased acidity for the microemulsions subjected with moist heat sterilisation.
Discussion. This study revealed that heat sterilization may not have a detrimental effect on the lecithin microemulsions. Long term stability studies, more detailed investigation using imaging techniques (microscope, etc), and characterizing each of the microemulsion components after heat sterilisation needs to be investigated. Future studies on the stability of drugs in microemulsion using moist heat sterilisation process can be explored.

## 596

**UV imaging and flow through Raman spectroscopy: information-rich tools for characterising the dissolution behaviour of furosemide**

Sarah Gordon[1], Line Hagner Nielsen[1], Kaisa Naelapää[1], Jukka Rantanen[1], Jesper Østergaard[1], Anette Müllertz[1,2]. [1]Dept of Pharm, Univ of Copenhagen, Copenhagen, DK; [2]Bioneer:FARMA, Dept of Pharm, Univ of Copenhagen, Copenhagen, DK

Introduction. The increasingly poorly soluble nature of new drug candidates makes thorough characterisation of drug solubility/dissolution behaviour vital. The use of visualisation techniques and biorelevant dissolution media may enhance the value and predictive capacity of dissolution testing methods.
Aims. To investigate and compare the biorelevant dissolution behaviour of different furosemide polymorphs, using UV imaging (figure) and Raman spectroscopy.
Methods. Furosemide amorphous acid and salt forms were prepared by spray drying. The dissolution behaviour of amorphous and also crystalline drug compacts in a simulated intestinal medium (10/2.5 mmol/L bile salt/phospholipid, pH 6. 5) was visualised using UV imaging. Effluent samples collected at various time points were analysed using UV spectrophotometry to obtain quantitative dissolution data. Raman spectroscopy was employed in a similar setup to investigate solid form transformations at drug compact surfaces during dissolution. The solid form of furosemide remaining following Raman experiments was identified using X-ray powder diffraction (XRPD).




Results. Real-time dissolution of furosemide forms could be visualised using UV imaging. UV images indicated a greater rate and extent of dissolution of amorphous furosemide salt as compared to both crystalline and amorphous furosemide acid. Analysis of effluent samples confirmed an approximately 10 fold higher dissolution rate of the amorphous salt as compared to the acid forms at measured time points. Raman spectroscopy and XRPD, however, showed a recrystallisation of the amorphous salt to a crystalline salt form during dissolution experiments.
Discussion. Using a combination of UV imaging and Raman spectroscopy, detailed information concerning the biorelevant dissolution behaviour of furosemide polymorphic forms could be gained.

## 597

**Higher apparent solubility and faster dissolution rate of amorphous furosemide salt leads to faster $T_{max}$ after oral dosing in rats compared to amorphous and crystalline furosemide acid**

Line Hagner Nielsen[1], Sarah Gordon[1], René Holm[2], Thomas Rades[1], Anette Müllertz[1,3]. [3]Bioneer:FARMA, [1]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark; [2]Preformulation, H. Lundbeck A/S, Valby, Denmark

Introduction. By forming a pharmaceutical salt of the amorphous form of a drug an enhancement of dissolution rate and solubility can be obtained

Aims. To prepare and characterise an amorphous furosemide sodium salt and evaluate its stability, biorelevant solubility and dissolution in comparison with both amorphous and crystalline furosemide acid. Furthermore, the *in vivo* properties of the amorphous salt and amorphous and crystalline acid were studied.

Methods. The two amorphous forms were prepared by spray drying. The stability of the two amorphous forms was studied using XRPD, and DSC was utilised to investigate glass transition temperature ($T_g$). The solubility of the three forms was determined in simulated gastric and intestinal media. The dissolution characteristics were studied using a $\mu$-Diss profiler. The *in vivo* properties of amorphous salt and amorphous and crystalline acid were studied by orally dosing rats.

Results. The amorphous salt was stable for 291 days, whereas the amorphous acid was stable for 4 days at 22°C and 33% RH. The increased stability found for the amorphous salt was also supported by the determined $T_g$ of 101.2°C; a value 40°C higher than that found for the amorphous acid. The apparent solubility of the amorphous salt in simulated gastric and intestinal media was significantly higher compared to the amorphous and crystalline acid. The intrinsic dissolution rate was found to be 8.8±0.6 mg/cm$^2$/min for the amorphous salt and 1.3±0.1 mg/cm$^2$/min and 0.45±0.07 mg/cm$^2$/min for the amorphous and crystalline acid, respectively. The amorphous salt was shown to have a $T_{max}$ of 23.3±5.2 min after oral dosing, which was significantly faster than that of the amorphous and crystalline acid.

Discussion. The faster $T_{max}$ of the amorphous salt correlates well with the significantly higher dissolution rate of this form compared to that of the amorphous and crystalline acid.

## 598

**Addition of hydroxypropyl methylcellulose to furosemide increases physical stability of the amorphous form of furosemide**

Line Hagner Nielsen[1], Thomas Rades[1], Anette Müllertz[1,2]. [2]Bioneer:FARMA, [1]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

Introduction. Converting poorly soluble drugs to an amorphous form improves dissolution rate, but amorphous forms are physically unstable. By adding a water-soluble polymer such as hydroxypropyl methylcellulose (HPMC) to the amorphous form it can be stabilised as the polymer inhibits crystal growth.

Aims. To determine the effect of spray drying furosemide with different solvents and ratios of HPMC on drug polymorphic form, stability and dissolution characteristics.

Methods. Furosemide and HPMC were spray dried with either water:methanol or water:NaOH as the solvent. The stability of the amorphous furosemide formulations was studied at 22°C and 33%RH using XRPD. Dissolution characteristics of the amorphous forms were studied using a $\mu$-diss profiler. Solid phase transformations of furosemide during dissolution were investigated by flow through dissolution combined with Raman spectroscopy.

Results. When utilising water:NaOH as the solvent no HPMC was needed to obtain an amorphous form of furosemide. This amorphous salt form was physically stable for 291 days. However, by adding 20, 50, or 80% HPMC (w/w%), physical stability was significant increased and the furosemide was stable in its amorphous form for at least 730 days. The formulations containing 50 and 80% HPMC (w/w%) further stabilised amorphous furosemide during flow through dissolution. When water:methanol was used as the solvent, addition of 80% HPMC (w/w%) was required to produce an amorphous furosemide form. In such a formulation furosemide remained amorphous for at least 730 days of storage and for the duration of flow through dissolution experiments. The addition of HPMC had no influence on the total amount released of furosemide.

Discussion. Spray drying furosemide together with HPMC resulted in an improved stability of the resulting amorphous furosemide, both under stored conditions and during dissolution. The addition of HPMC to amorphous furosemide affected the stability, but not the final release of furosemide as HPMC is a hydrophilic polymer.

## 599

**Comparison of two extraction methods prior to chromatographic determination of metabolic fragments of beta-endorphin within inflamed rat tissue**

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Introduction: Beta-endorphin (BE) is a prominent endogenous opioid peptide that has been shown to play an important role in pain, reward, stress and the immune system. During inflammation, the production of BE is increased and subsequently released in the inflamed tissue where it is metabolised. BE and its metabolites can be identified using liquid chromatography/ mass spectrometry (LCMS).Two protein precipitation methods are commonly used to clean up samples before HPLC: Trichloroacetic acid (TCA) precipitation and Acetonitrile (MeCN) precipitation.
Aims: Comparison of two extraction methods prior to chromatographic analysis of BE metabolic fragments
Methods: BE was incubated in homogenised inflamed tissue at pH 5.5, representing a localised acidic environment seen in inflammation. Protein precipitation/ sample clean-up was carried out using TCA and MeCN. The resultant fragments were separated by a C4 column and detected by mass spectrometry in total ion current (TIC) mode.
Results: In total, 29 fragments were identified after incubation of BE in inflamed tissue at pH 5.5. BE (19-31), BE (20-31), BE (5-18), BE (10-31), BE (10-28), BE (10-16) were major metabolites identified with both methods of sample preparation. However, fragments BE (16-30) and BE (5-24) were identified only with MeCN extraction. Also, the chromatograms were cleaner and the peak areas better with MeCN extraction.
Discussion: Using acetonitrile was found to be superior to TCA method due to the ability to concentrate samples and better removal of impurities. Cleaner samples are less prone to ion suppression in MS resulting higher peak intensities.

## 600

**Polarized light microscopy as a method for analyzing API precipitation in simulated gastric media**

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Introduction. For an increasing number of APIs, optimization of the solid form is a central part of the overall strategy to increase solubility and dissolution. This can lead to a supersaturation of API in the gastrointestinal-tract after oral administration and if the supersaturation cannot be retained the API will precipitate. It is therefore important to improve the knowledge of precipitation under simulated gastric conditions.
Aim.To develop a method for visualizing API precipitation in simulated gastric media (SGM) by use of polarized light microscopy.
Methods. Carbamazepine was dissolved in dimethylacetamide (DMA) and 0.5$\mu$L was placed on a microscope slide. 19.5$\mu$L SGM (0.1M HCl) was added to the DMA/drug solution. A camera attached to a polarized light microscope was used to capture videos of API precipitation, and the data was quantitatively analyzed, with respect to number of crystals and crystal growth. Results are given as Mean±SD.
Results. Using a 5x objective it was possible to film an area of approximately 1875$\mu$m x 1405$\mu$m (n=4). A typical needle growth of the hydrate phase was observed for all precipitated carbamazepine crystals. More than one hundred crystals were observed within the captured area (111±48) and no new crystals could be identified after 6.1±0.8 seconds. The average length of the precipitated crystals was 289±123$\mu$m, with 75% being shorter than 350$\mu$m, and crystal growth stopped after 28.5±9.6 seconds.
Discussion. The developed microscopy based method makes it possible to quantitatively analyze nucleation and crystal growth over time and it provides a fast method for evaluating API precipitation, using only very small amounts of API and media. The variation observed in the data may be due to the fact that only a small area was filmed by the camera and that nucleation and crystal growth are influenced by how well the DMA/drug solution is mixed with the SGM.

## 601

**Stability of risperidone in a novel polyol-based low-aqueous *in situ* gelling emulsion**

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Introduction. Risperidone is a benzisoxazole atypical antipsychotic used for the treatment of schizophrenia. To maintain therapeutic effects and to prevent rebound symptoms, patients are required to maintain a strict daily treatment regime. Current treatment options include daily tablets or fortnightly injections.
Aims. The aim was to determine the stability of risperidone in a novel *in situ* gelling formulation comprised of a hydrogel-containing polyol-peanut oil emulsion for use as a vehicle for sustained release of risperidone.
Methods. Formulations were prepared to contain 0.05 mg/ml risperidone and tested for stability over an 8-week period at real time (25 ± 1˚C/60% RH), accelerated (40 ± 1˚C/75% RH) and refrigerated (4 ± 1˚C) conditions.
Results. Differential Scanning Calorimetry results showed no changes in physical properties of risperidone within the formulation, however a slight shift in the endothermic peak from 170.6˚C to 166.2˚C was observed when exposed to peanut oil. HPLC results showed no significant loss of drug content over the 8-week study period when stored at room temperature or under refrigerated conditions ($n=3$, $p>0.05$), however a significant decrease in risperidone content was seen after 4 weeks ($n=3$, $p<0.05$) and after 8 weeks ($n=3$, $p<0.05$) when stored under accelerated conditions. There were no changes in physical appearance throughout the study period, with all formulations appearing homogenous, without any apparent change in colour or clarity, and no sign of caking or separation.
Discussion. These results indicate that the risperidone formulation is physically and chemically stable for up to 8 weeks when stored at or below room temperature. Higher temperatures are associated with significant loss in risperidone content, possibly due to physical incompatibility issues with peanut oil at higher temperatures. The physicochemical stability, in addition to the biocompatible excipients and recently demonstrated *in vitro* release capability of the formulation, shows potential for future clinical application in schizophrenia.

## 602

**Nicotine retention as the major determinant of percutaneous absorption of nicotine**

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Introduction. Nicotine patches have long been used as an aid in smoking cessation therapy. Despite differences in their design, total nicotine content and patch size, the major determinant of nicotine percutaneous absorption is still poorly understood.
Aims. To study the percutaneous absorption of nicotine and investigate the major determinant of nicotine flux while using water as a vehicle.
Methods. Percutaneous absorption of nicotine was studied using Franz cells with human epidermis. Thermodynamic activity was calculated from the partial vapour pressure data. Stratum corneum hydration was measured using a corneometer and the stratum corneum retention of nicotine was determined by tape stripping.
Results. The percutaneous absorption of nicotine, quantified as nicotine flux across the epidermis, showed a parabolic relationship with an increase in the donor concentration. However, the diffusion coefficients were unchanged across various donor nicotine concentrations. Nicotine thermodynamic activity increased as nicotine concentration increased; whereas stratum corneum hydration decreased. Nicotine retention in the stratum corneum showed a parabolic relationship with nicotine concentration.
Discussion. Nicotine stratum corneum retention has a good correlation with nicotine flux ($r^2=0.850$), which suggests that nicotine retention is the major determinant of the percutaneous absorption of nicotine. Conversely, stepwise regression analysis acquired a significantly improved prediction of nicotine flux by adding thermodynamic activity and skin hydration ($r^2=0.972$). This work identified the key determinants of percutaneous nicotine absorption and it may help to predict the skin absorption of nicotine patches and contribute to a better design of transdermal preparations.

## 603

**Oral delivery of nanoparticles to the colon for tumour targeting**

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Introduction. Targeted nanoparticle (NP) delivery to the colon shows potential for improving chemotherapy efficacy for colorectal cancer treatment by raising local drug concentrations at the tumour site and avoiding widespread systemic distribution following intravenous injection. However, various barriers such as the physicochemical characteristics of the carrier and active and the physiological environment of the upper gastrointestinal track can promote aggregation of nanoparticle carriers and premature drug release prior to reaching the colon.
Aims. Deliver high concentrations of anti-cancer drug-loaded nanoparticles to the colon for local drug delivery at tumour sites using alginate capsules.
Methods. Indomethacin, a model anti-cancer drug, was loaded into Eudragit S100 nanoparticles using a nanoprecipitation method and the NPs were incorporated in alginate hydrogel capsules using the drop technique. The drug load potentially available for release in the colon was determined by investigations of release behavior in simulated intestinal fluids (SIF).
Results. Eudragit nanoparticles (116 nm in diameter) were loaded with indomethacin to a level of 5%, w/w. Drug-loaded NPs were encapsulated in hydrated alginate capsules (2 mm in diameter) resulting in indomethacin loading of 0.01%. In vitro release testing in SIF demonstrated that around 60% of the drug load would potentially be available for release in the colon following transit through the stomach and small intestine.
Discussion. Our findings demonstrate that drug-loaded nanoparticles incorporated in alginate capsules may be useful for delivering high concentrations of anti-cancer agents to the colon for improved cancer therapy.

## 604

**Implications of the use of thickening agents to aid swallowing of altered medicines: *in vitro* study**

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Introduction**:** People who have difficulties swallowing solid medications, often crush and mix them with food or thickened fluids. Thickened fluids are comprised principally of polysaccharides, and must have the appropriate viscosity in order to form a bolus which stimulates the swallowing reflex. As a result, the use of these agents may have consequences for the oral bioavailability and pharmacotherapy of drugs.
Aim: To examine the *in vitro* implications of the use of thickening agents on the release/dissolution of atenolol immediate release (IR) tablets.
Methods: Atenolol 500 mg (IR) crushed tablets (Atehexal® Sandoz) and four commercial thickening agents: Janbak F (xanthan gum), Karicare (maltodextrin, starch, carob bean gum), Nutilis (maltodextrin, modified starch, tara gum, xanthan gum, guar gum) and Viscaid (guar gum) at three different viscosity levels (L150, L400, L900) were tested using the USP standard apparatus 2 (Varian VK 7000) at 37°C, 50 rpm in simulated gastric fluid pH 1.2. Concentration levels of dissolved/released drug samples were analysed by UV spectroscopy.
Results: While whole and crushed atenolol tablets delivered with water reached 96 and 97% dissolution within 30 min respectively, less than 80% of atenolol was released in 30 min for the xanthan gum based formulations at L150 and L400. At L900, all four thickeners remained in a big lump in the dissolution vessel and exhibited a restriction in the release of atenolol with no more than 60% being dissolved in 30 minutes.
Discussion: It was found that *in vitro* performance of solid altered medications with thickening agents depends on the composition of the polymer and viscosity level of the mixtures. Although these formulations have the viscosity profile appropriate for safe swallowing, the delay in drug release raises questions about the concomitant administration of thickening agents with crushed tablets and how these may affect the efficacy of the drug.

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**A mechanistic based approach for enhancing buccal mucoadhesion of chitosan**

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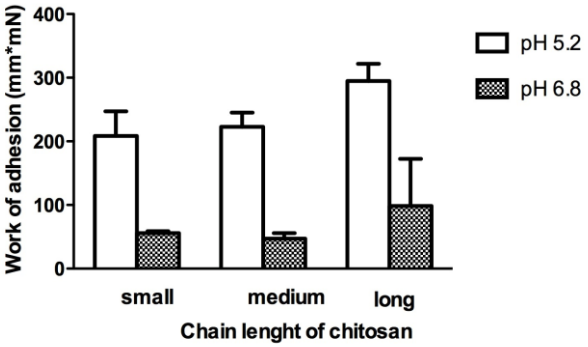
Introduction. Mucoadhesive formulations for drug administration into the oral cavity have attracted increased attention the last decades, as drugs absorbed in the oral cavity avoid presystemic degradation and/or hepatic first pass metabolism compared to the oral route. The mucoadhesion of these formulations can facilitate increased retention time of the formulation as well as steeper concentration gradient of the drug compound.




Aims. To study the pH dependent interactions between chitosan polymers of increased molecular weight and porcine gastric mucin.
Methods. Mucin-chitosan complex coacervate formation was measured by sample turbidity at UV-VIS (400 nm) and titration with hydrochloric acid (pH range: 6.5-2.7). Mucoadhesive strength between compressed chitosan dics and mucin wetted absorbing paper was tested using a texture analyser with a 30 kg load cell.
Results. Coacervation experiments showed a clear polynomial curve (turbidity vs. pH), with turbidity maximum around pH 5 for all three chitosan grades. A higher mucoadhesive strength at pH 5.2 compared to pH 6.8 was measured using tensile force method. The effect of pH was significant for all three chitosan grades (n=4, $p<0.05$)
Discussion. The potential of pH dependent mucoadhesion of oromucosal formulations could have an important impact on the performances of drug delivery from these formulations due to the intra-day pH variation of human saliva. This gives opportunities to adjust formulations in order to obtain the maximum mucoadhesion leading to potential higher performance such as faster onset and prolonged effect, hence increased bioavailability.
Conclusion. A pH dependent interaction between chitosan and mucin was confirmed in this study by 2 complementing techniques, with an optimal pH for interaction around 5.1.

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**Validation of Microtitre plate assay for *Haemophilus influenzae spp.*Biofilm**

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Introduction: The microtitre plate biofilm assay is a method for quantitatively measuring static biofilm formation. This assay has been most widely investigated using *Pseudomonas aeruginosa* and adapted for other organisms including non-typeable *Haemophilus influenzae* (NTHi).
Aim: This study aims to establish a baseline for reproducibility of the microtitre biofilm assay for subsequent investigation of the effect of other variables.
Method: 4 different clinical isolates of NTHi were selected with a robust biofilm producer*Pseudomonas aeruginosa* (PAO1) as a positive control and un-inoculated broth as a negative control. Strains were grown within supplemented broth for 18-22 hrs at 37°C in 5% $CO_2$, the cell density adjusted and broth inoculated into 4 wells of a 24 well microtitre platethen incubated for 20 hrs. Final growth (FG) and Biofilm (BF) was quantified by measuring absorbance. The relative biofilm production (BF/FG) for each well obtained. The method was evaluated over 13 episodes, where one episode represented a single plate. The average BF/FG for each of the 4 wells determined for each strain which is occurred over 5 different days, with 2 episodes per day on 4 days and one day with 5 episodes
Result: The mean and SD of the BF/FG ratio (n=13) and the mean of the SD of the BF/FG between episodes on single days (n=5) for each of the 4 NTHi strains and control are as follows. Strain 1 was (1.58, 0.73, 0.40), strain 2 (0.58, 0.23, 0.11), strain 3 (0.15, 0.08, 0.03), strain 4 (0.16, 0.17, 0.10) and for PAO1 (0.29, 0.07, 0.03).
Discussion: There is significant variation in BF/FG ratio of replicates across different days compared to the same day which been poorly reported in the literature on biofilm assays. This observation highlights the need for publications on in vitro biofilm production to explicitly describe their methodology.

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**Stability of key antioxidant compounds in pūhā (*Sonchus oleraceus* L.) leaf extracts under different post-harvest processes and storage conditions**

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Introduction. Pūhā (*Sonchus oleraceus* L.) leaves are rich in antioxidants and show potential to be formulated as nutritional supplements. We have identified three key antioxidants, caftaric acid, chlorogenic acid and chicoric acid, in pūhā leaf extracts (Ou et al. 2012).
Aims. To investigate the effects of different post-harvest processes and storage conditions on the stability of the three key antioxidants.
Methods. The antioxidants in pūhā leaves were extracted in 70% aqueous methanol. The mixture was centrifuged and the solvent was removed by rotary evaporation. Fresh pūhā leaves were subject to oven-drying (60!C), freeze-drying or air-drying (~25!C) for 6 h, 24 h and 3 days, respectively, until constant weight. Fresh leaves were used as control. Freeze-dried pūhā leaves and leaf extracts were stored at different temperatures (4°C, 25°C, 50°C) and relative humidity (0, 43 and 75%). A design of experiments approach was applied to design the stability study. Concentrations of the key antioxidants were quantified by a HPLC-DPPH (1, 1 - diphenyl-picrylhydrazyl) post-column derivatization method (Ou et al. 2012). Antioxidant activity was assessed by a DPPH free radical scavenging capacity assay.
Results. The three key antioxidants degraded to an unquantifiable level after 6 h in the oven. In contrast, they were retained after freeze-drying and air-drying. Within the storage period of both leaf and extracts, samples were stable, except those at 75% relative humidity. The concentration of chlorogenic and chicoric acid decreased over time while caftaric acid increased because it is a breakdown product of chicoric acid.
Discussion. The data suggested that humidity plays a critical role in storing pūhā material. Low humidity and moderately high temperature can preserve the key antioxidants. Although the degradation profiles were similar, the key antioxidants were less stable in extracts than in leaf.

Ou, Z.-Q., et al. (2012). *Journal of Pharmacy and Pharmacology* (in press).

## 608

**Optimization of simulated gastric media (FaSSGF) based on rheological characterization of human gastric fluid**

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Introduction. The Noyes-Whitney equation predicts that an increased viscosity decreases the dissolution rate of drugs. In the developed gastric simulated media (FaSSGF) the viscosity of the human gastric aspirates (HGA) has not been considered.
Aims. To optimize FaSSGF based on *in vivo* relevant physicochemical characteristics and rheological properties.
Methods. Fasted HGA were collected from 19 healthy volunteers. pH, osmolality, buffer capacity, surface tension, protein content, and bile salt concentration were measured. Rheological characterization of the aspirates was conducted using the cone and plate geometry on an AR-G2 rheometer, TA Instruments.
FaSSGF was chosen as a starting point for the creation of viscous simulated gastric media. Different amounts of methyl cellulose (MC) were added (0.5-0.7%). Dissolution of Cinnarizine in FaSSGF containing MC was investigated using the µDISS Profiler.
Results.The pH, osmolality, surface-tension, bile salt concentration, and protein content determined were in correlation with literature values. Rheological examination of HGA showed shear-thinning behaviour with predominant elastic behaviour in the linear range. The elastic modulus, G' was 0.08-4.39mPa at an oscillation torque of 0.01mN/m. The shear viscosity of HGA was measured to be 0.6–45.5Pa·s at rest corresponding to a shear rate of $0.01s^{-1}$. At shear rates of $50s^{-1}$ corresponding to the antrum, the measured viscosity interval for HGA was 1.7-9.3mPa·s. The FaSSGF and HCl pH 1.2 have no shear thinning properties and showed lower viscosity (1.1mPa·s). Addition of 0.5–0.7% MC to FaSSGF resulted in a media with shear thing behaviour and viscosities similar to that of HGA. An increased viscosity of FaSSGF decreased IDR of Cinnarizine.
Discussion. HGA showed shear thinning behavior and variable elasticity and viscosity indicating different amounts of mucus and proteins present. A media with similar rheological properties can be obtained by adding 0.5-0.7% MC and was observed to influence IDR of Cinnarizine.

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**The effect of haematocrit on insulin levels from dried blood spots for use in clinical studies.**

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Introduction. Hyperglycaemia is a common problem in preterm neonates. A major barrier to conducting pharmacokinetic trials in neonates is the relatively large volume of blood required by most assays. The use of dried blood spots in place of plasma samples has the potential to enable the use of smaller sample volumes and simplifies processing and handling. Haematocrit in neonates varies significantly and can reach values as high as 0.65 or more (Holub M et al, 2006).

Aim. To determine the effect of haematocrit on insulin concentrations from dried blood spots.

Method. A previously developed method (Butter NL et al, 2001) was used to measure insulin concentration from dried blood spots. Samples of varying haematocrit (0.25-0.65) were prepared at three different plasma concentrations (10, 25 and 50mU/L). 50uL was spotted onto filter paper and left to dry. Two 3mm filter paper discs were punched into the wells of the assay plate before analysing with an Invitron insulin chemiluminescent immunoassay (IV2-101).

Results. Chemiluminescence signals were significantly lower at higher haematocrit values, at all three plasma concentrations (p-values<0.05). All results showed high variability (CV% = 9-61%). Whole blood concentration was calculated using the equation: whole blood concentration=(1-haematocrit)(plasma concentration) and plotted against chemiluminescence. An exponential function ($y = ae^{bx}$) was fitted using Origin Pro: a = 350±24, b = 0.05±0.001, $r^2$ = 0.891.

Discussion. Haematocrit has a significant effect on plasma insulin concentration measured by chemiluminescence, from bloodspots. When whole blood concentration is calculated it is then possible to calculate the plasma concentration if the haematocrit value is known. However, using bloodspots to measure insulin concentrations in neonates for clinical studies is not ideal due to the high variability of this method.

Butter NL et al (2001) Clinica Chimica Acta 310:141-50.
Holub M et al (2006) Clinica Chimica Acta 373:27-31.

## 610

**The effect of formulation on the penetration of coated and uncoated zinc oxide nanoparticles into the viable epidermis of human skin *in vivo***

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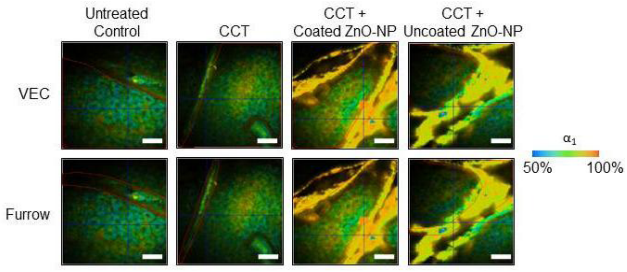
University of Queensland, [1]Therapeutics Research Centre, Woolloongabba, QLD; Universidade Federal de São Paulo, Instituto de Ciências Ambientais Químicas e Farmacêuticas[2], Diadema SP, Brasil; Claude Bernard University, Faculty of Pharmacy[3], Lyon, France; University of Queensland, Dermatology Research Centre[4], Woolloongabba, QLD; University of Queensland, ARC Centre of Excellence for Functional Nanomaterials[5], Australian Institute for Bioengineering and Nanotechnology, Brisbane, QLD; The University of Queensland, Australian Microscopy and Microanalysis Research Facility[6], Brisbane, QLD; University of South Australia, [7]School of Pharmacy and Medical Science, Adelaide, SA (introduced by Washington Y. Sanchez, Therapeutics Research Centre, University of Queensland, Woolloongabba, QLD).

Introduction. The use of nano particulate zinc oxide (ZnO-NP) in sunscreens and other cosmetic products has raised public health concerns. The two key issues are the extent of exposure to ZnO-NP and the likely hazard after the application of ZnO-NP in sunscreen and cosmetic products to humans in vivo.

Aims. Our aims were to assess exposure by the extent of ZnO-NP penetration into the viable epidermis and hazard by changes in the viable epidermal redox state for a number of topical products. Of particular interest is the role of the particle coating, formulation used and the presence of any enhancers.

Methods. Multiphoton tomography with fluorescence lifetime imaging microscopy (MPT-FLIM) was used to simultaneously observe ZnO-NP penetration and potential metabolic changes within the viable epidermis of human volunteers after topical application of various ZnO-NP products.




Results. Coated and uncoated ZnO-NP remained in the superficial layers of the SC and in the skin furrows. We observed limited penetration, of coated ZnO-NP dispersed in a water-in-oil emulsion formulation, which was predominantly localised adjacent to the skin furrow. However, the presence of ZnO-NP in the viable epidermis did not alter the metabolic state or morphology of the cells.

Discussion. Our data suggests that some limited penetration of coated and uncoated ZnO-NP may occur into viable stratum granulosum epidermis adjacent to furrows but that the extent is not sufficient to affect the redox state of those viable cells.

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**Allosteric modulation of regulatory protein recruitment to the glucagon-like peptide-1 receptor**

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Introduction. The glucagon-like peptide-1 receptor (GLP-1R) is a class B G protein-coupled receptor (GPCR) that responds to multiple endogenous ligands including four variants of GLP-1 (the predominant form being GLP-1(7-36)$NH_2$) and oxyntomodulin. This receptor is also activated by the exogenous peptide exendin-4 and allosteric ligands such as the Novo Nordisk Compound 2 and Eli Lily BETP. The GLP-1R has an essential role in nutrient regulated insulin release and is a potential therapeutic target for the treatment of type II diabetes mellitus and obesity.

It is already widely accepted that insulin secretion downstream of GLP-1R activation is critically dependent on cAMP formation, but recent evidence is also emerging for an essential role of regulatory proteins such as beta arrestins (β-Arr1) and G protein-coupled receptor kinases (GRK). The canonical role of these regulatory proteins is to terminate GPCR signaling and promote receptor internalization. However, more recently, roles as scaffolding proteins that can regulate G protein-independent signaling have emerge (Gurevich et al, 2012).

Aim. To assess the recruitment of regulatory proteins by the GLP-1R receptor.

Methods. In this current work, we have applied bioluminescence energy transfer to measure agonist-induced recruitment of β-Arr1, β-Arr2, GRK2, -3, -5 and -6 to the GLP-1R.

Results. We have established recruitment profiles of these regulatory proteins for multiple peptide and non-peptide ligands and have also assessed the ability of the allosteric ligands, Compound 2 and BETP, to modulate orthosteric ligand-mediated β-Arr and GRK recruitment. This revealed βArr1, βArr2, GRK2 and -3 (but not GRK5 or -6) recruitmentby GLP-1(7-36)$NH_2$, exendin-4 and oxyntomodulin could be positively modulated by both classes of compounds (albeit weakly by BETP), but the degree of modulation varied depending on the orthosteric ligand present.

Discussion. These data provide further insight into the cellular mechanisms of GLP-1R action.

Gurevich E.V. et al., (2012) PharmacolTher. 133(1):40-69

## 612

**Targeting β-alanyl aminopeptidase in *Pseudomonas aeruginosa***

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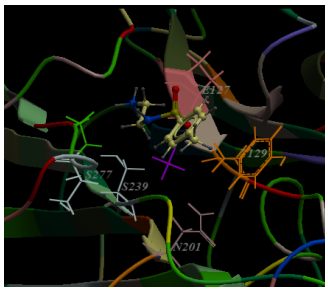
Introduction. ChromID™ *P. aeruginosa* enables the rapid identification of this pathogen in immunodeficient patients. Aggressive early directed treatment with the appropriate antibacterial agent(s) can then limit the severity of infection in patients suffering from burns, cystic fibrosis, and cancer. ChromID™ *P. aeruginosa* employs a chromogenic substrate for β-alanyl aminopeptidase (an enzyme specific to *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*).




Aims. To perform homology modelling of the β-alanyl aminopeptidase sequence of *P. aeruginosa*, and to use the model obtained in the design and synthesis of inhibitors in order to evaluate the cellular role of this enzyme.

Methods. A 3D model of β-alanyl aminopeptidase was derived and evaluated using Maestro (version 9.1, Schrödinger). Virtual database screening was then conducted in order discover inhibitors which would be predicted to bind to the active site of the enzyme. The hit compounds with the highest docking scores were synthesized, purified and and the *in vitro* antimicrobial activities for these synthesized agents, against both Gram negative and Gram positive strains, were evaluated using both disc diffusion and microdilution assays.

Results. Six compounds from a series of non-classical sulfonamides exhibited greater activity against *P. aeruginosa* than against the other organisms, with compound MS-17 having the greatest activity against *P. aeruginosa* (MIC 31.25 μg/ml) and no effect upon a human (prostate cancer) cell line.

Discussion. The sulfonamides (and their β-alanyl derivatives) represent new leads in the search for antimicrobial agents for the treatment of *P. aeruginosa*, which has developed such multidrug resistance that clinical isolates have emerged which are susceptible to only one class of antibacterial agent.

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**Variability of anti-inflammatory diterpenoids from the Northern Kaanju medicinal plant, *Dodonaea polyandra***

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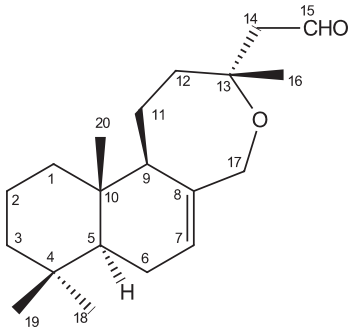


Introduction. It is well understood that chemical profiles of secondary metabolites in plants may vary because of factors like season, soil type, geographical location the age and sex of the plant.

Aims. To evaluate the variability of anti-inflammatory clerodane diterpenoids found in the dioecious Northern Kaanju medicinal plant, *Dodonaea polyandra* from leaf resin of female and male individuals.

Methods. Plant samples were collected from three different sites on Northern Kaanju traditional homelands, Cape York Peninsula. After shade drying the material, resin was scraped from the leaf surface. The quantities of three diterpenoids from the leaf resin were quantified using a validated isocratic reverse-phase HPLC-UV method. Chromatographic and NMR spectroscopic methods were employed to elucidate the chemical structures of several new constituents isolated from a male individual.

Results. All 3 compounds showed noticeable variation in each of the samples tested. DP5 was the most abundant, although it was detected at very low amounts in at least 30 % of samples. Interestingly, none of the diterpenoid markers were detected in a male individual sample. This subsequently led to a separate structural elucidation study of the male sample in which 4 labdane diterpenoids were isolated and characterised as major constituents.

Discussion. The variability study suggests that individual plants of similar age produce different levels of the active components found in *D. polyandra*. There is some evidence which indicates location as being a factor that affects the quantity of bioactive diterpenoids produced by the species. Moreover, this study showed that the sex of the species may be an influencing factor on the class of compound synthesised. Follow up studies are required in order to determine whether the labdane diterpenoids identified possess similar anti-inflammatory activities previously described for the clerodane diterpenoids.

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**Genetic polymorphisms of the CNS immune and opioid signalling pathways are associated with morphine requirements after caesarean delivery.**

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Introduction. Opioids such as morphine are the first line therapy for moderate to severe postoperative pain. Severity of postoperative pain, and opioid dose requirements for pain relief, can vary significantly between patients and ethnic groups.

Aims. To investigate if genetic polymorphisms in neuronal and CNS immune pathways implicated in pain processing and opioid activity are related to variability in pain and analgesia following caesarean surgery.

Methods. Chinese (n=598), Malay (n=230) and Indian (n=133) women undergoing elective caesarean delivery were genotyped for 21 SNPs in 15 genes: *CASP1, BDNF, CRP, LY96, IL6, IL1B, TGFB1, TNF, IL10, IL2, TLR2, TLR4, MYD88, IL6R* and *OPRM1*. Subject genetics, surgery duration, weight, age and postoperative visual analog scale (VAS) pain scores were investigated as predictors of patient controlled analgesia morphine requirements (mg/24 h), using stepwise linear regression model selection by Akaike Information Criterion.

Results. SNP frequencies differed significantly between ethnic groups (Chi-squared $P<0.05$ after Bonferroni correction) for all genes except *TNF*. In addition to surgical and demographic factors, several CNS immune and opioid signalling SNPs were associated with morphine requirements in Chinese (*TLR2, OPRM1,* VAS, surgery duration: model adjusted $r^2 = 0.04$), Malay (*IL2, OPRM1*, age: model adjusted $r^2 = 0.04$) and Indian (*IL6*, *IL1B, IL10, TLR4, CASP1*, age, weight: model adjusted $r^2 = 0.17$) subjects.

Discussion. Genetic variability in CNS immune and opioid signalling pathways plays a role in interpatient variability in morphine requirements following caesarean surgery. However, the genes and SNPs involved differ between ethnic groups, and only explain a small portion of variability. Genetic variability in CNS immune and opioid signalling pathways should be considered alongside other polymorphisms influencing pain processing and opioid pharmacokinetics. Bringing together multiple mechanisms in a pathway based approach will help to better predict variability in pain and opioid requirements and improve postoperative pain management.

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**The pharmacogenomics knowledge, education, practice and attitudes of hospital pharmacists in Adelaide, South Australia**

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Introduction. The lack of widespread use of pharmacogenomics among pharmacists is partly attributed to pharmacists' lack of knowledge and education on the subject. Currently, there is very limited literature of pharmacists' understanding, education and practice of pharmacogenomics, especially in Australia. Although previous surveys have addressed some of these issues, directly interviewing pharmacists to gain an in depth and broader understanding of these topics, has, to our knowledge, not yet taken place (Clemerson et al, 2006; McMahon et al, 2011).
Aims. To interview hospital pharmacists to investigate their knowledge, education, practice and attitudes with respect to pharmacogenomics.
Methods. Ethics approved semi-structured interviews were carried out with hospital pharmacists in Adelaide, South Australia. The framework approach of qualitative research was used to analyse the data.
Results. Twenty-one pharmacists from 4 public hospitals were interviewed over a 6 month period. Analysis of the data revealed themes including: whether a pharmacist pharmacogenomics role is possible given other activities and time constraints, whether such a role is warranted, a lack of confidence and willingness to engage in pharmacogenomics, and the importance of having timely and relevant pharmacogenomics education.
Discussion. Overall, study interviewees thought that pharmacists could have a greater participation in pharmacogenomics in the future. However, they questioned whether this would be possible at the moment owing to existing models of pharmacy practice and current workloads. Respondents strongly believed that leadership, guidance, regulations and an active interest in pharmacogenomics, would all be essential for the realisation of a pharmacist pharmacogenomics role.

Clemerson JP et al (2006) Pharm World Sci 28:126-130
McMahon T et al (2011) Pharmacy Practice 9:141-147

## 617

**A pharmacogenomic study investigating outcomes in advanced non-small cell lung cancer patients receiving paclitaxel and carboplatin therapy with a focus on ethnic differences**

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Introduction. Genetic variability can influence response and toxicity to paclitaxel and carboplatin in patients with non-small cell lung cancer (NSCLC). Additionally, the prevalence of variations can differ between ethnic groups and may account for observed interethnic variability in drug efficacy.
Aims. To undertake a PG investigation to account for differences in patient variability between Caucasians and Asians in order to improve dosing and patient selection in NSCLC patients.
Methods. 70 advanced NSCLC patients from Caucasian (n = 51) and Asian (n = 19) descent receiving paclitaxel and carboplatin at CRGH from 2007-2011 participated in a candidate gene study associating response and toxicity to 31 SNPs selected from 17 candidate genes. Patient outcomes were assessed according to CTCAE v 4.0 and PK data for paclitaxel and carboplatin was obtained (n = 62). SNPs were assessed for allele frequency differences between Asians and Caucasians and then regression analysis was undertaken to associate SNPs with toxicities, response and drug PK.
Results. Regression analysis identified 6 SNPs in genes *ERCC1, XRCC1, GSTP1, ATP7A* and *CCND1* that associated with toxicity (leukopenia, gastrointestinal and muscle pain). SNP rs2227291 in *ATP7A* was associated with response. SNP rs776476 in *CYP3A5* was associated with paclitaxel CL. Of the SNPs identified in regression only rs776476 in *CYP3A5* had differences in SNP prevalence between Asians and Caucasians ($\chi^2 = 12.4$, $p < 0.01$).
Discussion. The PG study identified SNPs affecting various toxicities, response and PK of paclitaxel, these SNPs could be integrated into future personalisation efforts of paclitaxel and carboplatin to improve drug efficacy. SNP rs776476 in *CYP3A5* may account for some interethnic variability in outcomes as it had different prevalence rates between Asian and Caucasians and had effects on paclitaxel PK. Continuation of the study is anticipated with a larger patient cohort required to further validate the results.

**Impact of recipient and donor multidrug resistance protein 2 genetic variability on mycophenolic acid pharmacokinetics following kidney transplantation**

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Introduction. Multidrug resistance protein 2 (MRP2), a membrane efflux transporter expressed on hepatocyte canalicular membranes, is encoded by *ABCC2*, for which a number of single nucleotide polymorphisms (SNPs) (*C-24T, G1249A, C3972T*) have been reported (Haenisch et al, 2006; Naesens et al, 2006). MRP2 plays an important role in enterohepatic recirculation of the immunosuppressant mycophenolic acid (MPA) and its metabolites (Kobayashi et al, 2004), therefore *ABCC2* polymorphisms may affect MPA pharmacokinetics. There are conflicting reports regarding the role of MRP2 on MPA pharmacokinetics, however the role of *ABCC2* haplotypes associated with high (CAC) or low (CGT, TGC, TGT) protein expression/activity (Laechelt et al, 2011) has not been considered.

Aims. To investigate the impact of recipient and donor *ABCC2* haplotypes on MPA pharmacokinetics in the first two weeks following kidney transplantation.

Methods. This was a retrospective study in 97 transplant recipients and 67 donors. *ABCC2* genotyping (C-*24T*, *G1249A*, *C3972T*) was performed with PCR-RFLP using DNA extracted from blood or graft tissue. Pharmacokinetic analysis was based on therapeutic drug monitoring data from recipients in whom abbreviated AUC (0-6 hr) monitoring had been carried out within 14 days of transplantation (n=43).

Results. Genotype frequencies conformed with Hardy-Weinberg equilibrium ($P>0.1$). Linkage disequilbrium was observed between the *C-24T* and *C3972T* SNPs ($D'=0.72$, $r^2=0.29$, $P<0.0001$). There was no significant difference between recipients and donors in allele, genotype or haplotype frequencies ($P>0.1$). Although there was no difference in dose-corrected average MPA plasma concentrations, dose-corrected MPA trough concentrations were 2-fold higher in recipients with high- (n=5) compared to low-expressor (n=27) *ABCC2* haplotypes ($P<0.05$). There was no effect of donor haplotypes on MPA pharmacokinetics.

Discussion. Higher MPA trough concentrations in recipients with high expressor haplotypes is consistent with increased enterohepatic recirculation. Further investigation is required to determine whether this observation translates into a significant effect on clinical outcomes.

Haenisch et al (2006) Pharmacogenomics J 7:56-65
Kobayashi et al (2004) J Pharmacol Exp Ther 309:1029-1035
Laechelt et al (2011) Pharmacogenomics J 11:25-34
Naesens et al (2006) Transplantation 82:1074-1084

## 620

**Characterisation of spontaneous activity in the human prostate gland**

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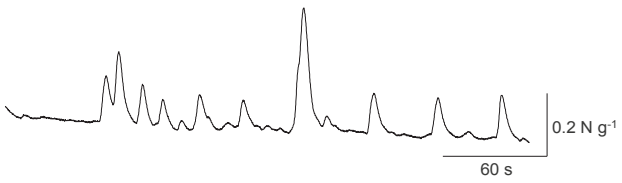


Introduction. Changes in spontaneous electrical activity promote an increase in prostatic tone and contractility in the guinea pig prostate gland (Dey A et al, 2009). These contractions are likely to be regulating the resting smooth muscle tone of the prostate gland, a major component implicated in Benign Prostatic Hyperplasia (BPH); the most common neoplasm in men. BPH occurs in the transition zone (TZ), as opposed to the peripheral zone (PZ). However, the aetiology of BPH remains poorly understood, and the fundamental reason there is an increase in prostatic smooth muscle tone with age remains unknown. Our overall hypothesis is that age-related changes in the mechanisms regulating spontaneous activity of the prostate gland, significantly contribute to the pathogenesis of BPH.

Aims. In this study, we characterised the spontaneous contractile activity in prostate specimens from 12 men.

Methods. TZ and PZ specimens were obtained from consenting patients undergoing a prostatectomy. Subsequent recordings were made from prostatic preparations (3mmx10mm) using conventional tension recording experiments.

Results. All specimens from the TZ, as shown, displayed spontaneous contractions at 1.94±0.20 $min^{-1}$, with a resting tone of 4.86±0.39 mN (n=12). Spontaneous contractions were abolished in 71% of TZ preparations by an L-type $Ca^{2+}$ channel blocker, 1μM nifedipine (n=7). Preliminary results using neurotransmission blockers, 1μM tetrodotoxin (n=3), 1μM guanethidine (n=4), and 1μM atropine (n=5), had no significant effects on frequency of spontaneous contractions in the TZ ($P>0.05$). Spontaneous contractions in the PZ (n=4) were significantly more frequent at 4.47±0.59 $min^{-1}$ ($P<0.05$), and at a significantly lower resting tone of 2.27±0.33 mN ($P<0.001$), in comparison to the TZ in this preliminary study.

Discussion. This study suggests that spontaneous contractions in the TZ may be myogenic in nature. Furthermore, mechanisms regulating spontaneous contractility may be zone-specific. This study provides novel insight into the basic physiology of the human prostate gland.

Dey A et al (2009) J Urol 181(6):2797-2805

## 621

**Effects of stinging nettle leaf extract on smooth muscle contractility in the isolated rat prostate gland.**

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Introduction. *Urtica dioica* (stinging nettle) is used worldwide as an alternative to conventional pharmacotherapies for benign prostatic hyperplasia (BPH). The root extract is thought to decrease the size of the prostate gland, while the leaf extract has also been used in traditional medicine for the relief of lower urinary tract symptoms (Sezik et al, 2001), as well as hypertension (Ziyyat et al, 1997). This vasorelaxant effect may infer a relaxant effect in the prostatic smooth muscle.

Aims. This study aimed to investigate the acute effect of stinging nettle root and leaf extract on prostatic contractility, and to elucidate the bioactives.

Methods. Liquid-liquid partitioning was employed to separate the extract into aqueous and organic phases. Isolated organ bath studies were conducted to investigate the effect of stinging nettle extracts (500mg/ml in 25% ethanol), and the partitioned phases on electrical field stimulated (EFS) (1 ms pulse duration, 60V, 10 pulses at 0.1 – 0.5 Hz, 10 seconds at 1.0 – 20.0 Hz) and agonist induced contraction in rat prostates.

Results. Whole leaf but not root extract attenuated EFS ($n = 6$; $P < 0.001$), adenosine 5'-triphosphate (ATP) (10 nmol/L – 1 mmol/L) ($n= 6$; $P < 0.001$) and αβmethylene ATP (3 nmol/L – 10 $\mu$mol/L) ($n = 6$; $P < 0.001$) induced contraction of the isolated rat prostate gland. The aqueous phase of leaf extract exhibited similar results, whereas the organic phase did not elicit any biological activity.

Discussion. Attenuation of ATP and αβmethylene ATP induced contraction implies the extract engenders an effect either at $P2X_1$-purinoceptors or along the intracellular pathway activated by ATP.

Sezik E, Yesilada E, et al (2001) J Ethnopharmacol 75:95-115.
Ziyyat A, Legssyer A, et al (1997) J Ethnopharmacol 58:45-54.

## 622

**Depressed contractile responses of the bladder detrusor and urothelium/lamina propria following luminal administration of the cytotoxic agent gemcitabine**

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Introduction. Intravesical administration of agents for the treatment of superficial bladder cancer often cause urological side effects (urgency, frequency, dysuria) and for doxorubicin these adverse effects are associated with enhanced urothelial contractions and also enhanced neurogenic detrusor contraction. Intravesical treatment with gemcitabine has similar efficacy to doxorubicin, but is associated with fewer side effects.
Aims. To investigate the effects of luminally applied gemcitabine on bladder responses.
Methods. To mimic intravesical treatment, isolated porcine bladder was incubated in gassed Krebs-bicarbonate solution containing a therapeutic concentration (40mg/mL) of gemcitabine administered to the urothelial surface at 37°C for 1 hour. Following this luminal treatment, strips of intact bladder, strips of detrusor smooth muscle and strips of urothelium with lamina propria were set up in organ baths and responses to carbachol and/or electrical field stimulation (EFS, 1-20Hz) were obtained. Responses of gemcitabine-pretreated tissues were compared to tissues receiving control-pretreatment.
Results. In all tissue types (detrusor, urothelium/lamina propria and intact bladder), responses to carbachol in gemcitabine pretreated tissues were depressed compared to controls. While the pEC50 between the control and treated bladders were similar, the maximal responses were significantly reduced in the detrusor (57.66±8.50g control vs. 31.26±3.40g gemcitabine, n=8, $P<0.05$) and urothelium (73.86±7.42g control vs. 39.34±6.98g gemcitabine, n=8, $P<0.01$). Nerve mediated contractile responses of the detrusor muscle to electrical field stimulation were also reduced by approximately 50% at each frequency by gemcitabine, although these changes were not statistically significant ($P>0.05$).
Discussion. . Contractile responses of the detrusor and urothelium/lamina propria were depressed after pretreatment with gemcitabine. These results contrast with the enhanced responses previously reported with doxorubicin and may explain the fewer adverse effects observed in patients following intravesical treatment with gemcitabine.

## 623

**Distribution of 5-HT receptors and interacting proteins in human colonic tissue layers**

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Introduction. Several disorders of the gastrointestinal tract are associated with abnormal serotonin (5-HT) metabolism and/or signalling where the serotonin receptors of clinical relevance include 5-$HT_3$ and 5-$HT_4$ receptors. 5-$HT_3$ receptors interact with RIC3, whereas 5-$HT_4$ and 5-$HT_7$ receptors interact with GRKs and Lin 7 homologues.
Aims. To examine the distribution of 5-$HT_3$, 5-$HT_4$ and 5-$HT_7$ receptors in the human colon and how this is associated with RIC3, GRKs and Lin 7 homologues to extend previous observations limited to the sigmoid colon.
Methods. Human colon samples from the ascending (n=3), transverse (n=3), descending (n=3) and sigmoid colon (n=7) were dissected into 3 separate layers (mucosa, longitudinal and circular muscles). In addition, ileum (n=4) samples were dissected into mucosa and muscle layers. RNA samples were extracted and amplified by RT-PCR and expression was determined quantitatively or by end point PCR.
Results. 5-$HT_4$ and 5-$HT_7$ receptors were expressed throughout the colon with possibly less transcripts evident in the transverse colon which also correlates with receptor expression level in the ileum. Similar levels of expression of GRKs (2, 3, 5 and 6) and Lin 7 (A, B and C) homologues were observed in all regions of the colon. 5-$HT_{3A}$ receptor expression was detected throughout the colon while $5HT_{3E}$ receptor was mainly found in the mucosa or longitudinal muscle layers and the 5-HT3B and C subunits were observed less frequently.
Discussion. We have previously described the expression pattern of 5-HT receptors and GRKs in the human sigmoid colon (Chetty et al. 2009). This study extends these findings to develop a distribution map of the clinically relevant serotonin receptors and their interacting proteins throughout the colon that can be used to inform future studies.

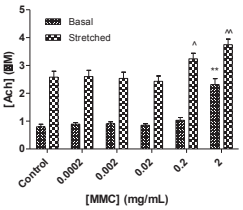
Chetty N et al. 2009 Neurogastroenterol Motil 21:551–58.e15

## 624

**Mitomycin C alters urothelial ATP, acetylcholine and $PGE_2$ release *in vitro***

Sung-Hyun Kang[1], Russ Chess-Williams[1] & Catherine McDermott[1]. Bond University[1], Gold Coast, Queensland.

Introduction. Intravesical chemotherapy is commonly used for the treatment of superficial bladder cancer. There is evidence of significant local adverse effects including contact dermatitis and symptoms of bladder overactivity. Doxorubicin has been shown to increase Ach and $PGE_2$ release in human urothelial cells (Chess-Williams et al, 2012). Effects of mitomycin C (MMC) have not been investigated despite evidence of greater toxicity.




Aims. This study investigates the immediate effects of the chemotherapeutic agent MMC on basal and stimulated mediator release (Ach, ATP and $PGE_2$) from a human urothelial cell line (RT4).

Methods. RT4 cells were treated with range of MMC concentration for 2 hours at 37°C. Immediately following treatment samples were prepared for analysis of basal and stimulated mediator release by incubating cell cultures in normal or hypotonic (50% normal [NaCl]) Krebs solution respectively for 15 minutes. The level of Ach, ATP and $PGE_2$ in these samples was measured using commercially available kits and compared to release from untreated vehicle control.

Results. Immediately following MMC treatment, basal Ach release from RT4 cells at its clinical concentration (2 mg/mL) increased significantly compared to the untreated vehicle control. Stimulated Ach release also increased significantly compared to the untreated vehicle control at MMC concentration $\geq$ 0.2 mg/mL (n=5, *P<0.05 and **P<0.01). A concentration dependent decrease in both basal and stimulated ATP release was observed from RT4 cells immediately following treatment. A similar decrease in basal and stimulated release of $PGE_2$ from RT4 cells was also observed immediately following treatment.

Discussion. The findings indicate that release of Ach, ATP and $PGE_2$ from RT4 cells is affected immediately following MMC treatment at clinically relevant concentrations and durations of treatment. Changes in urothelial signalling may relate to the adverse effects elicited by MMC treatment in patients.

Chess-Williams R (2012) Proc. Int. Cont. Soc. (Beijing) Abstract number 14306

## 625

**Effects of acrolein, a metabolite of Cyclophosphamide and Ifosfamide, on cultured human urothelial cells**

Kylie A Mills[1], Catherine McDermott[1], Russ Chess-Williams[1]. Faculty of Health Sciences & Medicine, Bond University[1], Gold Coast, QLD.

Introduction. Cyclophosphamide and ifosfamide are commonly used anticancer agents. A major limiting factor in their use is the resulting bladder toxicity which can result in ongoing bladder pain, urgency and dysuria. These drugs and their metabolites come into contact with the urothelium when they are excreted in the urine, potentially damaging the urothelium.

Aim. To investigate the effects of cyclophosphamide, ifosfamide and acrolein, on human urothelial cell viability and function.

Methods. Human urothelial cells (RT4) were treated with cyclophosphamide or ifosfamide (0.01–100$\mu$M) or acrolein (0.01–100$\mu$M) for 24 hours. Following treatment, cell viability and ROS formation were measured. Basal and hypotonic stretch-stimulated ATP and acetylcholine release were also determined.

Results. Treatment with acrolein resulted in a significant decrease in cell viability and a 2.5-fold increase in ROS formation at a concentration of 100$\mu$M. Basal and stimulated acetylcholine release was not altered by acrolein treatment, however at a concentration of 100$\mu$M acrolein caused a 5-fold increase in basal and 2.5-fold increase in stimulated ATP release (Figure 1).

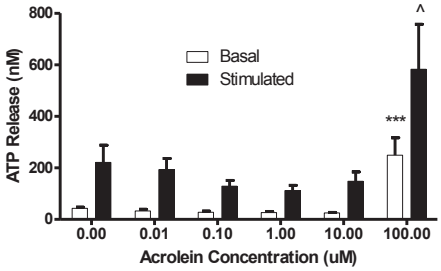



*Figure 1: Effect of 24hour acrolein treatment on basal and stimulated release of ATP from human urothelial cells. *** P<0.001 compared to control basal, ^ P<0.05 compared to control stimulated.*

Discussion. Acrolein (100$\mu$M) alters urothelial cell viability and ROS production, while the parent drugs (100$\mu$M) do not. Stretch of the urothelium during bladder filling is known to stimulate the release of ATP which acts on low threshold Aδ sensory nerve fibres in the suburothelium to initiate the micturition reflex. At high concentrations it may act on high threshold nerve fibres to give rise to perceptions of pain. Exposure of urothelial cells to acrolein also caused a large increase in the basal and stimulated release of ATP and this may contribute to the bladder pain, urgency and dysuria seen after cyclophosphamide or ifosfamide treatment.

## 626

**P-glycoprotein expression level in treatment - resistant *Helicobacter pylori* patients**

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Introduction. There appears to be an increasing incidence of *Helicobacter pylori* becoming more resistant to antibiotic therapy, which is resulting in a reduction in complete *H. pylori* eradication in patients.
Aims. We aimed to assess the P-glycoprotein expression levels among subjects who were *H. pylori*-positive and received multiple courses of eradication therapy (resistant group) to determine whether the presence of *H. pylori* increased the expression of this efflux protein. The profile of the *MDR1 C3435T* polymorphism also been investigated.
Methods. Eleven subjects were recruited for this study during their hospital visit for upper gastrointestinal examinations. *H. pylori* infection status was confirmed by rapid urease test and bacterial culture. Antibiotic sensitivity testing was performed by E-test. P-glycoprotein expressions from the antral and duodenal biopsies were measured by Western Blot. Genotyping for *MDR1 C3435T* of each resistant subject was performed using polymerase chain reaction and restriction fragment length polymorphism analysis. The data was compared with two other groups, recruited from our previous study, namely *H. pylori*-negative (n=54) and *H. pylori*-positive but treatment naive (n=22).
Results. The resistant group did show higher P-glycoprotein expression levels (antrum over duodenum ratio) compared to the *H. pylori*- negative group (p = 0.0361). The levels of P-glycorprotein expression in the resistant group was observed to be similar to *H. pylori*-positive but treatment naive group (p=0.319). In the resistant group, all three *MDR1 C3435T* genotypes showed an increasing trend of P-glycoprotein expression with the presence of *H. pylori*. Most subjects demonstrated resistance to clarithromycin (72%), metronidazole (63.6%) or both (54.5%).
Discussion. *H.pylori* infection induces the expression of P-glycoprotein in antrum. Increasing P-glycoprotein at the gut level may assist antibiotic therapy for *H. pylori* if the drug regime chosen consisted of P-glycoprotein substrate due to the increased duration and drug levels outside the cells where the bacteria resides.

## 627

**Nerve-evoked and phasic contractions of the rat bladder: effects of low testosterone and treatment with the selective androgen receptor modulator trenbolone**

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Introduction. Overactive bladder is prevalent with aging and recent evidence suggests androgen deficiency may play a role (Koritsiadis et al., 2008). Androgen receptors are expressed throughout the bladder (Chalvamane et al., 2010), although the role of testosterone in bladder function remains unclear.
Aims. To investigate the effect of low testosterone and treatment with a selective androgen receptor modulator on contractility of rat bladder strips.
Methods. Wistar rats (8 weeks) were orchiectomised (5% isoflurane). 8 weeks later, half received trenbolone acetate (2mg/kg/day for 8 weeks, sc.). Sham-operated controls received vehicle. Isolated bladder strips were mounted in tissue baths (Krebs-bicarbonate solution, 1.5g tension, 37°C). Amplitude and frequency of phasic contractions (PCs) and nerve-evoked contractions (EFS) (1-50Hz, 0.01ms duration, 40V, 5s every 100s) were examined.
Results. Orchiectomised rats had low serum testosterone vs controls and trenbolone-treated (0.24±0.05 vs 1.68±0.18 vs 0.21±0.04ng/ml, $P<0.001$). Amplitude of PCs was increased in orchiectomised rat bladder strips (0.0266±0.0025 vs 0.0016±0.0027g/mg, $P<0.05$), whilst frequency was reduced (32±5 vs 50±5 events/5mins, $P<0.05$). Trenbolone-treatment prevented the increased amplitude, but not the decreased frequency. EFS contractions were depressed in orchiectomised bladder strips and α,β-methylene-ATP (10$\mu$M) produced greater inhibition vs controls (80.3±2.8% vs 6.6±3.8%, $P<0.01$). Trenbolone-treatment did not prevent depressed EFS contractions, but did prevent the increased purinergic component. Atropine (1$\mu$M) plus α,β-methylene-ATP completely abolished EFS responses in orchiectomised bladder strips, but not in control and trenbolone-treated, where the remaining response was unaffected by L-NNA (100$\mu$M).
Discussion. Orchiectomy causes increased phasic contractions of rat bladder strips and depressed nerve-evoked contractions, in which ATP plays a greater role, supporting a role for testosterone in normal bladder function. Trenbolone prevented only some of these alterations, suggesting the actions of testosterone may be partly mediated via conversion to other sex steroids.

Chalvamane et al. (2010) J Sex Med 7(8):2698-713
Koritsiadis et al. (2008) BJU Int 101:1542-46

## 628

**Low prevalence of Helicobacter pylori infection among patients with ulcerative colitis in Ukraine**

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Introduction. The current prevalence of H. pylori infection (HPI) in Ukraine is more than 70%. In particular, the possibility that Helicobacter organisms play a role in human UC has been debated but not comprehensively investigated. Although a number of recent studies in Western countries have reported increased prevalence of enterohepatic Helicobacter species in the intestinal tracts of IBD patients, the role of these organisms remains controversial.
Aims. The aim of this study was to determine with what is connected the low prevalence of HPI among patients with UC and to investigate the prevalence of Helicobacter species in the intestinal mucosae.
Methods. We have examined 105 adult patients (56 female and 49 male) with UC and 103 non-UC adults. The median age of patients was 38,3 ±12,8 years. For detecting Helicobacter organisms we used blood antibody test, urea breath test, stool antigen test, polymerase chain reaction (PCR), gastro-intestinal mucosal biopsies.
Results. HPI was determined in 26, 67 % of UC patients compared to 44.3% in controls. There was no correlation between the age, gender or extent of disease. The prevalence of Helicobacter enterohepatic species was significantly higher 32 of 105 (31%) in UC group versus 8 of 103 (8%) in controls ($p < 0.0001$). The HI rate in UC patients who had previously used metronidazole or ciprofloxacin was considerably lower (21,5%) than the rate in controls (56%) ($p<0,005$). Intake of other drugs (5-ASA, corticosteroids, and immunosuppressants) had no significant influence on HPI and H.enterohepatic ($p> 0.001$).
Discussion. The lower prevalence could be attributed to previous antibiotic treatment, and contrary, lower prevalence of UC in Hp-infected patients may be due to protective effect of Hp or non-pylori Helicobacter organisms. These relationships may open new avenues to study the pathogenesis of IBD.
References. Sonnenberg A (2012) Alimentary Pharmacology & Therapeutics Vol. 35(4):469-476.

## 630

**Complexity in 5-$HT_3$ receptors: An optimized expression system for electrophysiology studies**

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Introduction. 5-$HT_3$ receptors fall in the family of ligand-gated cation channels. Their presence in both central and peripheral nervous systems has been implicated a range of clinical disorders such as irritable bowel syndrome, chemotherapy-induced nausea, schizophrenia and autism. 5-$HT_3$ receptors are composed of different types of subunits (A, B, C, D and E) and the complexity is increased as SNPs in the subunits have been proposed to contribute to the clinical associations (Yaakob et. al 2009).
Aims. To establish a recombinant cloning system for efficient expression of multiple 5-$HT_3$ receptor subunits in mammalian cells. This is to ultimately examine whether different 5-$HT_3$ receptor compositions and clinically-relevant SNPs in recombinant 5-$HT_3$ receptors produce different electrophysiological activities.
Methods. cDNA encoding 5-$HT_{3C}$, 5-$HT_{3E}$ and 5-$HT_{3A}$ subunits plus GFP were all cloned into one multi-cistronic vector using the Multisite Gateway® System. The genes were linked via viral 2A sequences to produce non-fused subunits and GFP as a marker of successful expression. Constructs were transiently transfected into HEK293T and COS7 cells and expression studied by western blotting and confocal microscopy. Quantification of GFP expression was performed using FACS. Receptor electrical activity is being studied using patch-clamp.
Results. Western blots confirmed expression of unfused receptor subunits and GFP at correct sizes. Confocal images showing GFP expression were indicative of successful expression of all receptor subunits at cellular level. Approximately 20% of cells were GFP positive in both HEK293T and COS7 transfections.
Discussion. Optimization of gene expression with a definitive marker prior to patch-clamping studies is vital to ensure electrical recording from the cells do represent our receptors of interest. We obtained robust GFP expression level of ~20% which is sufficient for patch-clamping reliably in each population of transfected cells.

Yaakob N et al. (2011) Current Molecular Medicine 11:57-68

## 632

**Changes in vimentin distribution accompany acrolein toxicity in epithelial lung cells: Association with protein adduct distribution**

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Introduction. The toxic α,β-unsaturated aldehyde acrolein is a common environmental air pollutant. It is highly concentrated in smoke and is a major edematogenic compound during smoke inhalation injury (SII) pathogenesis. Exactly how acrolein causes SII-related pulmonary edema is poorly understood. Since acrolein is highly reactive with cytoskeletal proteins, one potential mechanism involves the disruption of cytoskeletal function in lung epithelial cells. At present, little information relates chemical damage to individual cytoskeletal proteins with changes in their subcellular distribution.
Aims. This project used immunochemical approaches to assess adduction and distribution of the key cytoskeletal proteins actin, vimentin and tubulin in lung epithelial cells following exposure to acrolein.
Methods. A549 human lung epithelial cells were exposed to various concentrations of acrolein (25 to 100 $\mu$M) for 30 minutes. Western blot analysis of cell lysates was performed for protein carbonyls, actin, tubulin and vimentin. For immunocytofluorescent microscopy, A549 cells were grown on glass coverslips, exposed to a range of acrolein concentrations and subsequently stained for microfilaments, tubulin, vimentin or protein carbonyls.
Results. Western blot analysis revealed vimentin adduction occurred at low acrolein concentrations, whilst tubulin and actin were damaged at higher concentrations. Immunocytofluorescent microscopy revealed strong co-localisation of protein carbonyl adducts and vimentin staining, with the latter becoming increasingly perinuclear with increasing acrolein concentrations. Acrolein-induced changes in tubulin or actin distribution were less obvious.
Discussion. The finding that vimentin is vulnerable to acrolein concurs with previous findings from our group (Burcham et al., 2010). If it occurs in intact tissue, damage to this key cytoskeletal component may contribute to the loss of watertight properties in respiratory epithelium during intoxication with acrolein-containing smoke.

Burcham PC et al (2010) Antioxid Redox Signal 12: 337-47

## 633

**Acute acrolein exposure produces molecular, morphologic and functional changes in airway epithelium as investigated in a novel *ex vivo* mouse tracheal perfusion system**

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Introduction. Acrolein is a primary toxic constituent of smoke and is implicated in smoke inhalation injury. However, our understanding of acrolein toxicity arises principally from cell culture models.
Aims. To examine the effect of acute, high-dose acrolein exposure on molecular, morphologic and functional characteristics of intact airway epithelium using a novel *ex vivo* perfusion system.
Methods. Perfused murine tracheal segments were exposed to acrolein (or vehicle), and examined for evidence of molecular, morphologic and functional changes utilising immunohistochemical staining for protein carbonyls, PAS staining for mucin and isometric tension changes.
Results. Exposure to acrolein (200μM for 30min) caused pathologic changes to the epithelium including loss of cilia (at 24h post-exposure) and regional sloughing (48h). Acrolein-induced effects on epithelial morphology were preceded by elevated immunostaining for protein carbonyls (marker of oxidative damage) in the nuclei of epithelial and smooth muscle cells. In functional studies, quantitative analysis of PAS-stained sections revealed an acrolein induced dose-dependent reduction in epithelial mucin stores (75% reduction, n=3-5 mice, $p<0.01$ compared to vehicle), similar to the levels of mucin release induced by the known mucin secretogogue ATP (100mM, n=3). In addition, acrolein exposure impaired formation of new mucin stores (n=3, $p<0.001$). In isometric tension recording studies, acute exposure of murine tracheal segments to acrolein (200mM for 30min) did not affect responses to the epithelial-dependent relaxant substance P or the smooth muscle relaxant $PGE_2$ (n=4-6 mice).
Discussion. Acrolein causes significant dose- and time- dependent molecular, morphologic and functional changes in airway epithelium, as revealed using an innovative *ex vivo* mouse tracheal perfusion system. Results from this novel study may lead to a clearer understanding of the acute toxicological effects of acrolein responsible for the pathobiology of smoke inhalation injury.

## 634

**Expression of TLRs and GFAP in the rat intestine following chemotherapy for cancer and relationship to gut toxicity and central pain behaviour**

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Introduction. Gastrointestinal mucositis (GM) and pain are major clinical problems caused by the cytotoxic effects of chemotherapy. Previous research has indicated that toll-like receptor (TLR) expression may be altered following chemotherapy and correlate with severity of GM and pain.
Aims. To determine if TLR expression and activation of gut glial cells (GFAP expression) is related to GM and pain behaviour in our tumour-bearing rat model.
Methods. Female DA rats received irinotecan (175 mg/kg, ip n=35) or vehicle control (n=5) and assessed over 5 days for markers of GM (diarrhoea, weight loss) and pain (facial grimace). Groups of rats (n=5-8) were killed between 6 and 120 h. Immunohistochemistry for TLRs 2, 4, 5, and 9, and GFAP was conducted on sections of jejuna and colon.
Results. Irinotecan caused bi-phasic GM, with maximal diarrhoea at 72 h. Similarly peak weight loss occurred at 72 h (11.1±6.6%) before recovery at 120 h (-0.25±6.7%, $P<0.0001$). Irinotecan also elevated pain scores peaking at 72 h: median (range) 5 (0-5) versus 0 (0-0) in control animals, $P<0.0001$. At 96 and 120 h irinotecan significantly decreased jejuna expression of TLR4 and 5 (both $P<0.001$), but TLR2/9 expression was unchanged. Jejunum GFAP expression also increased significantly, with peak expression by 96 and 120 h ($P=0.017$). Jejunum expression of TLR4, 5 and GFAP was significantly associated with occurrence of diarrhoea and facial pain scores ($P<0.001$), and rats with diarrhoea had higher facial pain scores compared to those without: median (range) of 2 (0-5) versus 0 (0-5), $P=0.01$.
Discussion. Intestinal innate immunity activation and inflammation caused by chemotherapy potentially modifies central inflammation manifested as pain. As TLR4/5 expression decreased during the GM healing phase, TLR pharmacological inhibition may promote healing in the small intestine following chemotherapy. Impact of tumour-burden on gut TLR expression and glial activation requires further investigation.

## 635

**Safety and toxicity profile of fenugreek**

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Introduction. Fenugreek has been traditionally used by breastfeeding mothers to stimulate milk production. However, there is no current safety data on the use of fenugreek during breastfeeding. It is a concern that the active components of fenugreek may be passed on to the breastfed infants through ingestion of breast milk.
Aims. This study examined the effects of methanol, ethanol and aqueous extracts of fenugreek on 3T3 and MCF-7 cells to assess its toxicity profile. The effects of fenugreek on P-glycoprotein were studied using Caco-2 cells because the transporter plays an important part in the excretion of xenobiotics into the breast milk.
Methods. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay was employed to investigate the effect of fenugreek extracts on various cell lines. Cells response to different concentrations of fenugreek extracts was also determined by examining cell morphological changes. The transport study and multidrug resistance direct dye efflux assay (MDR1 efflux assay) were used to examine the impact of fenugreek on P-glycoprotein activities.
Results. Methanol extract of fenugreek (MEF) induced signs of toxicity in 3T3 and MCF-7 cells at high concentrations (50$\mu$g/mL, 100$\mu$g/mL, and 250$\mu$g/mL), with cell viability down to 10% at 100$\mu$g/mL. However, Rh123 efflux was unchanged with a 6 fold efflux ratio for both normal Caco-2 monolayers and those incubated with 50$\mu$g/mL fenugreek extract. Rates of cellular removal of Rh123 and DiOC2(3) were also not affected by fenugreek in our MDR1 efflux assay.
Discussion. At high concentrations, MEF can indiscriminately cause cell damage to both cancerous and non-cancerous cell lines. However, low concentrations of MEF did not result in substantial cell toxicity. This study shows that fenugreek may not be a P-glycoprotein substrate. It is also unlikely that fenugreek will interact with P-glycoprotein inhibitors, inducers or substrates in terms of their relationships with the transporter.

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**Structure toxicity studies of drugs implicated in immune mediated idiosyncratic hepatotoxicity**

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Introduction. Idiosyncratic drug induced liver injury (DILI) can have serious human, economic and commercial consequences and investigation of its mechanisms is hindered by its rare and unpredictable nature (Uetrecht, 2007). The prominent hypotheses regarding mechanisms and risk factors for DILI involve either the parent drug or its metabolites instigating toxic reactions. The immune system is thought to be involved, with 25-30% of idiosyncratic DILI presenting with the classic immunogenic features fever, rash and eosinophillia (Czaja, 2011). Aim. To identify pharmacophoric elements associated with immune-mediated hepatotoxicity (IMDILI). Methods. Drugs implicated in IMDILI were identified using Australia's Adverse Drug Reaction reporting system (ADRS) database. Disproportionality between expected and observed odds for reporting a combination of immune and hepatic terms indicative of IMDILI was determined using multivariate logistic regression analysis. The IMDILI potential of the identified toxic drugs was confirmed by reviewing published literature. Each toxic drug was grouped with its metabolites for subsequent pharmacophore modelling using Schrödinger's PHASE program. External validation was conducted on a set of drugs attrited due to DILI and non-toxic drugs (each with >300 records in the ADRS database and no reports of IMDILI). Drugs which lacked literature reports of idiosyncratic hepatotoxicty with immune features were excluded from the toxic validation set. Drugs which had literature reports of DILI of any nature were excluded from the non-toxic validation set. Results. Of 249 drugs investigated, 16 drugs were identified as significantly associated with IMDILI ($p<0.0002$). 4 of these drugs were excluded from further analysis. PHASE returned a number of 4-point pharmacophore hypotheses which matched 8 of the 12 drug/metabolite groups identified as toxic. External validation of these hypotheses yieled specificies and sensitivies of approximately 37% and 88% respectively. Discussion. Further investigation of structural similarities and potential targets may aid in developing useful predictive tests for currently unpredictable DILI.

**Mulga snake (*Pseudechis australis)* envenoming: a spectrum of myotoxicity, anticoagulant coagulopathy, haemolysis and the role of early antivenom therapy - Australian Snakebite Project (ASP-18)**

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Introduction. Mulga snakes (*Pseudechis australis*) are venomous snakes with a widespread distribution in Australia.
Aims. To describe the clinical effects of mulga snake envenoming and the response of envenoming to antivenom therapy.
Methods. Definite mulga bites, based on expert identification or venom specific enzyme immunoassay, were recruited from the Australian Snakebite Project. Demographics, information about the bite, clinical effects, laboratory investigations and antivenom treatment were recorded for all patients. Blood samples were collected to measure venom concentrations pre and post antivenom by ELISA.
Results. There were 17 patients with definite mulga snake bites. The median age was 37 years old (6 to 70y). Thirteen patients had systemic envenoming with systemic symptoms (11), anticoagulant coagulopathy (10), myotoxicity (7) and haemolysis (6). Antivenom was given to ten patients; median dose was one vial (1 to 3 vials). Three patients had systemic hypersensitivity reactions. Antivenom immediately reversed the coagulopathy in all cases, and appeared to prevent myotoxicity in 3 patients with high venom concentrations, each given antivenom within two hours of the bite. Median peak venom concentration in 12 envenomed patients with samples was 29ng/mL (Range: 0.6 to 624 ng/mL). There was a good correlation between venom concentrations and area under the curve of the creatine kinase for patients receiving antivenom after two hours. Higher venom concentrations were also associated with coagulopathy and haemolysis. Venom was not detected after antivenom administration except in one patient who had a venom concentration of 8.3ng/ml after one vial of antivenom, but immediate reversal of the coagulopathy.
Discussion. Mulga snake envenoming is characterised by myotoxicity, anticoagulant coagulopathy and haemolysis and has toxicity that is venom dose dependant. This study supports a dose of one vial of antivenom, given as soon as systemic envenoming is identified, rather than waiting for the development of myotoxicity.

## 638

**Resveratrol does not protect against paracetamol-induced cell death in mouse primary hepatocytes**

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Introduction. Current treatment of paracetamol-induced hepatotoxicity is the glutathione pre-cursor N-Acetyl Cysteine (NAC) (Daly et al, 2008). Resveratrol has been identified as a potential agent for protecting against paracetamol toxicity in animal studies (Sener et al, 2006), but it has not been tested *in vitro*.
Aims. To investigate resveratrol as an intervention to prevent acute high dose paracetamol toxicity in primary hepatocytes from mice.
Methods. Hepatocytes were isolated from male C57BL/6 mice by collagenase perfusion and plated on Collagen I coated dishes, with Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum. After 2.5 hours the medium was changed to serum-free media for 16 hours. The hepatocytes were treated with paracetamol (20mM), ethanol (0.25%), NAC (25mM), resveratrol (50$\mu$M in ethanol) or a combination of these treatments in medium. Hepatocyte survival was measured with the MTT assay 24 hours post-treatment.
Results. Preliminary results show that cell viability for ethanol (0.25%), NAC (25mM) and resveratrol (50$\mu$M) treatment did not differ from control. 20mM paracetamol resulted in 40.0±12.3% cell death compared to control ($p<0.010$), and concurrent treatment with 25mM NAC maintained cell viability at control levels ($p<0.001$). Concurrent treatment of resveratrol (50$\mu$M) and paracetamol (20mM) resulted in cell viability of 29.6±66.2%, although this was not significantly different from control ($p=0.08$).
Discussion. The unexpected finding that 50$\mu$M resveratrol does not protect against paracetamol induced toxicity in mouse primary hepatocytes may imply a different mechanism of action of resveratrol *in vitro* compared to *in vivo*. Current studies are testing different doses of paracetamol and resveratrol, and investigating possible mechanisms for this finding.

Daly F et al (2008) Med J Aust 188: 296-301
Sener G et al (2006) Hepatology Research 35: 62-68

## 640

**Isolation and characterisation of a procoagulant serine proteinase from the venom of the Eyelash pit viper, *Bothriechis schlegelii*.**

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Introduction. Procoagulant toxins are common components of many Latin American snake venoms and, when isolated, have proven to be useful both clinically and therapeutically.
Aims: In the present study we have used successive steps of reverse phase HPLC in order to isolate the first procoagulant toxin from the venom of the Central American 'Eyelash pit viper', *Bothriechis schlegelii*. This study also aimed to determine whether Instituto Clodomiro Picado (ICP) polyvalent antivenom, which is raised against related species of snakes, would bind and neutralise *B. schlegelii* venom and the toxin.
Methods. Assay driven isolation of the toxin was achieved using an *in vitro* turbidometric clotting assay with human plasma (O'Leary et al, 2010), with clotting activity confirmed following each step of venom fractionation. SDS-PAGE and MALDI-TOF MS were utilised to confirm purity and toxin molecular weight.
Results. The single chain toxin has a molecular weight of 32 kDa as determined by SDS-PAGE, and 27 kDa as determined by MALDI-TOF MS. The partial N-terminal amino acid sequence is VVGGDECNINEHRFL, indicating that the toxin is a snake venom serine proteinase. The toxin represents 2% of *B. schlegelii* venom. Crude venom (3.9-250$\mu$g/mL) and the isolated toxin (0.08-5$\mu$g/mL) induced concentration-dependent shortening of the clotting time of human plasma. Western blotting indicated that ICP polyvalent antivenom binds most venom components, including the toxin. ICP polyvalent antivenom delayed clotting times of plasma in the presence of *B. schlegelii* venom 7-fold (n=6; $P<0.001$) and in the presence of the isolated toxin 5-fold (n=5; $P<0.05$).
Discussion. This study has revealed a novel procoagulant toxin and suggests that ICP polyvalent antivenom could be considered for treatment of *B. schlegelii* venom-induced coagulopathy.

O'Leary MA & Isbister GK (2010) J Pharm Toxicol Methods 61(1):27-31

## 641

**The teratogenic effect of dofetilide during rat limb development and association with drug-induced bradycardia and hypoxia in the embryo.**

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Introduction. Dofetilide is an $I_{Kr}$ blocker ($I_{Kr}$ = rapid component of the delayed rectifying potassium current). Previous studies show dofetilide causes bradycardia in GD13 rat embryonic hearts and teratogenic effects (Abela et al, 2010 and Webster et al 1996). GD13 is a critical time of limb development.
Aims. Test the hypothesis a teratogenic dose of dofetilide administered to GD13 pregnant rats induces embryonic bradycardia causing the embryonic limbs to become hypoxic.
Methods. GD13 rats were treated with dofetilide (single oral, 5 mg/kg) and embryonic heart rates assessed by ultrasound (Vevo770) 2 hours later. Fetuses were examined for malformations at GD20. In a separate experiment, dofetilide treatment of GD13 pregnant rats was followed 2, 4, 12, or 24 hours with iv dosing with the hypoxic marker, pimonidazole (60mg/kg). Embryos were collected and heart rate was assessed *in vitro* and hypoxia in embryo limbs analysed.
Results. A teratogenic dose of dofetilide at a susceptible stage of development (GD13) caused bradycardia on the embryonic heart, temporary hypoxia in the developing limbs (GD13) and abnormal limb development (GD20).
Discussion. Hypoxia may result in abnormal limb development. It is uncertain whether dofetilide would be teratogenic in humans if taken during early pregnancy it's unknown if the human embryonic heart is sensitive to dofetilide.

Abela et al (2010) Birth Defects Research Part B Devel ReproTox 89(5) 429-440.
Webster et al (1996) Teratology 53(3) 168-175

## 642

**Chronic low dose exposure to STX inhibits neurite outgrowth**

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Introduction. The neurotoxin saxitoxin (STX) is produced in both marine and freshwater environments. Its production by cyanobacteria in Australian freshwater makes it a potential public health issue. STX blocks voltage gated sodium channels (VGSC), stopping the inflow of sodium ions and subsequently the generation of action potentials. Acute exposure leads to paralysis and death by respiratory depression and an acute drinking water guideline of 3$\mu$g/L exists. Yet in drinking water the likely pattern of exposure is chronic low doses, about which little is known despite the fact that VGSCs have previously been shown to play a role in proper neurodevelopment (Brackenbury et al., 2008).
Aims. We aimed to determine if chronic low dose exposure to STX could have adverse effects on developing neurons using model neuronal cells.
Methods. PC12 and SHSY5Y cells were grown on poly-L-lysine coated coverslips and treated with STX (0.25-3$\mu$g/L) for 7 days with toxin and growth medium replaced on day 4. Concentrations were chosen based on the 3$\mu$g/L Australian drinking water guideline. Following exposure cells were stained with TRITC-Phalloidin and the number and length of cellular projections were measured.
Results. After 7 days control cells developed a neuronal habit with long axonal like extensions. Following exposure to STX cells remained in a circular habit with numerous short filopodia. Axonal like extensions were significantly reduced ($p<0.05$). These effects were seen in a concentration dependent manner with PC12 cells being more affected then SHSY5Y cells.
Discussion. The results suggest that chronic low dose exposure to STX can inhibit neurite outgrowth. The results are of particular significance as adverse effects were seen even at the lowest concentrations, which are well below the current Australian drinking water guideline and could have implications for the safety of drinking water.

Brackenbury WJ et al (2008) J Neurosci 28:3246-3256

## 643

**Intravenous lipid emulsion does not improve haemodynamics or survival and increases drug concentrations in a rodent model of oral amitriptyline poisoning.**

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Introduction. Lipophilic drugs such as amitriptyline have the potential to cause cardiotoxicity in overdose. Severe poisoning is often resistant to traditional treatments. Intravenous lipid emulsion (ILE) is recommended as rescue therapy for the treatment of such overdoses (possibly via a lipid sink effect), however little is known about the effects of ILE-infusion on drug concentration and haemodynamics in the early/absorptive phase after oral poisoning.
Aims. To assess the effects of ILE on survival, haemodynamics and blood toxin concentrations in an orally poisoned rodent model.
Methods. Thirty minutes after orogastric administration of amitriptyline (70mg/kg), one of 20% Intralipid (ILE), 8.4% sodium bicarbonate (BIC) or Hartmann's solution (HAR) were infused to anaesthetised (pentobarbital, 85mg/kg, i.p.) and ventilated male Wistar rats (n=10 per group). Heart rate (HR), systolic blood pressure, mean arterial pressure, cutaneous ECG-QRS-duration and survival were monitored over 120mins. Blood drug concentrations were also collected during this period.
Results. ILE-infusion significantly decreased survival compared to other treatments (10% ILE v 70% BIC v 70% HAR, $p=0.001$). This was associated with significantly increased blood amitriptyline concentrations at T60, T90 and T120mins compared to the other treatments ($p\leq0.02$); amitriptyline area-under-curve was also significantly greater ($p\leq0.01$). ILE-treatment resulted in wider QRS durations compared to BIC-treatment, significant at T105, 120min ($p\leq0.01$) after commencement of treatment. No differences in blood pressure were observed.
Discussion. Early administration of ILE after oral amitriptyline overdose did not improve survival or haemodynamics compared to controls. Additionally, blood amitriptyline concentrations were higher in the ILE-treated group suggesting that drug absorption from the GI-tract may be augmented if given too early after oral poisoning, with potentially detrimental effects. Similar investigations should be made in oral poisonings with other lipophilic cardiotoxic drugs.

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***In vitro* assessment of chemical sensitisation potential using the human cell line activation test (h-CLAT)**

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Introduction. Allergic contact dermatitis (ACD) is a delayed-type hypersensitivity immune reaction which is mediated by T lymphocytes. Currently, the murine local lymph node assay (LLNA) is the 'method of choice' for screening potential skin sensitisers. However, increasing emphasis on the 3Rs principles of reduction, refinement and replacement in animal testing has gained political and economic momentum. Therefore, there is an urgent need for a panel of validated *in vitro* cell-based assays that can accurately identify skin sensitising agents and so replace the *in vivo* LLNA test in mice.
Aims. Evaluate skin sensitisation potential of chemicals using the *in vitro* human cell line activation test (h-CLAT) assay.
Methods. An *in vitro* cytotoxicity test was used to select concentrations of test chemicals for use in the h-CLAT assay. Briefly, monocytic leukaemia THP-1 cells were incubated with a range of concentrations of the test chemicals for 24 h. Concentrations that resulted in cell viability of 75-95% were selected for use in the h-CLAT assay. Subsequent to test chemical incubation, augmentation of surface molecules, CD54 and CD86 on THP-1 cells was monitored using flow cytometry.
Results. Relative fluorescence intensity (RFI) for both CD54 and CD86 were measured using flow cytometry. Chemicals at any given concentration that resulted in an RFI >200% for CD54 and/or >150% for CD86 were classified as sensitisers. Our data confirm that 2,4-dinitrochlorobenzene (DNCB) and eugenol are sensitisers whereas hexyl cinnamic aldehyde (HCA), methyl salicylate and isoeugenol are non-sensitisers.
Discussion. The *in vitro* h-CLAT assay is an effective method for identification of the sensitisation potential of chemicals. Limitations that remain to be addressed include detection of weak sensitisers and prohaptens.

Ashikaga T et al (2006) Toxicol in vitro 20:767-773.
Sakaguchi H et al (2006) Toxicol in vitro 20:774-784.